Appendix V1.1: Conceptualisation paper

N.B. This is a full manuscript that has been submitted to a scientific journal for review. Inclusion as an output in this technical report doesn't preclude the ability to publish.

Blue, green and in-between; setting objectives for and evaluating wetland vegetation responses to environmental flows

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Abstract

Floodplain and wetland vegetation communities have a high intrinsic value and play a critical role in supporting a wide range of ecosystem functions, services and human values. Throughout the world, changes to flow regimes resulting from river regulation, water extraction and other human activities (e.g. land clearing) have compromised many of these values, leading to widespread efforts to restore wetland vegetation through the delivery of environmental flows. Setting appropriate objectives and targets for, and evaluating wetland vegetation responses to, environmental flows is challenging. This is because of the inherently variable and dynamic nature of wetland vegetation, and the human values it supports, in space and time. Here, we propose four principles to guide the development of robust objectives and evaluation approaches for the adaptive management of environmental flows with respect to vegetation outcomes. First, we assert a need for more explicit, direct and defensible alignment of vegetation management objectives, targets and indicators to broader ecological, socio-cultural and economic values. Second, we propose a framework for indicator selection across multiple scales and levels of ecological organization. Third, we emphasize the necessity of evaluating vegetation condition and responses to watering in relation to a more nuanced understanding of temporal dynamics, nested flow regime components, and long-term trajectories of change. Finally, we discuss the importance of considering the effects of non-flow modifiers on vegetation responses to environmental flows. We highlight key knowledge needs required to support the implementation of these principles, particularly the urgency of improving our understanding of human values of wetland vegetation and the attributes which support these.

Highlights

- Evaluating wetland vegetation responses to flows is challenging but critical.
- Objectives need more explicit alignment with management goals and values.
- Multiple spatial and temporal scales need to be incorporated.
- Linking vegetation structure to function and human values is a key knowledge need.

Keywords: ecological function; restoration; riparian vegetation; vegetation condition

Declaration of interest:

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1. Introduction

The degradation and loss of inland wetlands, including rivers, streams, backwaters, floodplain wetlands and lakes, are of significant concern globally (Davidson 2014). Wetlands are widely recognized as disproportionately valuable components of the landscape, attributed with high intrinsic values and supporting a broad range of critical ecosystem functions and services (Capon et al. 2013, Capon and Pettit 2018). Wetlands are also amongst the most modified ecosystems on the planet and are highly vulnerable to a wide range of human pressures, including climate change (Zedler and Kercher 2005, Capon et al. 2013). Wetland degradation can be attributed to many anthropogenic influences (e.g. clearing, grazing, cropping, urbanization, pollution) but the alteration of hydrological regimes due to river regulation and water extraction is often a major driver (Kuiper et al. 2014, Kingsford et al. 2015, Reis et al. 2017).

Environmental flows are increasingly being used as a strategy globally to protect and restore wetland ecosystems by managing flows and allocating water to generate environmental outcomes (Arthington 2012). Management of environmental water, however, is complex and presents many challenges (Harris and Heathwaite 2012, Bond et al. 2014). Decreased water availability, coupled with increased water demands for consumptive use, can escalate competition for water, not only between consumptive and environmental users, but between different environmental users. In addition, there are often perceived risks associated with the delivery of environmental flows, including flood damage to property and infrastructure (MDBA 2013), potential water quality degradation, e.g. hypoxic black water events (Whitworth and Baldwin 2016), increased bank erosion (Vietz et al. 2018) and promotion of exotic species' invasions (Howell and Benson 2000, Taylor and Ganf 2005, Colleran and Goodall 2014). Consequently, there is a growing need to both clearly articulate the values that wetlands support and to provide rigorous evidence of the outcomes of environmental flows in achieving management goals and informing adaptive management. To achieve this, the development of appropriate objectives, quantitative targets and relevant indicators that are sensitive to environmental water management is critical. The importance of setting clear objectives within an adaptive management framework is not a new issue and there is a body of literature around this field (Kentula 2000, Lindenmayer and Likens 2010, Lauber et al. 2011, Lindenmayer et al. 2012, King et al. 2015, Horne et al. 2017, Gawne et al. 2018). Here we focus on the challenges specific to wetland vegetation.

Vegetation is often a key focus of wetland restoration projects because of its intrinsic value as well as its role in supporting a wide range of ecosystem functions and services, such as provision of habitat for fish and birds, riverbank stabilisation and the cycling of nutrients. These ecosystem functions and services in turn support many environmental, socio-cultural and economic values (Capon and Pettit 2018). Wetland vegetation is also typically sensitive to hydrologic changes and relatively straightforward to monitor, both in the field and via remote sensing technologies, making it an ideal indicator of wetland condition and response to management interventions. On the other hand, wetland vegetation can be highly dynamic, responding to watering, as well as many other non-flow pressures and stressors (e.g. grazing, fire, salinisation), over a range of spatial scales and levels of ecological organization, i.e. from individual plants to landscapes. As a result, setting appropriate objectives and targets, and selecting effective indicators for monitoring and evaluating wetland vegetation responses to environmental water management presents some significant challenges (Matthews et al. 2009, Capon and Capon 2017).

Here, we propose four key principles to guide the development of robust and defensible management objectives and targets for wetland vegetation as well as the selection of appropriate indicators for monitoring and evaluating wetland vegetation responses to environmental flows. First, we provide context by outlining some of the main challenges involved in the design and implementation of vegetation monitoring and evaluation programmes. We then assert the need to consider: 1. alignment of vegetation indicators to management objectives, wetland function and the delivery of ecosystem services; 2. multiple scales and levels of ecological organization; 3. temporal context and the influence of nested flow regimes; and 4. non-hydrologic modifying factors. We conclude by identifying major knowledge needs to support the implementation of these principles.

2. Key challenges to monitoring and evaluating wetland vegetation responses to environmental flows

Common objectives and targets for wetland vegetation condition and responses to environmental flows focus on indicators that emphasize stability or improvements in attributes (e.g. increased/maintained presence, extent, canopy condition), often associated with dominant (woody) iconic or threatened taxa (e.g. Moss 2007, Matthews et al. 2009, Overton et al. 2009, MDBA 2014). Many Ramsar wetlands, for example, have management objectives associated with maintaining specific extents of key vegetation types, usually in relation to the areas occupied by particular vegetation communities at the time of listing, or some other reference condition (OEH 2012, Gell et al. 2016). Spatial or temporal variation in population or community structure within vegetation communities, which may be functionally significant (e.g. variation in stem density), are often overlooked. This 'vegetation map' driven approach often disregards the potential for landscape-scale patterns in wetland vegetation to fluctuate over time in response to changing conditions. Such 'dynamic patch mosaics' of vegetation communities are characteristic of arid and semi-arid floodplain landscapes and probably contribute to the function and long-term ecological resilience of these systems, such as by providing a dynamic range of temporally variable habitat types for fauna (van Coller et al. 2000).

Condition of wetland vegetation communities is often described with respect to the diversity of native plant species and especially the richness and abundance of aquatic and amphibious plant taxa (Casanova 2011). Consequently, there is often an implicit expectation that restoring environmental flows to wetlands should lead to an increase in the richness of native aquatic and amphibious plant species. However, it is also the case that many highly valued and relatively unmodified wetlands support either near monocultures (e.g. *Phragmites* reed beds) or highly dynamic vegetation communities which shift rapidly in composition and structure making accurate assessments of changes in species diversity very difficult (Capon 2003, James et al. 2007). Furthermore, restored wetlands may support fewer species than degraded wetlands (Wassens et al. 2017). Where inundation regimes are restored to wetlands previously subject to drying as a result of river regulation, for example, vegetation responses may include a gradual decline in species richness over longer periods of time because of the establishment and expansion of dominant clonal aquatic plants (Matthews et al. 2009, Wassens et al. 2017).

The presence, extent and abundance of exotic plants, or terrestrial taxa, is widely used to infer degraded conditions in wetlands (Catford and Jansson 2014, Bino et al. 2015b). In dryland wetlands of inland Australia, for example, the presence and increased abundance of chenopod shrubs is a widespread and regular vegetation response to drier conditions which tends to rapidly reverse in response to rewetting (Capon 2003, Capon and Reid 2016). Such ‘encroachment’ of terrestrial shrubs during dry periods may not necessarily represent ecological degradation, therefore, but rather different phases of highly variable systems. Furthermore, encroachment of terrestrial shrubs could play an important functional role by protecting top soil (Potts et al. 2010), and therefore wetland soil seed banks, from wind erosion and may trap wind-blown propagules as well as provide physical habitat for fauna (e.g. Read 1995). On the other hand, such ‘terrestrialisation’ might be considered as indicative of a decline in ecological conditions following prolonged dry periods if functional wetland values are altered (Catford et al. 2011, Bino et al. 2015b).

Current approaches to monitoring wetland vegetation include both large scale assessments of vegetation extent and condition (e.g. greenness) via remote sensing techniques, as well as a variety of on-ground approaches that range from rapid assessments to detailed floristic surveys (Cunningham et al. 2007, Cunningham et al. 2009, Lawley et al. 2016). Selection of indicators and evaluation approaches applied to wetland vegetation therefore tends to reflect the focus and constraints of the methods employed (e.g. resolution of available data) or legacies of past monitoring projects (e.g. the need to align with historic datasets). As a result, much wetland vegetation monitoring focuses on relatively generic compositional and structural attributes such as species richness, vegetation cover and community composition. Processes contributing to the survival of individual plants (e.g. recruitment) or the dynamics of populations and communities (e.g. dispersal, competition and facilitation) are less commonly considered (Lawley et al. 2016).

Evaluation of wetland vegetation responses to environmental flows is further complicated by problems associated with attribution. Effects of environmental flows are often inferred, for example, from BACI (i.e. before-after-control-impact) sampling designs or, where this is not possible because of a lack of control sites, before and after comparisons (e.g. Wassens et al. 2017). The potential influences of seasonal variation, antecedent conditions (e.g. time since last wet) and longer-term trajectories of wetland dynamics (e.g. legacies of past land management practices) are difficult to disentangle as are the effects of a wide range of non-hydrological modifiers such as fire, grazing and other human interventions (e.g. weed control). Furthermore, wetland vegetation responses to watering are likely to be shifting in many parts of the world in relation to a range of climatic changes such as carbon dioxide fertilization (Saintilan and Rogers 2015).

3. Principles to guide robust evaluation of wetland vegetation responses to environmental flows

3.1 Align indicators to management objectives, ecological functions and human values

Wetland vegetation supports a wide range of critical ecological functions that deliver ecosystem goods and services including: 1) regulating (e.g. of climate, water, soil etc.), 2) habitat (e.g. nurseries, corridors), 3) production (e.g. food, raw materials) and 4) information (e.g. cultural, recreation etc.) functions (de Groot et al. 2002, Capon et al. 2013). The functions and services supported by wetland vegetation and the role of vegetation in delivering these, however, vary widely in relation to vegetation attributes across multiple scales as well as other modifying factors, e.g. wetland type, location, anthropogenic pressures etc. (Capon and Pettit 2018). Functions and values associated with a particular plant species or vegetation community can therefore shift considerably within its range and/or over time.

High level goals for conserving, managing and restoring wetland vegetation typically encompass both the implicit value placed on floristic biodiversity itself, as well as the role of plants and vegetation in supporting highly valued ecosystem functions, goods and services. In the Environmental Watering Strategy for Australia's Murray-Darling Basin Plan (MDBA 2014), for example, the rationale for watering wetland vegetation includes its capacity to provide food and habitat for fauna, maintain water quality, promote soil and bank stability and support a range of social, economic and cultural values. Explicit definition of vegetation objectives for environmental flows that will support these broader environmental, socio-cultural or economic values, however, are often lacking with evaluation typically focusing on a relatively narrow suite of indicators that represent aspects of the intrinsic values of vegetation or 'naturalness'. By default, this selection of indicators tends to be influenced more by the adherence to standard monitoring approaches than their relevance to specific management goals or human values.

In the case of the Murray-Darling Basin Plan's Environmental Watering Strategy, for instance, 'expected outcomes' of environmental watering mostly concern the maintenance or improvement of the extent and condition of key wetland vegetation community types, with condition associated mainly with tree health and species diversity (MDBA 2014). Rarely do the objectives relate to more specific ecosystem functions, despite these often having more restrictive requirements if they are to be achieved. For example, some waterbirds are dependent on vegetation such as the shrub, tangled lignum (*Duma florulenta* (Meisn.) T.M.Schust.) for nesting (Maher and Braithwaite 1992, Kingsford and Johnson 1998). This species exhibits desirable structural attributes that only arise where the plant is inundated frequently for prolonged periods (e.g. large tangled clumps with open space in-between), despite the ability of that plant to also persist under much less frequent or prolonged flooding regimes (where it's structural attributes are completely different, e.g. small, scattered shrubs).

We propose that objectives for, and indicators of, wetland vegetation response to environmental flows should be more directly and defensibly related to ecological functions and environmental, sociocultural and economic values (see Table 1 for examples). In most cases, this would include indicators which refer to the presence, extent and condition of key iconic or threatened plant taxa and/or vegetation communities which are intrinsically valued. However, we suggest that a greater emphasis also be given to indicators that describe the attributes of vegetation that help support their associated ecosystem functions, goods and services (Capon and Pettit 2018). For instance, where a specific vegetation type plays a role in providing important habitat for fauna (e.g. waterbird breeding), indicators are needed that explicitly describe shifts in the presence, extent and condition of patches of the vegetation that display the attributes necessary to support this function (e.g. a certain size and density of shrubs). Effects of environmental flows on some human values of wetland vegetation could be similarly evaluated by focusing more explicitly on specific indicators within relevant areas. Provision of shade in important recreational sites, for example, could be considered by investigating changes in canopy cover within these areas. Cultural indicators could be similarly developed (Tipa and Nelson 2008), e.g. presence and abundance of particular sedge species used for traditional basket weaving, with the further benefit of facilitating the incorporation of traditional ecological knowledge into adaptive management practices.

Table 1. Examples of wetland vegetation indicators of relevance to potential management objectives, ecosystem functions and human values associated with wetland vegetation across a range of spatial scales.

Potential management objectives / ecosystem functions or human values associated with wetland vegetation	**Relevant spatial scale[^]**	**Examples of relevant vegetation indicators**
Maintain health of a culturally significant tree	local	Physiological responses to flow (e.g. new tip growth, leaf die-off, bark cracking), crown density and extent
Provide habitat for arboreal fauna	local, wetland	Presence and abundance of large, hollow-bearing floodplain trees
Provide cultural resources (e.g. sedges for harvesting for basket weaving)	local, wetland	Presence, extent and biomass of particular plant taxa used in basket weaving
Provide habitat and food resources for small-bodied fish	local, wetland	Cover and structural complexity of wetland plants (e.g. submerged, floating leaves, emergent sedges)
Provision of healthy water quality to support recreational activities (e.g. swimming, boating)	local, wetland	Cover, richness and structural complexity of wetland plants
Maintain and improve population status of threatened species	jurisdictional scale of listing	Population demographics (e.g. age structure), reproductive success (e.g. flowering, seed production, viability), distribution and extent of populations
Provision of waterbird breeding habitat	landscape	Presence of contiguous, different vegetation communities to support critical life stages (e.g. nesting habitat, feeding habitat)

Provide longitudinal connectivity for movement of terrestrial fauna	landscape	Continuity and width of riparian vegetation, longitudinally and with upland vegetation

^ Spatial scales are not prescriptive and are provided as examples of potentially relevant scales. Scales will need to be defined on a case-by-case basis by organisations responsible for management.

Hence, improving the management relevance of monitoring and evaluation of wetland vegetation responses to environmental flows in many cases may mostly be a matter of analyzing conventional indicators at more applicable and nuanced scales and levels of ecological organization. In other cases, such an approach may necessitate the collection of data to inform new indicators, e.g. recording the presence of tree hollows or mistletoe for bird habitat. Such explicit links between vegetation indicators and management objectives would greatly improve our understanding of wetland ecosystems and strengthen our capacity to inform adaptive management and learning.

We recognize that an emphasis on functional traits may be seen as overly 'anthropocentric'. Indeed, such an approach can certainly lead to some unconventional outcomes for evaluating vegetation condition or choosing to implement management actions. For instance, the presence of exotic species may promote functional characteristics that are deemed to be of management significance, e.g. protection of top soil (Capon and Palmer 2018). Consequently, the presence, extent and richness of exotic species might not necessarily imply degraded wetland condition or the need to implement management actions in this context. This may rely on evaluating the value of the functional service provided by the exotic species, against the value of the 'naturalness' of the wetland and the risk of further invasions. If 'nativeness' or 'naturalness' are themselves management objectives, or represent human values for a wetland, however, these will also need to be clearly associated with particular vegetation attributes that can be similarly evaluated (e.g. proportion of native species). The challenge therefore becomes one of identifying values, unpacking them into explicit objectives and assessing trade-offs between potentially conflicting objectives or values. A functional approach at least makes such trade-offs more transparent.

3.2 Consider multiple spatial scales and levels of ecological organisation

Monitoring and evaluating wetland vegetation condition and responses to environmental flows often occurs at a relatively local scale, typically defined by sampling units, with a focus on community level attributes, e.g. species richness, canopy cover, dominance of exotic species etc. Diversity in particular is frequently used as a key indicator of wetland vegetation condition and intervention response with associated objectives usually concerning the increase or maintenance of species richness and abundance, either overall or amongst particular plant groups (e.g. aquatic and amphibious taxa). Consideration of vegetation diversity at other levels of ecological organization is comparatively rare but may be highly significant to many management objectives. For example, a diverse 'patch mosaic' of vegetation community types (and states within community types), which may include the presence of some near-monocultural communities as well as more species rich vegetation types, is likely to be ecologically important at a landscape scale (Bino et al. 2015a) and may be captured via measures of beta and gamma diversity. Waterbirds, for example, may require different types of vegetation patches in proximity to each other to support both breeding and feeding functions (Kingsford and Norman 2002).

At the population and species level, assessing processes (e.g. recruitment) and status requires evaluation at the appropriate scale and often consideration of multiple scales. For example, lack of tree recruitment within established patches of woody wetland vegetation may not necessarily be indicative of degraded conditions if recruitment is constrained in these places by shading from dense canopies (Righi et al. 2016) or potential allelochemical effects of leaf litter (Capon et al. 2017). In these circumstances, recruitment of such a tree species might be expected to occur more frequently at edges or in unwooded patches, akin to tree fall gap dynamics in rainforests albeit at a much larger scale. Maintaining or promoting a diversity of age cohorts within a woody species across a wetland landscape, rather than at the patch scale, may therefore be critical to the long-term resilience of that species in a variable landscape (George et al. 2005). Similarly, if we anticipate species' ranges to shift significantly at regional scales in response to the changing climate (James et al. 2017), assessment of the local extirpation or invasion of a particular species should be evaluated in relation to changes in its overall distribution so as to avoid local interventions (e.g. control of newly establishing populations) threatening the broader survival of a species.

To reflect current ecological understanding and limit perverse outcomes of adaptive management decisions, we propose that a greater range of spatial scales, temporal hydrological regimes and levels of ecological organisation need to be considered when setting environmental flow objectives for wetland vegetation and selecting relevant indicators to assess vegetation responses to watering (Tables 1 and 2). In general, wetland vegetation indicators will align with one of five levels of ecological organization: 1) individual plants; 2) populations (within species); 3) communities (multi-species assemblages); 4) landscapes ('vegscapes'); and 5) species (e.g. threatened species). Following Noss (1990), we further suggest that each of these hierarchical levels can be described by a range of compositional, structural and process indicators (Table 2). Appropriate scales and levels of ecological organization for particular attributes will depend on the particular management objectives, functions and values being assessed as these are also likely to shift depending on scale. At a local scale, for example, the health of individual trees (e.g. trees of cultural significance) may be important while maintaining a certain overall proportion of trees in good condition or a proportion of wooded patches in good condition may be more meaningful for managers operating at the landscape scale.

Table 2. Library of potential indicators of wetland vegetation responses to environmental flows across multiple hierarchical levels of ecological organization (individual, population, community, landscape and species) and temporal scales (flow pulse, short-term and long-term flow regimes). Following Noss (1990), indicators are categorized as being either compositional, structural or process. Relevant hydrological metrics at each hydrological regime scale are also provided. N/A indicates not applicable.

Hydrological regime scale	**Level of ecological organisation[1]**	**Examples of relevant functions, services and values**	**Vegetation indicators**		
			Composition	Structure	Process
Flow pulse (days to months) Relevant flow metrics: depth, duration, seasonal timing, magnitude, rate of rise and recession, velocity, soil moisture	I	Cultural significance, genetic relics, habitat	N/A	Canopy extent and architecture	Physiological responses to the flow (e.g. new tip growth, leaf die-off, bark cracking, water status), reproductive responses to the flow (e.g. flowering, seed production)
	P	Persistence biodiversity and species-specific functions (e.g. cultural, habitat, production)	Age structure, % dead, % flowering/ seeding, % viable seed	Cover, density, extent, height	Physiological responses to the flow (e.g. Δ in biomass, shoot length), reproductive responses to the flow (e.g. flowering, seed production)
	C	Biodiversity, habitat, regulating functions	Species composition, species richness, dominant species, functional groups, nativeness - applicable to extant and	Number of strata, structural attributes (e.g. cover, density, extent, height, age) of species, strata and other distinct compositional groups	Turnover (Δ in strata/compositional traits), physiological responses to the flow (e.g. Δ in biomass) within strata/compositional

Hydrological regime scale	Level of ecological organisation[1]	Examples of relevant functions, services and values	Vegetation indicators		
			Composition	Structure	Process
			seedbank communities, % viable seed		groups, reproductive responses to the flow (e.g. flowering, seed production) within strata/compositional groups
	L	Habitat, biodiversity, regulating, information functions	Composition and richness of vegetation community types, dominant communities	Extent, evenness, condition / greenness	Turnover (Δ in compositional and structural traits), terrestrialisation, contiguousness, hydrological connectivity between communities
	S	Persistence, biodiversity and species-specific functions (e.g. cultural, habitat, production)	Age structure, % dead, % flowering/ seeding, % viable seed	Cover, density, extent, height	Physiological responses to the flow (e.g. Δ in biomass, shoot length), reproductive responses to the flow (e.g. flowering, seed production)
Short-term flow regime (year(s) to decade)	I	Cultural significance, genetic relics, habitat	N/A	Canopy cover, extent and architecture, number and shape of hollows, biomass, height, age measurements (DBH*, tree cores)	Growth rate (e.g. Δ in height, biomass), Condition (e.g. Δ in canopy cover/density, bark cracking, new tip growth, leaf die-off, sapwood thickness)
Relevant flow metrics: time-since-last inundation, frequency of	P	Persistence, biodiversity and species-specific functions (e.g.	Age structure, % dead, % flowering/ seeding, % viable seed	Cover, density, extent, height	Growth rate (e.g. Δ in height, biomass, extent), Condition (e.g. Δ in cover, density), Mortality, Δ in seed viability,

Hydrological regime scale	**Level of ecological organisation**[1]	**Examples of relevant functions, services and values**	**Vegetation indicators**		
			Composition	Structure	Process
inundation, seasonal patterns of inundation, spatial patterns of inundation (magnitude / connectivity)		cultural, habitat, production)			recruitment / germination / Δ in age structure, dispersal (extent, distance, abundance, # of dispersal opportunities)
	C	Biodiversity, habitat, regulating functions	Species composition and richness, dominant species, functional groups, nativeness - applicable to extant and seedbank communities, % viable seed	Number of strata, structural attributes (e.g. cover, density, extent, height, age) of species, strata and other distinct compositional groups	Turnover (Δ in strata/compositional traits), trajectories, Δ in seed viability, dispersal (extent, distance, abundance, # of dispersal opportunities), germination
	L	Habitat, biodiversity, regulating, information functions	Community composition and richness, dominant communities	Extent, evenness, condition / greenness,	Turnover (Δ in compositional and structural traits), trajectories, terrestrialisation, contiguousness, hydrological connectivity between communities
	S	Persistence biodiversity and species-specific functions (e.g. cultural, habitat, production)	Age structure, % dead, % flowering/ seeding, % viable seed	Cover, density, extent, height	Growth rate (e.g. Δ in height, biomass, extent), condition (e.g. Δ in cover, density), Mortality, Δ in seed viability, recruitment / germination / Δ in age structure, dispersal (extent, distance, abundance, # of dispersal opportunities)
Long-term flow regime	I	Cultural significance, genetic relics, habitat		Canopy cover and architecture, number and	Growth rate (e.g. Δ in height, biomass), Condition (e.g. Δ

Hydrological regime scale	**Level of ecological organisation[1]**	**Examples of relevant functions, services and values**	**Vegetation indicators**		
			Composition	Structure	Process
(decade(s) to centuries) Relevant flow metrics: time-since-last inundation, frequency of inundation, seasonal patterns of inundation, spatial patterns of inundation, wet/dry sequence, maximum period dry				shape of hollows, biomass, height, age measurements (DBH, tree cores)	in canopy cover/density, bark cracking, new tip growth, leaf die-off, sapwood thickness)
	P	Persistence biodiversity and species-specific functions (e.g. cultural, habitat, production)	Age structure, % dead, % flowering/ seeding, % viable seed	Cover, density, extent, height	Growth rate (e.g. Δ in height, biomass, extent), Condition (e.g. Δ in cover, density), Mortality, Δ in seed viability, recruitment / germination / Δ in age structure, dispersal (extent, distance, abundance, # of dispersal opportunities)
	C	Biodiversity, habitat, regulating functions	Species composition and richness, dominant species, functional groups, nativeness - applicable to extant and seedbank communities, % viable seed	Number of strata, structural attributes (e.g. cover, density, extent, height, age) of species, strata and other distinct compositional groups	Turnover (Δ in strata/compositional traits), trajectories, Δ in seed viability, dispersal (extent, distance, abundance, # of dispersal opportunities), germination
	L	Habitat, biodiversity, regulating, information functions	Community composition and richness, dominant communities	Extent, evenness, condition / greenness,	Turnover (Δ in compositional and structural traits), trajectories, terrestrialisation, Δ in contiguousness, patterns of hydrological connectivity between communities

Hydrological regime scale	**Level of ecological organisation[1]**	**Examples of relevant functions, services and values**	**Vegetation indicators**		
			Composition	Structure	Process
	S	Persistence biodiversity and species-specific functions (e.g. cultural, habitat, production)	Age structure, % dead, % flowering/ seeding, % viable seed	Cover, density, extent, height	Growth rate (e.g. Δ in height, biomass, extent), condition (e.g. Δ in cover, density), Mortality, Δ in seed viability, recruitment / germination / Δ in age structure, dispersal (extent, distance, abundance, # of dispersal opportunities)

[*] DBH Diameter-at-breast-height (measured at 1.3m)
[1] Level of ecological organisation: I = individual, P = population, C = community, L = landscape, S = species

3.3 Consider temporal dynamics, trajectories and uncertainties

Wetland vegetation is inherently dynamic and responds to flow inundation regimes over multiple temporal scales within which key hydrological determinants themselves also vary (Table 2). For example, understory vegetation composition at any particular time may reflect recent antecedent conditions (e.g. time since last inundation, flood pulse timing, rates of rise and recession) while the composition and structure of soil seed banks is typically shaped by flood histories over longer periods (e.g. flood frequency) and population structures of long-lived woody species will reflect even longer hydrological regimes (e.g. spatially patterning of flood extent, inter-flood dry periods) (George et al. 2005, McGinness et al. 2013). This has several significant implications for the selection and evaluation of indicators of wetland vegetation condition and response to environmental flows.

First, vegetation indicators must be evaluated in relation to short-term antecedent and prevailing conditions. An increased extent and abundance of terrestrial plants during dry phases, for example, might not necessarily imply a degraded wetland condition but rather a relatively natural phase shift. Indeed, because of the potential functional significance of such plants during dry periods (e.g. provision of structural habitat), an absence of terrestrial invaders and a lack of plant cover in general is likely to be of much greater management concern. Similarly, in ephemeral and temporary wetlands and on riverbanks, an increase in species richness during a drying phase can represent a beneficial outcome of preceding watering actions since establishment of many wetland soil seed bank species is favoured by moist, rather than submerged, conditions (Capon 2016).

Second, attention must be given to longer-term trajectories of wetland vegetation dynamics, flood history and landscape alteration. For example, wetlands that are inundated following long periods of drying may exhibit greater increases in plant species richness in response to watering than wetlands which have been regularly flooded over several years (Wassens et al. 2017). Similarly, wetland vegetation affected by past human activities, such as clearing or alteration of flow regimes to promote timber growth, may still be on trajectories of change with current watering responses reflecting lag or legacy effects of past disturbances at both local and landscape scales (Thompson et al. 2018). Future scenarios also need to be considered in setting watering objectives for wetland vegetation, especially with respect to projected climatic changes because these are likely to result in significant shifts in the extent and character of many wetland vegetation communities and, in turn, the mixture of vegetation types across wetland landscapes (Finlayson et al. 2017).

To tackle the challenges raised by temporal variation, we recommend that objectives for and evaluation of wetland vegetation responses to environmental flows avoid reliance on historical, pre-disturbance reference conditions as a baseline. Instead, we recommend developing objectives and targets that: i) draw on socio-ecological values as discussed above; ii) reflect expectations of the rates and magnitude of change following management (Kopf et al. 2015); iii) incorporate dynamic reference points relevant to hydrological phases (e.g. inundation, drawdown, drying) and iv) are explicit with respect to their temporal relevance. Initially these targets may rely on expert opinion until data is available.

We also propose that assessment of wetland vegetation condition and responses be conducted within more nuanced temporal frameworks that permit management targets to be adjusted as understanding of short- and longer-term phase shifts and trajectories of vegetation change improves. Furthermore, we promote the inclusion of process indicators at various scales (Table 2), including measures of resilience and adaptive capacity, to better understand and assess temporal dynamics and trajectories of vegetation responses in the face of the high levels of uncertainty wrought by climate change. Measuring and evaluating the composition and structure of soil seed banks, for example, may be more informative than solely observing extant vegetation dynamics with respect to assessing the vulnerability of herbaceous wetland plant communities to climate change (e.g. Grieger et al. 2019). Similarly, objectives for environmental flows could also include measures of resistance and/or recovery of various vegetation attributes to other disturbances (e.g. fire or grazing). Finally, we emphasise the need to evaluate selected indicators of vegetation responses to environmental flows in relation to hydrologic attributes at an appropriately aligned scale (Table 2), e.g. percentage of individual plants flowering within a population in response to the rate of recession of a flow pulse, or the change in extent of reed bed communities over time in relation to spatial and temporal patterns of inundation.

3.4 Consider multiple interacting drivers

While hydrology typically has an overriding influence on the character and dynamics of wetland vegetation, many other factors can influence vegetation responses to flow via a range of complex interactions. Shading and litter from mature canopies, for example, can significantly affect the composition and structure of establishing understory communities following watering (Capon et al. 2017). Similarly, the sediment regime in a river system can modify the responses of understory riverbank vegetation to inundation; sediment deposition combined with inundation can have a negative effect on plant growth and survival (Lowe et al. 2010). Vegetation responses to watering actions can also be modified by many local and landscape-scale pressures and disturbances, e.g. fire, feral animals etc. (Douglas et al. 2016). Altered patterns of connectivity at landscape and regional scales will also affect dispersal of plant propagules and therefore patterns of genetic diversity and vegetation resilience over the longer-term (Jansson et al. 2005, Nilsson et al. 2010, Akasaka and Takamura 2012). Furthermore, projected climatic changes can be expected to strongly alter wetland vegetation responses to watering. Warmer temperatures, for instance, are very likely to have substantial effects on inundation patterns and soil moisture responses to environmental watering actions with significant implications for wetland vegetation outcomes (Capon et al. 2013).

Objectives for and evaluation of wetland vegetation responses to environmental flows therefore need to reflect the potential influence of non-flow modifiers as well as spatial and temporal variation of these. Designing monitoring programmes which specifically seek to improve our knowledge of complex interactions between flows and other pressures may be particularly beneficial for effective adaptive management as this will inform better integration and alignment of environmental water management with other wetland management strategies, e.g. grazing management, weed and feral animal control.

4. Knowledge needs

Overall, the principles outlined here require a more direct consideration of wetland vegetation processes, functions and human values in setting objectives for and prioritizing, designing and evaluating environmental flows. We assert that the aim of monitoring and evaluation in this arena should be to better understand, determine and predict the status and trajectories of wetland vegetation and associated functions, and other specified values, rather than solely focusing on its assumed intrinsic value. To achieve this, it is essential that we explicitly identify the spatial and temporal scales at which the functions and values of wetland vegetation manifest themselves. Consequently, there is a significant need for greater research to elicit human values associated with wetland vegetation, and to link vegetation attributes to these, as well as to ecological functions supported across multiple scales. Such research will be inherently transdisciplinary and necessitate significant attention to engagement and communication with a wide range of stakeholders. In doing so, there is considerable potential to support complex decision-making regarding the allocation of scarce water resources. In particular, there is a need for approaches that clearly articulate objectives, increase transparency, elucidate trade-offs, and promote alignment between management outcomes for vegetation and those of other wetland values (e.g. maintaining water quality and supporting fish and waterbird populations). Finally, there is a need to align water and land management in complementary ways, by considering the interacting effects of flow with other stressors such as grazing and weed management.

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Appendix V1.2: Conceptualisation Research Activity Report

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Document purpose: Summary document to capture the outputs and outcomes from the Conceptualisation component of the MDB EWKR Vegetation theme. This document complements and refers to other outputs rather than duplicates information.

• Research Question

How do we define our vegetation response objectives to consider multiple trait responses, ecological levels of organisation, functions and values and spatio-temporal scales?

• Methods

This component evolved through the research planning phase. The overarching aim and priority research topics defined in the MDB EWKR program are very broad and do not easily lend themselves to a research portfolio that is achievable within the budget and timeframes of MDB EWKR. In order to focus the research direction while still being applicable to a range of locations and watering situations, we needed to focus and refine the research priorities.

We started by unpacking what is meant by water-dependent vegetation outcomes, particularly around diversity responses of understorey and wetland vegetation. Maintaining or improving vegetation condition or diversity are objectives of environmental water management common to wetlands across the Murray–Darling Basin. Targets associated with vegetation objectives tend to emphasise diversity and/or stability as desirable characteristics, despite recognition that many highly valued wetlands may support virtual monocultures or highly dynamic vegetation communities. Managers require a clear understanding of the vegetation response objective, the effect of flow on vegetation response, and an understanding of how modifiers or non-flow drivers (e.g. climatic conditions) influence predicted vegetation responses.

Research undertaken in this component will focus on defining and conceptually understanding the types of vegetation responses that occur across different vegetation traits (e.g. compositional, structural and process), levels of ecological organisation (e.g. species, community, vegscape), and spatial and temporal scales. Given the range of vegetation responses that could be assessed and the importance of assessing outcomes against clearly defined objectives, we wanted to develop a vegetation response framework to assist in the articulation of vegetation response objectives.

• Results

The following information was also reported on in the Murray-Darling Basin Environmental Water Knowledge and Research Project: Multi-Year Research Plan 2016-2019, Section 3.3.2. (MDFRC 2016).

Additional communication of these results can also be found in the following appendices within MDB EWKR Vegetation Theme summary report (the document to which this is an appendix).

- Paper: Campbell et al (submitted), *Blue, green and in-between; setting objectives for and evaluating wetland vegetation responses to environmental flows.* Submitted to Ecological Indicators
- Presentation: Campbell et al 2016. *Vegetation outcomes: what are we seeking and why?* Australian Society of Limnology Conference, Ballarat, 27th September 2016
- Article: *Grow with the flow*, RipRap V40, 2017, pp 16-18, Australian River Restoration Centre, Canberra

Vegetation responses — what are we watering for and why?

Wetland and floodplain plants are critical components of both aquatic and terrestrial ecosystems. They have intrinsic value, but also provide ecosystem functions that support economic, social and environmental values. Ecosystem functions include the supply of energy to support food webs, provision of habitat and dispersal corridors for fauna, (Bornette & Puijalon 2011; Boulton & Brock 1999), and contribute to other ecosystem services such as nutrient and carbon cycling, and water and sediment oxygenation (Aldridge & Ganf 2003; Baldwin *et al.* 2013; Boulton & Brock 1999; Brookes *et al.* 2005). Additionally, they have aesthetic, cultural and recreational values.

The diversity of species recorded in wetland and floodplain habitats across the Murray–Darling Basin is in excess of 800 species (Campbell and Nielsen 2014). These species take a range of structural forms, from floating ferns to 600-year-old trees, and provide a range of functions at different locations and different times. Given this complexity, it is critical that the following processes are considered in guiding management decisions: (i) vegetation management objectives need to be clearly expressed; (ii) relationships between management objectives and management interventions need to be represented in conceptual models along with the variables that modify these relationships; and (iii) the uncertainties around these relationships need to be expressed. This process underpins the development of sensitive and appropriate indicators and reveals key knowledge gaps that can be addressed through monitoring and research.

Vegetation outcomes from environmental flow management may seek to achieve objectives that are focused on compositional, structural or process responses. The situation is further complicated because objectives may be scale dependent, with objectives for a landscape providing context for smaller-scale objectives that will vary from location to location (e.g. improve condition of adult trees in some areas, or recruitment of juvenile trees, or control seedling recruitment in other areas). Once specific management objectives are defined, conceptual models can be developed that represent the relationships between the objective and the flow regime. These inform management actions and the expected outcomes. The clarification of objectives and development of conceptual models aid in both determining specific water requirements and the development of monitoring and research programs. Objectives and conceptual models are also useful tools in communicating the rationale of decisions and outcomes to stakeholders.

Vegetation response framework

Here we present a framework that aims to assist in the development of more specific vegetation objectives that support the function and services provided by vegetation. Delivering environmental water to achieve objectives requires conceptual models that summarise understanding of the relationships between a particular objective and environmental water delivery. We propose that in building these conceptual models, the influence of flow across temporal scales needs to be considered. In addition, we wanted this framework to consider the context in which environmental watering decisions are made, in terms of water availability, constraints to the delivery of flows and the influence of complementary management.

Response traits and levels of ecological organisation

When considering vegetation responses, there are three broad categories of responses that may be included in managers' objectives, specifically composition, structure and process. These responses may occur at different levels of ecological organisation, ranging from landscape to individual plant responses. These perspectives have been synthesised in the conceptual model adapted from Noss (1990) (Figure 1).

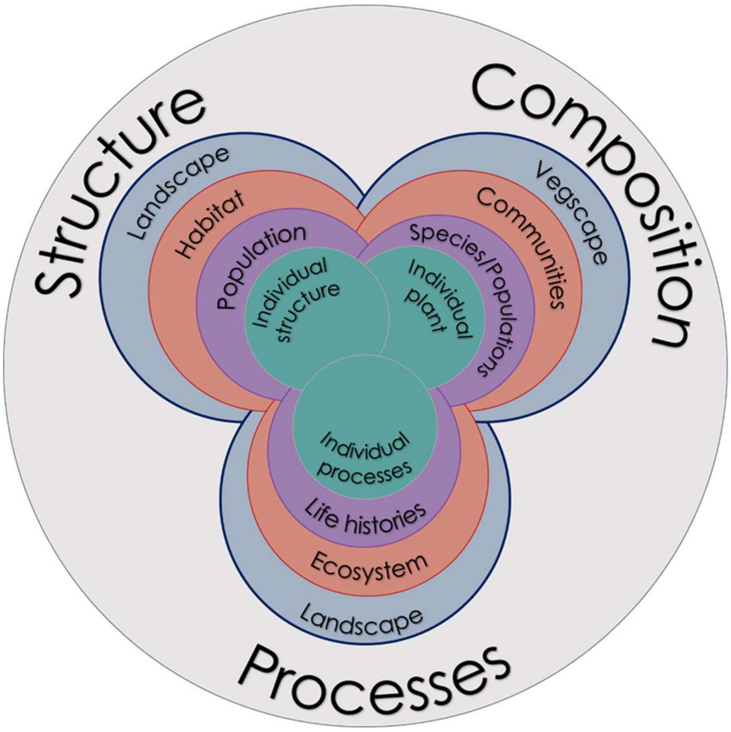

Figure 1. Vegetation attributes and levels of ecological organisation.

For example, objectives may be focused on:

- promoting high species diversity within a wetland following inundation (composition and communities)
- maintaining large, hollow-bearing River Red Gum trees at a particular floodplain location (structure and population)
- increasing the abundance of Moira Grass (composition and species)
- stimulating germination of Black Box trees (processes and life-histories) to improve the age-class structure at a site (structure and population)
- maintaining a spatial array of reed beds, open water, and woodland communities (composition and vegscape)
- increasing the abundance and complexity of structural wetland plants (e.g. submerged, floating leaves, emergent sedges) (structure and habitat)
- maintaining large, dense canopy cover in Lignum shrubland (structure and population).

Functions and services

When considering the functions and services provided by vegetation, these can be grouped into four different types: habitat, regulating, production and information (Figure 2) (adapted from de Groot *et al.* 2002 and Capon *et al.* 2013). Examples of the kinds of functions and services provided under each group are given in Figure 2. For example, vegetation can provide habitat in terms of nursery habitat for fish, corridor habitat for the movement of birds, or structural habitat for frogs. When setting objectives, this model allows scope to incorporate both ecological functions and services (largely included under habitat and regulating functions), as well as economic and social functions and services, such as food sources (e.g. honey production from River Red Gums), recreational values (e.g. improving the submerged habitat at important fishing locations) and cultural values (e.g. health of scar trees or the maintenance of totem species).

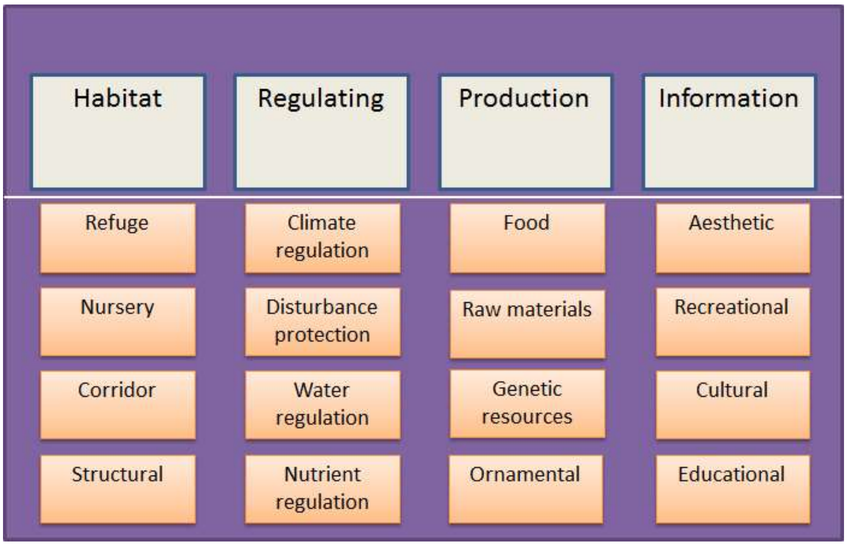

Figure 2. Structural grouping of potential functions and services provided by vegetation.

Nested flow regimes

Responses of vegetation to flows will be influenced by flow history. We propose that three temporal scales collectively shape vegetation communities including (i) flow pulses, representing inundation events lasting days to months, (ii) short-term flow regimes that characterise flow history over 1–10 years, and (iii) long-term flow regimes that characterise flow history over decadal time spans (Figure 3).

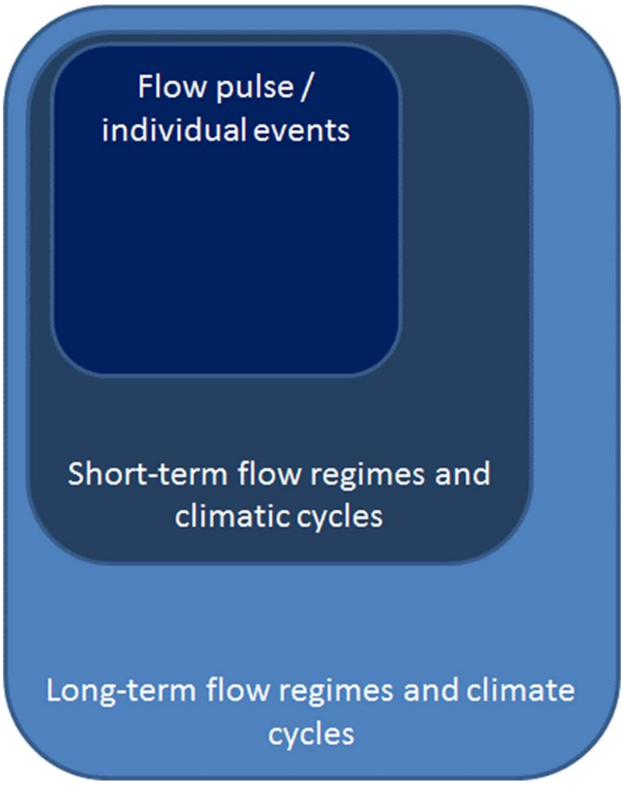

Figure 3. Model of nested flow regimes that can influence vegetation.

1. **Long-term** (decadal) cycles of wet and dry periods. At this scale, flow influences landscape patterns of vegetation, such as the types, distributions and relative abundance of different vegetation communities. The key flow characteristics that are expected to be important at this temporal scale are described below:

- average inundation frequency and patterns of frequency
    - an important determinate of community distribution on decadal time scales
- average and maximum period without inundation
    - an important disturbance for communities such as forests, marshes and reed beds
    - an important determinant of community distribution on decadal time scales
- wet sequence duration (number of sequential years in which inundation occurs)
    - an important opportunity for forests, woodlands and shrublands to expand their distribution
    - an important disturbance for some ecosystems
- average and maximum inundation depth and duration
    - a disturbance for some ecosystems
    - an important determinant of community distribution on decadal time scales
- magnitude and connectivity of inundation
    - an important determinant in species dispersal patterns and transport of nutrients and sediment
- patterns of inundation seasonality
    - an important determinant of species distribution on decadal time scales.

These flow regime characteristics interact with landform and key climate variables including average, maximum and minimum rainfall and temperatures to determine landscape vegetation composition, structure and processes.

2. **Short-term** (1–10 years) flow regimes. At this scale, flow influences the composition of ecosystems and the condition of populations within those systems. The important flow characteristics at this scale are similar to those that are important for long-term flow regimes; however, the vegetation responses are finer scale (in terms of the magnitude of the response) in recognition of the longer time frames over which landscape vegetation patterns change. The key flow characteristics that are important at this scale are:

- inundation frequency and the sequencing of inundation
    - for trees and long-lived shrubs, frequency is important in meeting water requirements for persistence and recruitment opportunities
    - flow frequency influences seedbank and rhizome viability
- maximum period without inundation
    - both prolonged inundation or prolonged drought may cause a decline in the health and persistence of trees and woody understorey species

    - both prolonged inundation or prolonged drought may reduce seedbank and rhizome viability

- time-since-last inundation
    - an important determinant of vegetation condition and seedbank and rhizome viability

- wet sequence duration (number of sequential years in which inundation occurs)
    - an important opportunity for trees and long-lived shrubs to expand their distribution
    - an important disturbance for species intolerant of inundation, which will influence seed availability over time
    - influences condition and recovery trajectories

- average and maximum inundation depth and duration
    - most species have limits to the depth or duration of inundation that they can tolerate and so this can act as a filter or disturbance

- magnitude and frequency of hydrological connectivity
    - an important determinate in species dispersal patterns and transport of nutrients and sediment

- seasonal patterns of inundation
    - species cued to germinate and/or grow in different seasons will be influenced by seasonality in their extant distributions and abundance of propagules

These flow regime characteristics interact with landform and key climate variables including average, maximum and minimum rainfall and temperatures to determine vegetation composition, structure and processes. There is also an important interaction with long-term flow regime characteristics, in that the establishment of long-lived vegetation will have an influence on the understory that develops at the site. The processes are not well understood, but it is likely to be due to a variety of factors including the changes in the microclimate under the canopy (e.g. light, temperature), changes in soil properties, competition for nutrients and water and allellopathic interactions.

3. **Flow pulses/individual events**. At this scale, key flow characteristics influence individual plant responses, which may include growth, reproduction, germination, dispersal, quiescence or death. The important flow characteristics include:

- depth
    - individual plants have limits to the depth of inundation that they can tolerate. Their tolerance will be influenced by species characteristics, but also the condition they are in when inundated. For individuals whose tolerance is exceeded, inundation will act as a disturbance leading to declines in condition or death.
    - for some species, depth is an important habitat characteristic providing resources and an opportunity to successfully compete for those resources

- duration

- individual plants have limits to the duration of inundation that they can tolerate. Their tolerance will be influenced by species characteristics, but also the condition they are in when inundated. For individuals whose tolerance is exceeded, inundation will act as a disturbance leading to declines in condition or death.
- duration is also important for species that require inundation to complete either their entire life-cycle or a particular stage. It is important that the duration is equivalent to the time required for the species to complete development, or there will be long term implications for the population.

- rate of recession
    - most aquatic plants have the capacity to tolerate a range of habitat conditions associated with cycles of wetting and drying. The capacity of plants to deal with the changes associated with drying is limited, and if the rate of drawdown is too rapid this will act as a disturbance for the plant, essentially shortening the duration of the inundation.

- season of inundation
    - timing is important as day length and temperature act as cues for germination and reproduction
    - seasonal timing is also important as temperature and light influence the plant's productivity or the productivity of competing species that may influence the plant's capacity to capitalise on the opportunity
    - timing may also influence external processes such as sediment microbial processes which may influence nutrient or oxygen availability within the sediments

- magnitude of hydrological connectivity
    - an important determinate in species dispersal patterns and transport of nutrients and sediment

- flow velocity
    - velocity exerts a physical stress on individuals that can lead to either scouring or sedimentation which may subsequently lead to death or reduced production
    - velocity may also influence the availability of nutrients and carbon dioxide in the water column, which may affect productivity

- turbidity/euphotic depth
    - turbidity affects the light available to submerged plants, which will affect their productivity
    - turbidity, combined with increased water depth, can reduce the light reaching submerged plants below the level required for plant growth
    - higher turbidity may result in the deposition of sediment on leaves and limit productivity.

Combining the components into a framework

Consideration of response traits and levels of ecological organisation, and functions and services and flow regimes on different temporal scales helps to shape objectives for targeted vegetation responses (Figure 4). The relationship between objectives and the proposed flow regime to meet the objectives is shown as a cyclic process to acknowledge that the flow design needs to incorporate/consider flow conditions (e.g. water availability), non-flow drivers, and any complementary actions, and that objectives may need to be revised/revisited if a suitably designed flow cannot be achieved because of constraints such as water availability.

With monitoring, the actual vegetation response can be compared to the predicted vegetation response and improve predictive capacity for future flows.

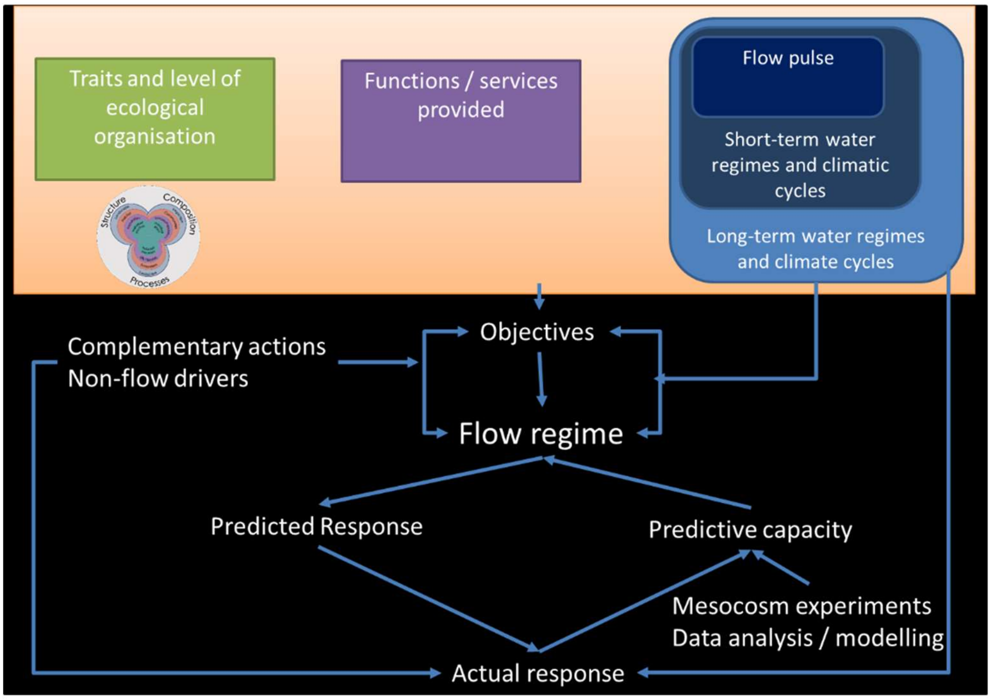

Figure 4. Framework incorporating different vegetation response traits, levels of ecological organisation, functions and services, and temporal scales of flow regime into an adaptive management framework.

Further work has refined our display of the vegetation response model as shown in Figure 5. This depiction provides more detail around the framework components; however Figure 4 provides a good representation of the links to the broader adaptive management process around planning, delivering and assessing responses to environmental flows.

Continued work, particularly associated with the development of our research paper, *Blue, green and in-between; setting objectives for and evaluating wetland vegetation responses to environmental flow*, lead to the identification of indicators for different trait responses (e.g. composition, structure or process) to environmental flows across multiple hierarchical levels of ecological organisation (individual, population, community, landscape and species) and temporal scales (flow pulse, short-term and long-term flow regimes) (Table 1).

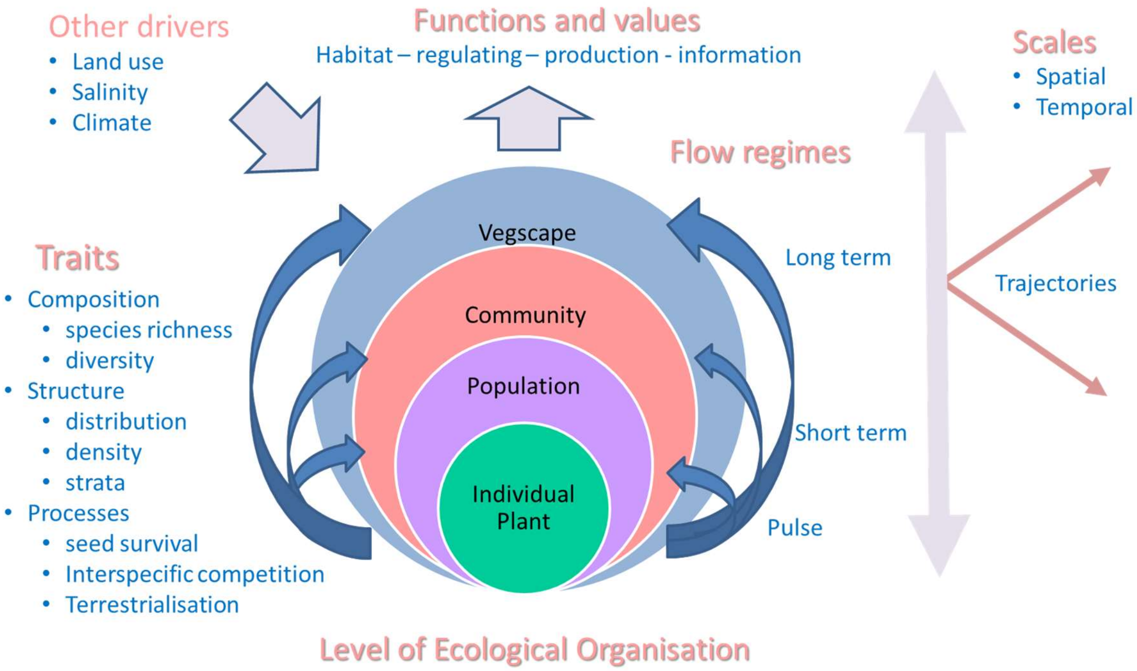

Figure 5. Vegetation response framework, incorporating five key components: 1) different levels of ecological organisation; 2) different trait responses at each of the levels of organisation; 3) ecological, socio-cultural and economic functions and values of different vegetation responses; 4) temporal dynamics including the influence of nested flow regimes on long-term trajectories of change; and 5) modifying effect of non-flow drivers.

• Discussion / applications

The concepts within this component feed directly into the planning stages of environmental water decisions at a range of management scales. Clearly defining the vegetation response objective has flow on effects in terms of designing flow regimes to meet the objectives, developing monitoring / research programs to detect a response and therefore evaluating the outcomes from the delivery of environmental water.

• Conclusions / further work

The challenge now is to operationalise the framework and guiding principles and develop them into useful decision support tools for water decision makers operating at a range of scales (e.g. local/ wetland scale, regional, State-based, Basin-scale). There is a range of research and consultation which could help inform this process, such as: i) workshop the utility of the framework with a diversity of water decision makers; ii) review existing processes to 'scale-up' information from plot to landscape scales from other disciplines; iii) develop consistent classification systems for non-woody vegetation at a range of levels of ecological organisation; iv) better understand relationships between function and value for vegetation responses; v) better alignment or development of response indicators for different vegetation trait responses at different levels of ecological organisation and different spatial and temporal scales; vi) develop better predictive capacity around response indicators, flow regimes and non-flow drivers.

Table 1. Library of potential indicators of wetland vegetation responses to environmental flows across multiple hierarchical levels of ecological organization (individual, population, community, landscape and species) and temporal scales (flow pulse, short-term and long-term flow regimes). Following Noss (1990), indicators are categorized as being either compositional, structural or process. Relevant hydrological metrics at each hydrological regime scale are also provided. N/A indicates not applicable.

Hydrological regime scale	**Level of ecological organisation**[1]	**Examples of relevant functions, services and values**	**Vegetation indicators**		
			Composition	Structure	Process
Flow pulse (days to months) Relevant flow metrics: depth, duration, seasonal timing, magnitude, rate of rise and recession, velocity, soil moisture	I	Cultural significance, genetic relics, habitat	N/A	Canopy extent and architecture	Physiological responses to the flow (e.g. new tip growth, leaf die-off, bark cracking, water status), reproductive responses to the flow (e.g. flowering, seed production)
	P	Persistence biodiversity and species-specific functions (e.g. cultural, habitat, production)	Age structure, % dead, % flowering/ seeding, % viable seed	Cover, density, extent, height	Physiological responses to the flow (e.g. Δ in biomass, shoot length), reproductive responses to the flow (e.g. flowering, seed production)
	C	Biodiversity, habitat, regulating functions	Species composition, species richness, dominant species, functional groups, nativeness - applicable to extant and	Number of strata, structural attributes (e.g. cover, density, extent, height, age) of species, strata and other	Turnover (Δ in strata/compositional traits), physiological responses to the flow (e.g. Δ in biomass) within strata/compositional

Hydrological regime scale	Level of ecological organisation[1]	Examples of relevant functions, services and values	Vegetation indicators		
			Composition	Structure	Process
			seedbank communities, % viable seed	distinct compositional groups	groups, reproductive responses to the flow (e.g. flowering, seed production) within strata/compositional groups
	L	Habitat, biodiversity, regulating, information functions	Composition and richness of vegetation community types, dominant communities	Extent, evenness, condition / greenness	Turnover (Δ in compositional and structural traits), terrestrialisation, contiguousness, hydrological connectivity between communities
	S	Persistence, biodiversity and species-specific functions (e.g. cultural, habitat, production)	Age structure, % dead, % flowering/ seeding, % viable seed	Cover, density, extent, height	Physiological responses to the flow (e.g. Δ in biomass, shoot length), reproductive responses to the flow (e.g. flowering, seed production)
Short-term flow regime (year(s) to decade) Relevant flow metrics: time-since-last inundation,	I	Cultural significance, genetic relics, habitat	N/A	Canopy cover, extent and architecture, number and shape of hollows, biomass, height, age measurements (DBH*, tree cores)	Growth rate (e.g. Δ in height, biomass), Condition (e.g. Δ in canopy cover/density, bark cracking, new tip growth, leaf die-off, sapwood thickness)
	P	Persistence, biodiversity and species-specific	Age structure, % dead, % flowering/ seeding, % viable seed	Cover, density, extent, height	Growth rate (e.g. Δ in height, biomass, extent), Condition (e.g. Δ in cover, density),

Hydrological regime scale	**Level of ecological organisation**[1]	**Examples of relevant functions, services and values**	**Vegetation indicators**		
			Composition	Structure	Process
frequency of inundation, seasonal patterns of inundation, spatial patterns of inundation (magnitude / connectivity)		functions (e.g. cultural, habitat, production)			Mortality, Δ in seed viability, recruitment / germination / Δ in age structure, dispersal (extent, distance, abundance, # of dispersal opportunities)
	C	Biodiversity, habitat, regulating functions	Species composition and richness, dominant species, functional groups, nativeness - applicable to extant and seedbank communities, % viable seed	Number of strata, structural attributes (e.g. cover, density, extent, height, age) of species, strata and other distinct compositional groups	Turnover (Δ in strata/compositional traits), trajectories, Δ in seed viability, dispersal (extent, distance, abundance, # of dispersal opportunities), germination
	L	Habitat, biodiversity, regulating, information functions	Community composition and richness, dominant communities	Extent, evenness, condition / greenness,	Turnover (Δ in compositional and structural traits), trajectories, terrestrialisation, contiguousness, hydrological connectivity between communities
	S	Persistence biodiversity and species-specific functions (e.g. cultural, habitat, production)	Age structure, % dead, % flowering/ seeding, % viable seed	Cover, density, extent, height	Growth rate (e.g. Δ in height, biomass, extent), condition (e.g. Δ in cover, density), Mortality, Δ in seed viability, recruitment / germination / Δ in age structure, dispersal

Hydrological regime scale	Level of ecological organisation[1]	Examples of relevant functions, services and values	Vegetation indicators		
			Composition	Structure	Process
					(extent, distance, abundance, # of dispersal opportunities)
Long-term flow regime (decade(s) to centuries) Relevant flow metrics: time-since-last inundation, frequency of inundation, seasonal patterns of inundation, spatial patterns of inundation, wet/dry sequence, maximum period dry	I	Cultural significance, genetic relics, habitat		Canopy cover and architecture, number and shape of hollows, biomass, height, age measurements (DBH, tree cores)	Growth rate (e.g. Δ in height, biomass), Condition (e.g. Δ in canopy cover/density, bark cracking, new tip growth, leaf die-off, sapwood thickness)
	P	Persistence biodiversity and species-specific functions (e.g. cultural, habitat, production)	Age structure, % dead, % flowering/ seeding, % viable seed	Cover, density, extent, height	Growth rate (e.g. Δ in height, biomass, extent), Condition (e.g. Δ in cover, density), Mortality, Δ in seed viability, recruitment / germination / Δ in age structure, dispersal (extent, distance, abundance, # of dispersal opportunities)
	C	Biodiversity, habitat, regulating functions	Species composition and richness, dominant species, functional groups, nativeness - applicable to extant and seedbank communities, % viable seed	Number of strata, structural attributes (e.g. cover, density, extent, height, age) of species, strata and other distinct compositional groups	Turnover (Δ in strata/compositional traits), trajectories, Δ in seed viability, dispersal (extent, distance, abundance, # of dispersal opportunities), germination

Hydrological regime scale	**Level of ecological organisation[1]**	**Examples of relevant functions, services and values**	**Vegetation indicators**		
			Composition	Structure	Process
	L	Habitat, biodiversity, regulating, information functions	Community composition and richness, dominant communities	Extent, evenness, condition / greenness,	Turnover (Δ in compositional and structural traits), trajectories, terrestrialisation, Δ in contiguousness, patterns of hydrological connectivity between communities
	S	Persistence biodiversity and species-specific functions (e.g. cultural, habitat, production)	Age structure, % dead, % flowering/ seeding, % viable seed	Cover, density, extent, height	Growth rate (e.g. Δ in height, biomass, extent), condition (e.g. Δ in cover, density), Mortality, Δ in seed viability, recruitment / germination / Δ in age structure, dispersal (extent, distance, abundance, # of dispersal opportunities)

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Vegetation outcomes: what are we seeking and why?

The Murray-Darling Freshwater Research Centre

Cherie Campbell, Sam Capon, Cassie James, Kay Morris, Jason Nicol, Daryl Nielsen, Rachael Thomas

Australian Society of Limnology Conference
Ballarat, 27th September 2016

LA TROBE UNIVERSITY

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Australian Government
Commonwealth Environmental Water Office

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  - SARDI

The Murray-Darling Freshwater Research Centre

Australian Government

Commonwealth Environmental Water Office

Vegetation Responses






Vegetation responses

- Vegetation response? What does it mean?
  - The term is ambiguous and the options are broad
  - Means different things to different people in different places

What are we watering for and why?

- Why is it important to articulate the targeted / desirable response?
  - Objectives incorporating function / services
  - Indicator selection
  - Capacity to consider:
    - Ability to deliver appropriate water events?
    - Trade-offs, why this response over another?
    - Different water availability scenarios?
  - Communication

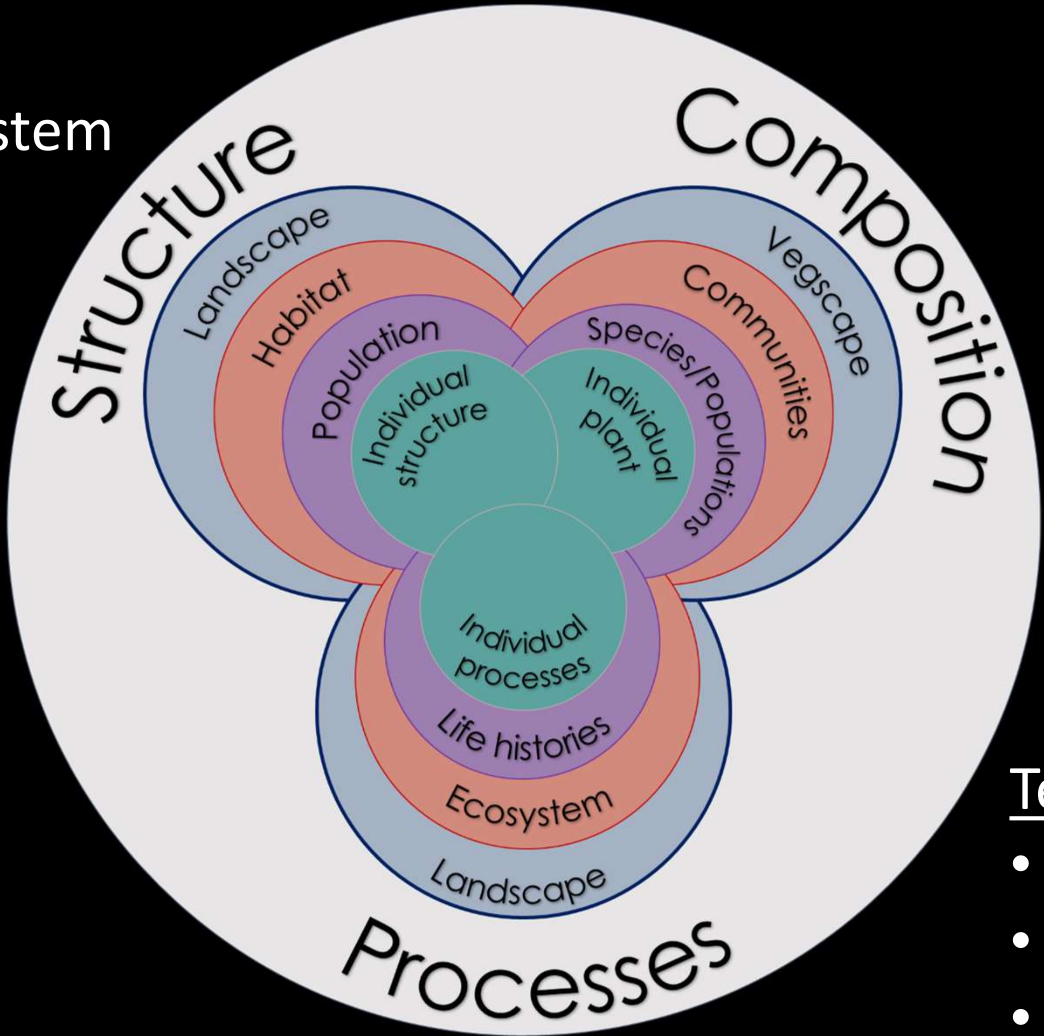
Vegetation Response Framework

- Can we provide a useful framework:
  - Incorporate objectives of function / services
  - Indicator selection
  - Capacity to consider objective trade-offs
  - Support communication of rationale and value of outcomes


Traits and level of ecological organisation

<u>Spatial scales</u>

- Wetland system
- Regional
- Basin-scale



<u>Temporal scales</u>

- Short term
- Medium term
- Longer-term

Adapted from Noss 1990

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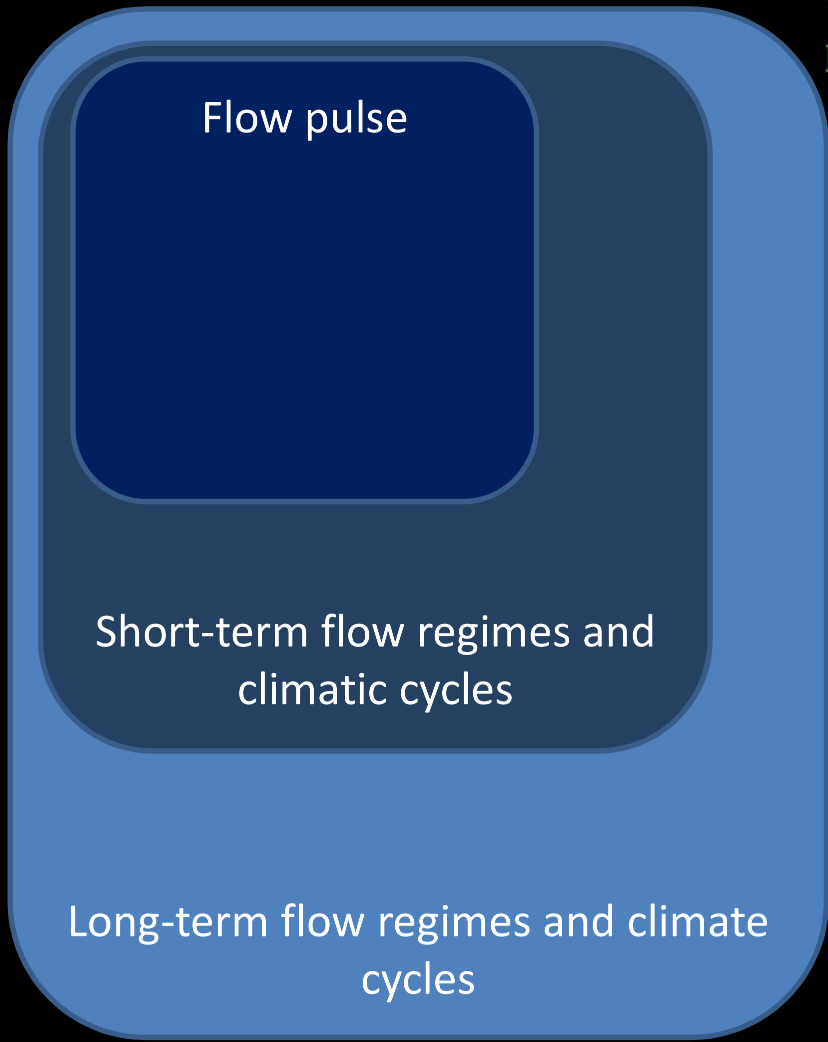
Functions and services provided

Habitat	Regulating	Production	Information
Refuge	Climate regulation	Food	Aesthetic
Nursery	Disturbance protection	Raw materials	Recreational
Corridor	Water regulation	Genetic resources	Cultural
Structural	Nutrient regulation	Ornamental	Educational

Adapted from de Groot et al 2002 and Capon et al 2013

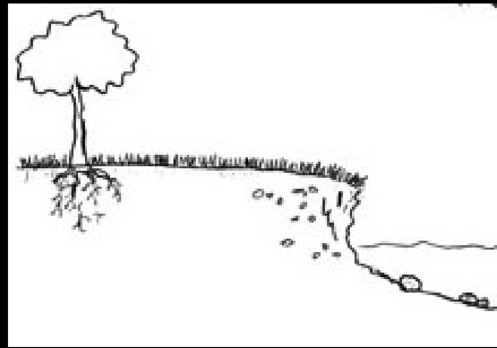
Nested flow regimes

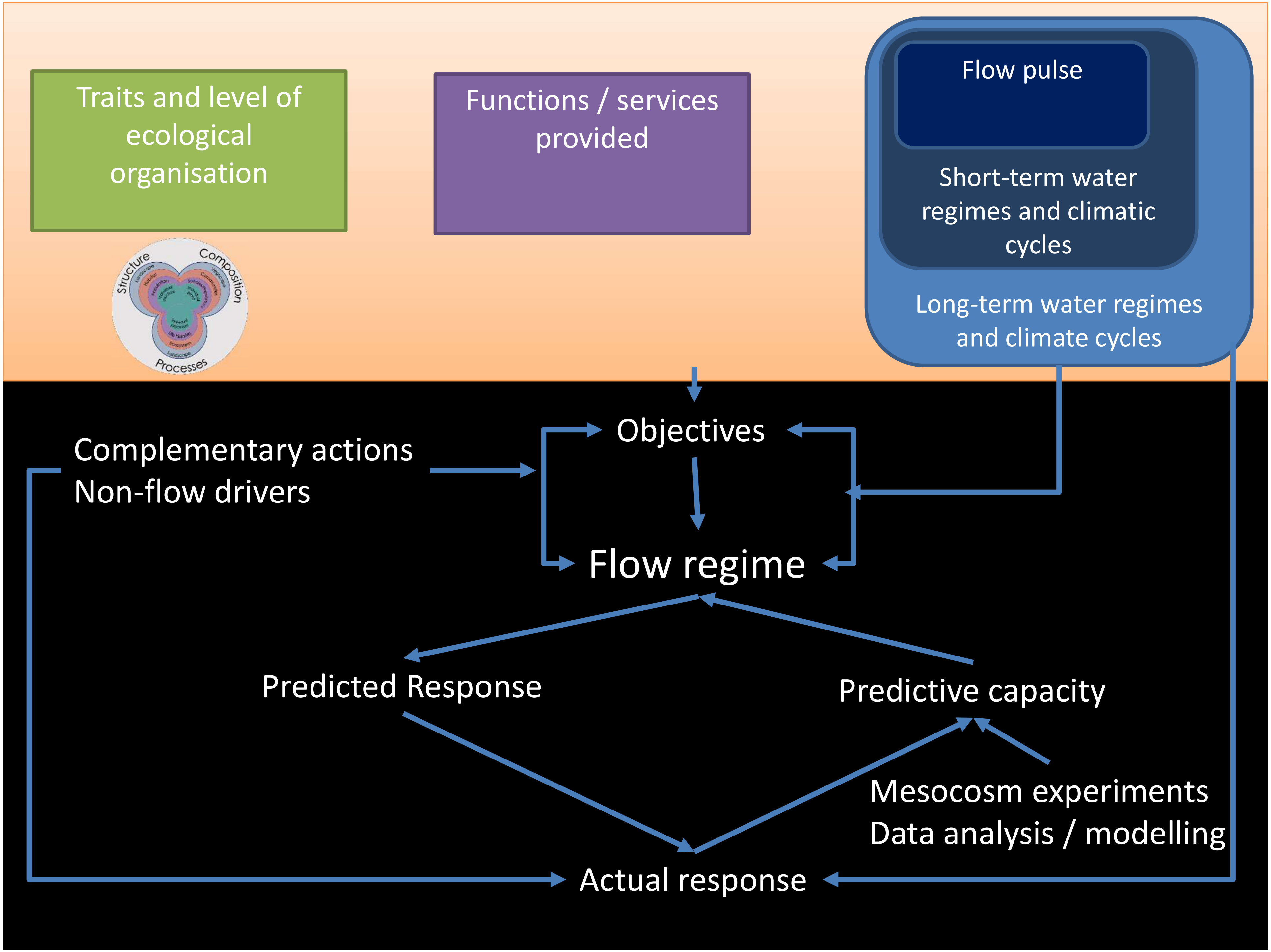
- Flow regimes
  - Flow pulse
    - Response to an event
  - Short-term regimes
    - Annual to decadal
  - Long-term regimes
    - Decades to centuries



When flow is not enough

- Landscape context
- Historical legacies
- Non-flow drivers
  - Season/temperature
  - Light/shading
  - Soils/substrate
  - Nutrient and oxygen availability
  - Competition, disturbance, herbivory




Traits and level of ecological organisation
Structure
Composition
Processes
Functions / services provided
Flow pulse
Short-term water regimes and climatic cycles
Long-term water regimes and climate cycles
Objectives
Complementary actions
Non-flow drivers
Flow regime
Predicted Response
Predictive capacity
Mesocosm experiments
Data analysis / modelling
Actual response

How would the framework apply?

- Local species composition response to an individual event – high species richness

Scotties Billabong, Lower Murray, Summer 2008/09

How would the framework apply?

- Maintain or improve structural habitat within a region

e.g. for Growling Grass Frogs, medium to long-term time-scales

How would the framework apply?

- Maintain ‘vegscapes’ to provide particular associations of communities within a spatial area

e.g. open water, reed beds, woodlands, shallow aquatic marsh

images: NSW OEH website + UNSW Centre for Ecosystem Science website

Filling in the gaps

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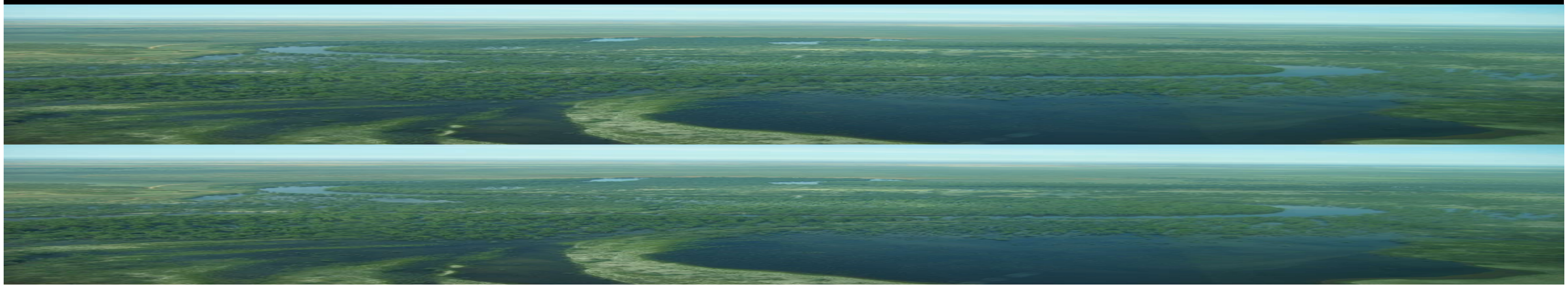
- Vegetation *response* framework
  - Conceptual models representing relationships
  - Need to be developed and tested



Predicted application

- To assist the development of objectives and predicted outcomes under different:
  - Water availability scenarios
  - Water management scenarios
- To contribute to water planning and management



Questions:



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Grow with the flow

CHERIE CAMPBELL ASKS WHAT OUTCOMES ARE WE SEEKING FOR WETLAND VEGETATION AND WHY?

Wetland and floodplain plants are critical components of both aquatic and terrestrial ecosystems, supplying energy to support food webs, providing habitat and dispersal corridors for animals and birds, and contributing to other ecosystem services such as nutrient and carbon cycling, water and sediment oxygenation. They are also beautiful parts of our river landscapes with aesthetic, cultural and recreational values, as well as intrinsic biodiversity value.

What, however, do you picture when you think of a wetland or floodplain plant? Is it a majestic 600 year old tree, a pond full of swamp lilies, tall reeds and grasses in which waterbirds build their nests, or is it a mass of green herbs that covers the floodplain after waters recedes?

The diversity of plants in Murray–Darling Basin wetlands and floodplains is tremendous, with more than 800 species. These take a myriad of structural forms, from floating ferns, to ancient trees, and provide a range of functions. Vegetation outcomes from environmental flow management may seek to achieve multiple objectives relating to composition, structure, and/or ecological processes that support other biota. These objectives are also scale dependent; with wider landscape objectives providing context for smaller site-scale objectives that will vary from location to location. For example, to improve adult tree condition in some areas, to recruit juvenile trees, or to control seedling recruitment in other parts of the floodplain. Clarifying multiple objectives allows managers to better define water requirements, monitor outcomes and communicate decisions and outcomes to stakeholders.

The Vegetation Theme of the Murray–Darling Basin Environmental Water Knowledge and Research (MDB EWKR) project aims to provide a framework to assist in clarifying objectives, by considering the functions and services provided by different vegetation responses and the influence of flow across temporal scales. In addition, we want this framework to consider the context in which environmental watering decisions are made, in terms of water availability, delivery constraints and the influence of complementary management. The framework we are using has three main components.

A carpet of Red Water-milfoil (*Myriophyllum verrucosum*) as Lake Boich draws down, Hattah Lakes, 2015. Photo Fiona Freestone.

Below: A diverse aquatic wetland community following environmental watering, Scottie's Billabong, Lindsay Island, 2009. Photo Cherie Campbell.

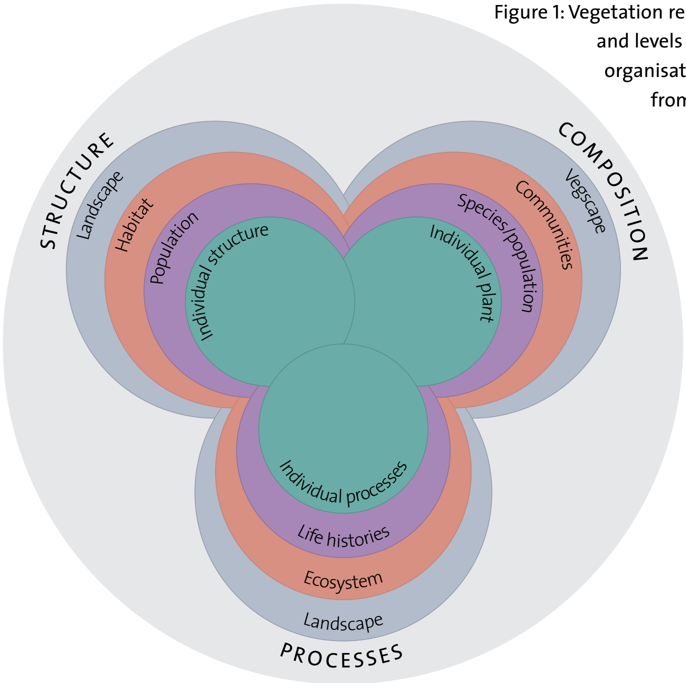


Figure 1: Vegetation response traits and levels of ecological organisation (adapted from Noss 1990).

Component 1: Response traits and levels of ecological organisation

There are three broad categories of vegetation response that may be included in managers' objectives; these are composition, structure and process. These responses may occur at different levels of ecological organisation, ranging from landscape to individual plant responses (Figure 1). For example, objectives may be focused on:

- promoting high species diversity (composition and communities)
- maintaining large, hollow-bearing River Red Gum trees (structure and population)
- increasing the abundance of Moira Grass (composition and species)
- stimulating germination of Black Box trees (processes and life histories) to improve age-class structure at a site (structure and population)
- maintaining a spatial array of reed beds, open water, and woodland communities (composition and vegscape)
- increasing the abundance and complexity of structural wetland plants (e.g. submerged, floating leaves, emergent sedges) (structure and habitat).

Component 2: Functions and services

Functions and services provided by vegetation can be grouped into four different types; habitat, regulating, production and information (Figure 2). For example, vegetation can provide nursery habitat for fish, corridor habitat for the movement of birds, or structural habitat for frogs. This model provides us with the scope to incorporate both ecological functions and services, as well as economic and social functions and services, such as food sources (e.g. honey production from River Red Gums), recreational values (e.g. improving submerged habitat at important fishing locations) and cultural values (e.g. health of scar trees or maintenance of totem species).

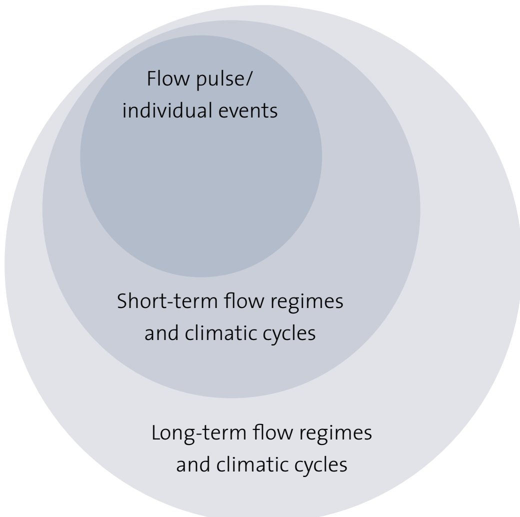
Component 3: Nested flow regimes

Vegetation responses to flows also occur at a variety of temporal scales that can be summarised into three broad flow regimes (Figure 3).

1. **Long-term** (decadal) cycles of wet and dry periods. At this scale, flow influences landscape patterns of vegetation such as the types, distributions and relative abundance of different vegetation communities. The key flow characteristics at this scale are:
   - average inundation frequency and patterns of frequency
   - average and maximum period without inundation
   - wet sequence duration (number of sequential years in which inundation occurs)
   - average and maximum inundation depth and duration
   - magnitude and connectivity of inundation
   - patterns of inundation seasonality.

Habitat	Regulating	Production	Information
Refuge	Climate regulation	Food	Aesthetic
Nursery	Disturbance protection	Raw materials	Recreational
Corridor	Water regulation	Genetic resources	Cultural
Structural	Nutrient regulation	Ornamental	Educational

Figure 2 (above). Structural grouping of potential functions and services provided by vegetation (adapted from de Groot et al. 2002 and Capon et al. 2013).
Figure 3 (right). Nested flow regimes influencing vegetation responses.



River Red Gum (*Eucalyptus camaldulensis*) trees at Hattah Lakes following flooding in 2010/11. Photo Caitlin Johns.

ALLELOPATHY is a biological phenomenon by which an organism produces one or more biochemicals that influence the germination, growth, survival and reproduction of other organisms.

2. **Short-term** (1–10 years) flow regimes. At this scale, flow influences the composition of ecosystems and condition of populations within those systems. The important flow characteristics at this scale are similar to those for long-term flow regimes, however, the vegetation responses are smaller scale in recognition of the longer time frames over which landscape vegetation patterns change. The key flow characteristics at this scale are:
   - inundation frequency and patterns of frequency
   - maximum period without inundation
   - time-since-last inundation
   - wet sequence duration (number of sequential years in which inundation occurs)
   - average and maximum inundation depth and duration
   - magnitude and connectivity of inundation
   - patterns of inundation seasonality.

   Both long-term and short-term flow regime characteristics interact with land form and climate variables including average, maximum and minimum rainfall and temperatures to determine vegetation composition, structure and processes.

   There is also an important interaction between these two flow regimes in that the establishment of long-lived vegetation will have an influence on the understory that develops at the site. This is likely to be due to a variety of factors including the changes in the micro-climate under the canopy (e.g. light, temperature), changes in soil properties, competition for nutrients, water and allelopathic interactions.
3. **Flow pulses/individual events**. At this scale key flow characteristics influence individual plant responses which may include growth, reproduction, germination, dispersal, quiescence or death. The important flow characteristics include:
   - depth
   - duration
   - rate of recession
   - seasonal timing
   - magnitude and connectivity of inundation
   - velocity
   - turbidity/euphotic depth.

This framework will help land and water managers to develop specific objectives for different types of vegetation responses across a range of spatial and temporal scales, and for a variety of functional outcomes. The framework and related information will be published on the MDB EWKR website over the coming year.

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FOR FURTHER INFORMATION

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MDB EWKR Story Space — www.ewkr.com.au

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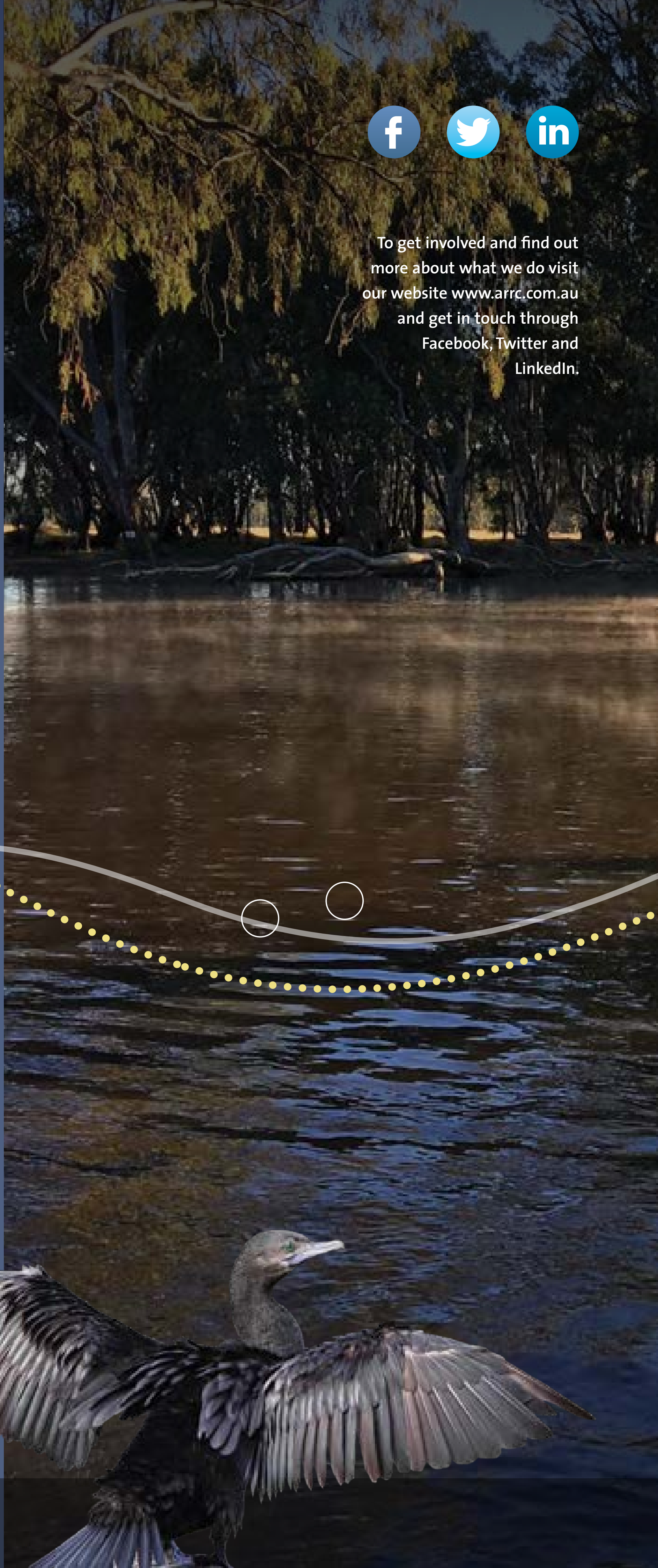

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RipRap

EDITION 40

Appendix V2.1: Data integration and synthesis component paper

N.B. This is a full manuscript in preparation for submission to a scientific journal for publication. Inclusion as an output in this technical report doesn't preclude the ability to publish.

Disentangling flow-vegetation relationships and antecedent legacies to inform environmental flows

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Introduction

Altered flow regimes are one of the greatest and most pervasive threats to wetlands globally (Kuiper et al. 2014, Kingsford et al. 2016). Environmental water provisions (or environmental flows) are intended to mitigate the impacts associated with reductions in the frequency, duration, or magnitude of flows and, protect and restore wetland ecosystems, although are usually constrained in terms of how, when and what water can be used due to a range of physical, technical, legal and social limits (Arthington 2012). Consequently, environmental flow provisions typically represent a compromise between environmental objectives and socio-economic considerations, particularly in times of water shortage (Pittock and Lankford 2009). Accordingly, water provisions for environmental benefits increasingly need to be justified to a wider audience with demonstrable outcomes that meet expectations of both water managers and the public. Development of robust explanatory and predictive models based on long-term monitoring data is therefore essential for better understanding variations in ecological responses to flow provisions as well as to inform environmental water policy and adaptive management (Overton et al. 2014).

Because of its high intrinsic value, as well as its crucial role supporting a wide range of ecosystem functions, services and human values (Capon et al. 2013, Capon and Pettit 2018), wetland vegetation is typically a key focus of environmental water provisions aimed at protecting and restoring wetlands (Campbell et al. In review). Vegetation responses to flows are highly complex, however, and there is considerable uncertainty in predicting vegetation outcomes of watering actions in both the short- and long-term (Moxham et al. 2019, Campbell et al. In review). Patterns in the composition and structure of wetland vegetation reflect both contemporary hydrological conditions and the legacy of past hydrological events, as well as a wide range of potential non-flow factors, e.g. soil characteristics, grazing etc. (Tabacchi 1995, Davies et al. 2014).

In the short-term, wetland vegetation responses to hydrology are strongly influenced by characteristics of recent flood pulses (e.g. timing, depth and duration of flooding) and reflect variation amongst plant species in their capacity to establish, grow and reproduce under particular hydrological conditions (Britton and Brock 1994, van der Valk 1994, Seabloom et al.

1998, Casanova and Brock 2000, Brock et al. 2006). Many ephemeral wetland plant species avoid unfavorable conditions (both wet and dry) by maintaining long-lived soil seed (or propagule) banks which enables them to persist in a dormant state and reestablish when suitable conditions return (Brock 2011). Like the extant vegetation, wetland soil seed banks are strongly shaped by hydrology, through its influence on processes of seed depletion (e.g. germination, seed mortality, scouring etc.) and replenishment (e.g. local reproduction, dispersal). For species residing in the soil seed bank, the influences of historical conditions are therefore encoded through the legacy of past events that contribute to, or deplete, the seed bank. As a result, contemporary soil seed banks represent the cumulative legacy of historical hydrological conditions at a site which, in turn, set the scene for future vegetation responses to flows - a kind of 'ecological memory' (Padisak 1992).

The influences of antecedent hydrological conditions on wetland vegetation responses to flows in the short-term can be expected to play out across a range of temporal scales. The time since last inundation, for example, is likely to have a significant effect on the current condition of vegetation (e.g. size, reproductive maturity etc.) and therefore its capacity to respond to subsequent flooding. Extended dry periods between flood events may result in a decline in the richness and abundance of wetland plants present and also alter soil seed bank composition in favour of species with more persistent propagules (Brock 2011). Conversely, long periods of saturated soils may reduce seed survival in some species (Mordecai 2012). The frequency of consecutive flooding and drying events may also alter soil seed bank composition, and therefore the capacity of vegetation to respond to subsequent events, through selective depletion of some species if there is insufficient time for the plants reproduce, set seed and replenish the seed bank between successive floods (Capon 2005).

Many other factors, in addition to hydrology, can influence the composition and structure of wetland vegetation, both directly as well as through interactions with flow and over short- and longer timeframes. Few flow-vegetation ecology studies, with the exception of some remote sensing studies, consider the independent effects of recent weather conditions despite the exposure of wetland vegetation to both local rainfall and air temperatures. However, local rainfall can have a direct influence on wetland vegetation patterns by eliciting germination and growth responses independently of flooding. Temperature may also shape vegetation directly as well as through interactions with hydrology. The seasonal timing of flooding, for example, can determine which species establish from wetland soil seed banks as a result of variation in temperature cues for germination (Capon 2007). Temperature may also shape soil seed bank composition if thermal extremes cause seed mortality (Nielsen et al. 2015). Over longer time periods, historical climate patterns can be expected to have a major influence on wetland vegetation dynamics, with broad patterns in vegetation distribution typically reflecting biophysical constraints imposed by climate (Kearney and Porter 2009), often resulting in biogeographical affinities such as that observed for the temperate and tropical aquatic floras of Australia (Jacobs and Wilson 1996). Other legacy effects which may modify contemporary vegetation responses to flows include soil conditions, past land management (e.g. vegetation clearing, grazing etc.) and other extreme events (e.g. fires).

Because of the strong 'ecological memory' of wetlands, i.e. 'the capacity of past states or experiences to influence present or future responses of the community' (Padisak, 1992), understanding wetland vegetation responses to flows in the short-term requires consideration of antecedent conditions, both with respect to hydrology and other factors, e.g. climate, as well as recent modifiers, e.g. weather. Here, we explore the relative influence of recent and longer-term antecedent conditions, both hydrological and climatic, on wetland vegetation composition and structure in the Hattah-Kulkyne Lakes, a Ramsar-listed wetland complex in Australia's dryland Murray-Darling Basin. Comprising over 20 temporary and perennial lakes, this site is ideal for disentangling complex flow-ecology relationships for wetland vegetation because of the range of historical trajectories present as well as the existence of a long-term data set (> 8 years) comprising floristics and accompanying hydrologic and weather data. Furthermore, recent weather conditions can be expected to have a particularly pronounced effect on vegetation responses in dryland wetland systems, because water availability is usually limiting, and temperatures are subject to extremes.

Dryland temporary wetlands, such as the Hattah-Kulkyne Lakes, are characterized by dynamic transitions between wet and dry phases that support predominantly hydric and xeric plant communities respectively. We hypothesized that the influence of antecedent hydrologic and climatic conditions would vary between these contrasting wetland plant communities because key determinants of patterns in the distribution and abundance of xeric species are likely to differ from those associated with more hydric floras. We predicted stronger effects of antecedent weather conditions on patterns amongst xeric species based on relationships with seasonal and mean annual rainfall for desert annuals (Ogle and Reynolds 2004) and the low tolerance of many arid and semi-arid plant species to waterlogged soils. Conversely, we expected that patterns of hydric species would be more closely linked to antecedent hydrological conditions and would be relatively insensitive to the direct effects of local weather having greater water needs than are usually provided by local precipitation. Instead, we anticipated that the effects of recent weather on hydric plant communities would most likely occur indirectly through interactions with hydrological drivers that influence rates of wetland and floodplain evaporation and drying.

Using annual vegetation monitoring data collected over eight years at each of 12 wetlands within the Hattah-Kulkyne Lakes complex, we applied novel modelling approaches to explore flow-ecology relationships across a temporal hierarchy (*sensu* Biggs et al. 2005) spanning: 1.) recent conditions in the immediate lead up to monitoring (i.e. 0- 3 months), 2.) short-term antecedent conditions capturing the year prior to monitoring (4 months to 1.25), 3.) medium term (i.e. 1.25 year to 3.25 years) and 4.) long term (i.e. 30 year flood frequency) conditions. We used boosted generalized additive models (GAMs) and boosted regression trees to incorporate the flexibility of generalized additive models and regression trees with automatic variable selection. In the first stage of the analysis, we employed the general modelling framework suggested by Hothorn et al. (2011) to decompose the variation in a number of wetland vegetation response metrics to local and global additive and interactive effects of environmental (hydrological and climatic), spatial and spatiotemporal variation. We then extended this framework to investigate the relative importance of hydrological and climatic variables both independently and together. Subsequent to model selection, we also explored the partial relationships of significant predictor variables using partial dependency plots for the best candidate model identified for each response variable. Finally, we used boosted regression trees to investigate interactions between predictors.

Methods

Study area and climate

Hattah Lakes, part of the Hattah-Kulkyne National Park, encompasses c. 13 000 ha of lakes and floodplain adjacent to the Murray River in north-west Victoria, Australia (Figure 1). Hattah Lakes are characterized by more than 20 intermittent and perennial freshwater lakes and creeklines. Typical vegetation communities include aquatic macrophytes and lake-bed herblands, *Eucalyptus camaldulensis* Dehnh. (River Red Gum) forests and woodlands, *E. largiflorens* F. Muell. (Black Box) woodlands and *Duma florulenta* (Meisn.) T.M.Schust. (Lignum) shrubland (MDBA 2012). The Hattah Lakes, based on their ecological, social and/or cultural importance, were selected as an icon site for The Living Murray program (MDBA 2011, MDBA 2012) and 12 of the wetlands are listed as Wetlands of International Importance under the Ramsar Convention (Butcher and Hale 2011).

Hattah Lakes are situated within a semi-arid climate zone, with mean annual rainfall of c. 300 mm (Nulkwyne Kiamal station, BOM 2019). Rainfall occurs year-round with the mean monthly rainfall ranging from 32.9 mm (October) to 19.3 mm (March). There is a high degree of variability, however, as evidenced by the 5^{th} and 95^{th} percentiles which range from no rainfall (0 mm) in a month to greater than 90 mm in a month (Nulkwyne Kiamal station, BOM 2019). Mean maximum/minimum temperatures range from 32.4/16.8°C (January) to 15.5/4.3°C (July). However daily maximum temperatures exceeding 40°C are common in summer with minimum temperatures below 0°C experienced during winter (Mildura Airport station, BOM 2019).

Hattah Lakes are naturally inundated by overbank floods in the Murray River, typically resulting from rainfall in upstream catchment areas. Water initially enters Hattah Lakes via Chalka Creek, an anabranch of the Murray River, before filling through the lake system in several directions (Butcher and Hale 2011).

The availability of surface water and the frequency of small to medium overbank flood events in the mid-lower reaches of the Murray River has decreased substantially due to the stabilizing effects of a series of large weirs and water storage dams upstream as well as the impacts of water extraction (Leblanc et al. 2012, CSIRO 2008). Due to the effects of reduced flooding (MDBA 2012), Hattah Lakes has a relatively long history of environmental water management beginning in 1972-73 with modifications to the channel of Chalka Creek

(Butcher and Hale 2011). Intermittent pumping via temporary pumps was undertaken as an emergency measure from 2005 to 2011 (MDBA 2012) prior to the construction of a pumping station and other associated works to enable managed inundation on a larger scale (MDBA 2012). Inundation via the pumping station has occurred on a number of occasions since 2013-14 (Freestone et al. 2014). Unregulated ('natural') overbank flooding occurred on three occasions in the last 20 years (2001, 2010-11, 2016) (MDBA 2019).

Vegetation data

The Hattah Lakes wetland monitoring data consisted of 3 – 4 transects surveyed at each of between 3 and 7 elevations, in each of 12 wetlands (number of transects = 213). The number of elevations surveyed varied depending upon the depth profile of each wetland, providing sampling coverage from the wetland base to the wetland edge, as defined by the mature tree line. At a transect, 15 contiguous 1x1m quadrats were sampled with dependent variables analysed at the transect level. For each quadrat the presence/absence of all species present was recorded. Hence, abundance is scaled between 1 and 15 with the maximum abundance in each transect being 15. The sites were surveyed annually usually in mid to late Australian summer eight times between 2008 and 2016 (data for 2015 is missing) for 9 wetlands with three added in later years (3, 4 and 5 years of data for the additional wetlands). In total there were 1459 transects surveyed (64-216 per wetland).

We selected response variables that describe the diversity of vegetation of ephemeral wetlands that are often used to infer the condition or health of wetland plant communities, the richness and abundances of native plants (Casanova 2011). Because species associated with dry and wet conditions are likely to respond very differently to environmental conditions, we modelled dry preferring (xeric) and wet (hydric) preferring species separately. We used the plant functional groups of Brock and Casanova (1997), to categorize species as hydric species and xeric species. The hydric categories included all species that are likely to respond to wet or damp conditions and so this category includes species classified as terrestrial damp species which germinate, grow and/or reproduce on saturated soils. Xeric species were determined as those species classified as terrestrial dry preferring species using the groupings of Brock and Casanova (1997). Species were assigned functional groups based on a literature survey and knowledge of their overall morphology as observed in the field. Group allocation in some cases could not be assigned confidently and these species were removed from subsequent analysis. Species were also allocated an exotic or native status based the Victorian Flora (VicFlora, 2016). A full species list with function group allocation and exotic status is provided in Appendix B. We also allocated a native or exotic status to each species. The final response variables, calculated at the transect scale, were: native plant species richness, native wetland plant species abundance (the accumulative frequency of all wetland species summed for each transect) and, native dryland plant species abundance (the accumulative frequency of all dryland species summed for each quadrat).

Covariates

Environmental metrics were derived for each transect describing site hydrology, rainfall and temperature conditions as well as spatial location (Table 1).

Hydrological information for this study have been generated using the Murray Darling Basin Authority (MDBA) Bigmod model of Hattah Lakes (calibrated to the MDBA MIKE21 hydrodynamic model of Hattah Lakes and verified/refined using measured water level data since 2013). Pumping rates for environmental watering prior to 2013 were estimated from pumping records and volume estimates from modelling and satellite images. The model generated daily water depths for each monitored site for the period between January 2005 and December 2016. Model outputs were checked against inundation observations recorded during vegetation monitoring. This enabled model refinement through the identification of mismatches and ensured that all pumping events, or alternatively, channel closures for works, were incorporated into the model.

Using the Bigmod modelled data we calculated a number of hydrological measures for each quadrat (Table 1). For the purposes of this analysis we defined an inundation event as a single wetting and drying cycle and stipulated a minimum time period between successive cycles of 5 days. However, cycles were predominantly separated by periods of weeks to months so this criterion is unlikely to affect the results.

We calculated metrics that defined the time since last inundated (days; TSLW), and the proportion of time wet and conditional water depth (depth >0) for recent (three months), short term (3 months to I.25 years) and, medium term (1.25 years to 3.25 years) flow events preceding each sampling date. We followed Beelsey et al. (2014) in not using nested antecedent flow metrics. The antecedent time periods used capture the main spring/summer growth seasons for the current, the previous year's growth seasons conditions and the flow conditions in the 2-3 years preceding the sample date.

The above metrics describe short to medium term hydrological conditions. For an estimate of long term flood frequency (based on the 30 year record period prior to each monitoring date and site combination) flow records from Euston flow gauge (No. 414203C) upstream of the Hattah Lakes complex were used for the period of time prior to the Bigmod modelled data (1/1/2005). Commence to flow (CTF) values for each quadrat were extracted from RMFIM model GIS layers for Hattah Lakes (Overton et al. 2006). Flood frequency was estimated as the number of independent flood events (with a time period of at least 5 days between events) over the thirty year period that exceeded the CTF. For periods of time post 1/1/2005 we used the Bigmod derived depth data to estimate flood frequencies as this data incorporated water management actions such as pumping events and channel closures and will more accurately reflect recent inundation frequency.

To describe antecedent rainfall we used local rainfall data collected from Hattah Lakes Information Centre, Victoria Parks and temperature data from the closest Bureau of Meterology station (Nulkwyne Kiamal BOM station no. 76043). We examined correlations between potential predictor climate variables. Because the antecedent rainfall variables all correlated strongly we selected rainfall total (d90 for the three months preceding each sampling date) to represent the rainfall occurring during the growing season prior to sampling and d365 for rainfall in the year prior to sampling. Temperature variables also correlated strongly so we selected temperature averages and extremes in the 3 months preceding each sampling date (MeanTemp90, MinTemp90, MaxTemp90, MeanTemp365, MinTemp365 and MaxTemp365) to represent the range of temperatures encountered during the current growing season and the year preceding the sampling event.

Three hundred and forty four records had missing values for one or more of the predictors and were removed from the analysis leaving n=1264 records for the analysis.

Modeling approach

We used boosted GAMs to model relationships between selected response variables and assembled predictor variables. Boosted approaches are a flexible modelling approach that can address issues both concerning the data itself (non-normal errors, nonlinear relationships and autocorrelation) and with the modelling process (overfitting, variable selection and prediction) (Maloney et al. 2012). This generally approach has been described and illustrated in detail by Maloney et al. (2012) and, more recently, Smith et al. (2018). One of the advantages of this method is that it incorporates variable selection within the model fitting process itself and can deal with complex, multidimensional data. In boosting, models are fitted iteratively to the data with increased emphasis or weighting upon those observations that are poorly modelled by the existing models. The main tuning parameter is the number of boosting iterations denoted by "mstop". This is a crucial stage in the modelling as it prevents overfitting the model. The number of iterations was optimized via internal validation using subsampling. For 25 subsamples of the data (of size n/2 from the original dataset) the model was refitted and a measure of model performance was assessed on the independent validation data to determine prediction accuracy measured by the negative log-likelihood of each model (mstop was determined as the lowest average empirical risk). See Mayer and Hofner (2018) for a short non-technical introduction to boosting.

For each response variable we investigated the use of seven candidate models to explore the effects independently and in combination: spatial and spatiotemporal variability (Spatial), hydrological and climate (Climate+Hydro), climate only (Climate), hydrological only (Hydro), hydrological, climate, spatial and spatiotemporal (Climate+Hydro+Spatial), interactions using tree-based learners (Tree) and, interactions, spatial and spatiotemporal (Tree+Spatial) effects (Table 2).

In the case of the candidate GAM models, for each of the continuous predictors we included two base leaners: a linear base-learner (bols) and a smooth non-linear base learner (bbs). This allows the selection of different functional forms (no effect, linear or/and smooth effects) for each covariate included in the model (Smith et al. 2019). The parameter df (degrees of freedom) controls the smoothness of the curve. The degrees of freedom was set to 1 for all base-learners and we omitted the intercept term for each base-learner to ensure that there was no bias in the variable selection process (Kneib et al. 2009). We then added a linear base-learner to the overall model as a model intercept. For the categorical variable (inundated True or False) we specified the linear base-learner only. For the spatial component, we followed Smith et al. (2019) in specifying linear functions for eastings and northings separately and, a smooth nonlinear function as a function of northings and eastings using the bspatial base-learner. For the spatio-temporal component we specified a smooth nonlinear function as a function of northings and eastings by water year (defined as 1 July to 30 June of the following year). We also included linear base-learners for eastings and northings with an interaction with water year. For the regression tree approach (that allows for higher order interactions and does not require explicit specification of interaction terms as in the case of the additive models), a tree base-learner (btree) was utilized with (Tree/Spatial) and without the additional spatial and spatiotemporal terms (Tree).

For abundance measures and richness of native hydric species' we used negative binomial distributions (Table 3) after exploring various options (e.g. poisson and zero truncated poisson and negative binomial) as this distribution was found to provide the best fit with respect to the responses. For the presence of aquatic species we used the binomial distribution. Standard diagnostic (residual) plots of the best models for each response variable were reviewed to check model fittings.

For each response variable, the seven candidate models were compared using resampling procedures where the negative log-likelihoods is a function of the number of iterations and lower negative log-likelihoods values indicating better performing models. The models were formally compared using multiplicity adjusted all-pairwise comparisons (Hothorn et al. 2008). For the best performing candidate model we used boot strapping to assess the model goodness of fit as we did not have a separate test dataset. For 100 bootstrap datasets (2/n of the full dataset) we determined the median and confidence intervals of the pseudo R^2 (Nagelkerke 1991) by comparing the log-likelihood values of the selected model with the null model (intercept only).

We further investigated the role of the environmental predictors in the best performing model for each response variable using a formal stability selection procedure after Meinshausen and Buhlmann (2010) to select predictors that have a high probability of influencing the response whilst controlling the family-wise error rate (Hothorn et al. 2011). This procedure measures how stable the model is with respect to different subsets of the data and which variables are consistently influential despite changes in the dataset. Where there were no significance differences in the 'best' candidate models we selected the most parsimonious model. The partial effects for selected predictors for each response metric were plotted to explore their effects on the response variables.

In a second set of analyses, we used boosted regression trees to explore potential interactions between drivers. Boosted regression trees are a machine learning technique in which a large number of simple models are fitted adaptively, the results of which are then combined to optimize the predictive performance of the final model (Elith et al. 2008). We used tree complexity equal to 2 (allowing for two-way interactions) and a Poisson error distribution. Model stochasticity is controlled though the out-of-bag fraction which is the number of data points to be selected at each step. This parameter was set to 0.5 consistent with the advice of Elith et al. (2008). Other parameters required (e.g. the learning rate or shrinkage which determines the contribution of each tree to the growing model) were determined through initial model runs (set at 0.005 as further decreases did not significantly improve the fit of the models). The models were built using the default settings of 10-fold cross-validation to estimate the optimal number of trees. During cross validation the dataset is divided into ten subsets with nine used for each model iteration and the remaining subset used to test the model and cross- validate the results. We retained some of the natural structure in the data by keeping all data from one site sampled repeatedly over time in the same fold. The relative importance of predictor variables is assessed as the number of times a variable is selected for splitting, weighted by the improvement to the model with each split averaged over all the trees. This measure includes both independent and interactive effects of variables. To examine the overall performance of the BRT models we used the cross validation deviance explained and the cross-validated correlation coefficient between the observed data and the fitted values. The former provides a measure of how well the model explains the portion of data left out during the cross-validation procedure.

These analyses were undertaken in the R system for statistical computing (R Development Core Team 2010) using the ‘mboost’ package (Hothorn et al. 2018) using code adapted from Maloney et al. (2012) and Hothorn et al. (2011). Boosted regression trees were performed with the package ‘dismo’ (Hijmans et al. 2016) and following the recommendations of Elith and Leathwick (2017).

Results

Boosted GAM

Examination of candidate models

For each response variable, we initially fitted eight independent candidate models. Many of the more complex models (those that included environmental predictors for climate and hydrology) failed to stop early and converged to the maximum likelihood estimates. According to Smith et al. (2018) this can happen in datasets with many observations and strong, complex effects. Large values of mstop indicate that the saturated model is appropriate and overfitting is not likely to be an issue (T. Hothorn pers. Comm.).

Each of the candidate models was fitted to the response variables and assessed using the bootstrap distribution of the negative log-likelihood (Figure 2). For all three response variables the candidate model with the hydrological data (as simple additive effects) was the best and most parsimonious model. The models including only climate data were not informative (out-of-bootstrap negative log-likelihoods were significantly higher) hence suggesting that climate variables did not strongly influence plant abundances or species richness. The models including the spatial effects and the legacy effects alone were generally the worst performing models across the responses suggesting that these effects were not important in explaining any of the response variables. Including spatial and climate terms with the hydrological terms did not significantly improve the hydrological model performance on the out-of-bootstrap observations. For plant species richness the interaction models (Tree and Tree+Spatial) performed significantly poorer than the simpler additive models including hydrological metrics suggesting that interactive effects were not important in explaining species richness. For the abundance response metrics, the interaction models (Tree and Tree+Spatial) performed similarly to the simple additive models including hydrological variables alone indicating that interactive effects, if present, are relatively subtle and did not have a strong influence on plant abundances relative to the simple additive effects.

Drivers of native wetland plant species richness

The hydrological model explained reasonable amounts of variation in the richness of native ‘wet’ wetland plants (peudo R2 = 0.55, Table 3). The stable selection procedure performed on the hydro model identified three variables that were important in explaining wetland plant species richness (Table 3).The estimated partial effects of the important variables, integrating out the effects of other variables, are shown in Figure 3. Negative linear relationships were found between native species richness and, the mean conditional water depth and proportion of time wet in the last three months. Species richness was nonlinearly related to time since last inundated with richness rising shallowly to a peak at around 100 -200 days since last inundation and then dropping gradually off with further increases in time after inundation.

Drivers of native wetland plant abundances

The hydrological model explained moderate amounts of variation in the abundance of native 'wet' wetland plants (peudo R^2 = 0.34, Table 3). Stable selection of the hydro model identified four variables that were important in explaining wetland plant native abundances (Table 4) the variables represent components across the recent, short term and medium term time-frames. The estimated partial effects of the important variables, integrating out the effects of other variables, are shown in Figure 4. Unimodal relationships were identified between wetland plant abundances and the time since last wet with abundances peaking at around 50 to 60 days after being inundated. Abundances also peaked when sites had been wet for around 50% of the period between 4 months and 1.25 years before sampling. Negative linear relationships were found between 'wet' wetland plant abundances and conditional mean water depth in both the shorter time scales (the three months prior to sampling) and the medium term (1.25 to 3.25 years prior to sampling).

The hydrological model explained moderate amounts of variation in the abundance of native 'dry' wetland plants (pseudo R^2=0.33, Table 3). Stable selection of the additive hydrological model identified three variables that were important in explaining terrestrial plant native abundances (Table 4) reflecting recent hydrological conditions and the time since last inundated. Linear negative relationships were identified with recent hydrological condition (conditional mean depth and proportion of time wet in last 3 months) and a non-linear relationship was found with time since last inundation (Figure 5 shows the partial effects of these predictors). Abundances of the drier preferring species peaked at between 90 and 200 days before decreasing with further increases in time since inundation.

Boosted regression trees

The boosted regression tree (BRT) models explained 61%, 55% and 59% of the cross-validated (CV) deviance for native species richness, abundances of 'hydric' species and abundances of 'xeric' species respectively. The CV correlation between the raw and fitted values for each model were 0.735 ± 0.024, 0.72 ± 0.02, and 0.623 ± 0.022 for native species richness, abundances of 'wet' species and abundances of 'dry' species respectively. Time since last inundation was the single most influential variable explaining all three response variables (Figure 6). Conditional mean water depth in the preceding 3 months before sampling was the second most important variable for both wetland species richness and the abundance of 'hydric' native wetland plants and the third most important variable for the abundance of 'xeric' native wetland plants. The shape of the partial responses was similar to that found for the boosted GAMs with a more complex nonlinear relationship between the response variables and time since last inundated and a generally linear negative relationship with recent conditional mean water depth (3 months prior to sampling).

Both flood frequency and mean temperature in the 90 days prior to sampling were also influential variables (in the top five important variables) in the BRT models for species richness and the abundance of 'hydric' wetland native plants. The influence of these variables, however, appears to be interactive with other predictors. For the species' richness models, eleven pairwise interactions were identified although the size of the interactions was generally weak (low effect size values). In the case of the species richness model, seven out of the eleven interactions identified included flood frequency whilst for the abundances of 'wet' wetland native plants, six out of the 11 most important pairwise interactions included flood frequency. The most important interaction identified between predictors of the native species richness was the effect of time since last inundation which was much more pronounced at high flood frequencies (> 25 over the 30 year period) compared with sites with lower flood frequencies (Figure 7). The interactive plots for the 'hydric' wetland plant abundance model including flood frequency also showed a marked increase in the effects of other predictors at flood frequency values in excess of approximately 25 floods in a thirty year period prior to sampling (Figure 8). It should be noted however, that the distribution of the observations is strongly affecting the model fit. There are relatively few sites with flood frequencies in excess of 25 and hence the interactions are being strongly driven by a relatively small number of potentially correlated records.

In contrast to the species richness and hydric wetland plant abundance models, flood frequency was only identified in three of the eleven most important pairwise interactions for xeric wetland plant abundances. Logically, for the drier preferring wetland plant abundances, where water had persisted during the recent time frame (3 months), terrestrial plants were generally absent or had low abundances and hence there was a clear threshold effect of the conditional mean water depth in the recent antecedent period (Figure 9).

Of the climate variables investigated, mean temperature in the 90 day period leading up to sampling was identified as the most influential variable (Tables 4,5 and 6) although relationships with climate variables directly or indirectly through interactive effects were not found to be particularly strong relative the hydrological effects. Local rainfall was relatively unimportant for all three response models.

Discussion

There is a crucial need for empirical relationships that link flow components to desired ecological responses that are both robust and transferable to guide the management of environmental water. Our analysis demonstrated the importance of hydrological drivers and considering antecedent conditions across a range of relatively recent temporal scales when deriving flow-vegetation relationships. For all three responses examined here, time since last inundated was consistently an important predictor of current vegetation state. The floristics of Hattah lakes wetlands are dominated by herbaceous species associated with saturated and drying muds, with obligate aquatic species being relatively rare hence the majority of species germination responses will be delayed until water levels start to recede. Recent conditional mean water depth was negatively related to our response metrics likewise reflecting the preferences of most species to damp soils rather than surface water presence. Conditions in this post inundation period are therefore likely to be important in predicting vegetation states and are likely to relate to subtle differences in microtopography and soils at a local scale (Deane et al. 2017, Vivian-Smith 1997) which reflect complex gradients in water availability, soil redox and associated changes in soil chemistry and microbial activity.

Short-term antecedent conditions were the dominant drivers of all the response metrics explored here. The effects of longer-term antecedent conditions were only detected for the wetland native abundance response with both the mean water depth over the 1 to 3 year time period prior to sampling and, and the proportion of time wet over the preceding year affecting abundances. The abundances of hydric wetland plants were maximized when the site was inundated for around 50% of the preceding year underlining the importance of periods of both inundation and, drawdown and drying in maximize wetland plant abundances (perhaps allowing for the relatively recent replenishment of the soil seed bank for both wet and drier preferring species). The xeric community appeared to be most strongly affected by recent antecedent conditions, namely the time since last inundation and conditional mean water depth in the recent 3 months. The negative relationship between recent water depth and xeric species makes sound ecological sense as the presence of any surface water in the preceding 3 months prior to sampling dictated that for most sites conditions were not likely to be sufficiently dry to promote germination and growth of xeric species. Maximizing native dry preferring species for temporary wetlands is not necessarily stipulated as a management goal for wetland managers. It can, however, represent a highly beneficial outcome of a watering actions through the removal of existing vegetation and the replenishment of nutrients and organic matter to wetland soils, and recognizes the natural phase shifts that occur in these dynamic systems (Campbell et al. In review).

Notably, significant direct relationships between the long-term flood frequency (FF) of the sites and the response metrics were not found. Boosted GAM models incorporating simple interactions did not perform significantly better than the model with simple additive effects and no interactions. Seed banks composition and structure of temporary wetlands typically reflects long term flooding histories (Capon 2007). We expected areas subject to more frequent historical flooding and therefore increased opportunities for replenishment of the native species propagule bank to have high wetland plant abundances and wetland plant species richness. The boosted regression tree (BRT) analysis identified FF as having an interactive effect with many of the shorter-term metrics with responses to more recent metrics much more pronounced for sites subject to higher historical flood frequencies. This result suggests that flooding history was constraining the responses to recent events but that these effects were relatively subtle. Our results may reflect difficulties disentangling the effects of recent hydrological conditions and long term FF where the drivers are likely to be at least partially correlated (i.e. areas with high FF are also likely to have been subject to different recent hydrological conditions compared with sites with low FF). Whilst many common species are well distributed across flood frequency gradients, low flood frequencies tend to have lower abundances and differ in composition from more frequently flooded habitats (Capon 2007, James et al. 2007) and, as such, changes in composition may not necessarily be reflected in composite metrics.

This study demonstrated the use of two relatively innovative techniques for exploring relationships between wetland vegetation and potential environmental drivers. Both the boosted GAMs and BRT models broadly agreed in terms of the most important drivers of the response variables species richness and the abundances of contrasting hydric and xeric floras of temporary wetland systems. Using boosted GAMs, and implementing the general approach of Hothorn et al. (2011), we evaluated the relative importance of local effects (spatial and spatiotemporal influences) on the response variables. Where local effects are important this would infer processes driving the response variables that are only applicable to the study area and time periods included in the model and would limit the transferability of the models. Local effects due to spatial and/spatiotemporal effects were, however, generally found to be unimportant for all three response variables suggesting that neither the geographic proximity of sites nor the repeated measures nature of the data were strongly influencing the relationships between the response variables and the environmental predictors.

Dispersal capacity is often considered an important predictor of spatial autocorrelation with high dispersal capacity resulting a higher degree of spatial autocorrelation in the response variable (Dirnböck and Dullinger 2004). Spatial autocorrelation has certainly been a feature of some temporary wetland vegetation data sets (Dean et al. 2017, Porter et al. 2007). Wetland plants exhibit a range of morphological adaptations to facilitate dispersal and differences in the degree of autocorrelation may be explained by the predominance of particular dispersal syndromes that tend to characterize wetland and dryland floras. The xeric flora, for example, included many species of the family Asteraceae and Poacecae (Appendix A) which tend to have small seeds effectively dispersed by wind. We would therefore expect greater spatial autocorrelation for the xeric wetland plant models. The model comparisons suggest that this is indeed the case with the model combining additively hydrological, climate and spatial effects being the best performing. However, this model was not significantly better performing that the model including only the hydrological predictors alone. Spatial parameters were, however, based on Euclidean distance rather than flow path distance (through the river/drainage network). Although, spatial autocorrelation is likely to be strongest between transects within the same wetland and hence geographical distance is a reasonable distance measure.

The view of hydrological influences as a temporal hierarchy (Biggs et al, 2005; Beesley et al. 2014) in which responses to recent conditions depend on past hydrological conditions suggests a strong role for antecedent conditions in predicting vegetation responses to flow provisions. This view was only partially borne out by the finding of this study. However, relationships between seed banks and extant vegetation communities are often complex and levels of correlation vary substantially between standing vegetation and seed banks (e.g. Leck and Simpson 1987, Grillas et al. 1993). Low correspondence between seed banks and extant vegetation may result from the spatial redistribution of seed banks where, for example, floods result in redistribution of surface seeds and/or soils within which seeds reside (Bourgeois et al. 2017). Alternately, long periods of dry conditions provide ample opportunities for secondary dispersal of seeds away from sites, through abiotic drivers such as wind dispersal and biotic agents such as ants and rodents. These factors may act to uncouple extant vegetation responses from local seed banks and weaken the role of antecedent hydrological conditions (James et al. 2007). It is significant that demographic studies have found surface plant populations to be poor predictors of long term population trends in annual wetland plants (Adams et al. 2005). Further research is needed to test the models developed here at other wetland locations and habitat types, and hence determine the transferability of predicted outcomes and key drivers between different locations and situations.

We did not detect a strong influence of antecedent rainfall or temperature on any of our native response variables. There are a number of possible reasons for this. In the first instance, climate data was derived from single gauged locations and hence localized variations in rainfall between different monitored sites were not captured and rainfall estimates may therefore not accurately reflect rainfall at any one site. Spatial variation in rainfall for flat semi-arid regions is, however, general low at the spatial scales of the wetlands modelled here (Augustine 2010). In semi-arid environments even small differences in rainfall can be ecologically important (Sala and Laurenroth 1982) and hence our inability to account for small scale variations in rainfall may account for some of the variation in responses observed. The wetland complex studied here provides only limited spatial climatic gradients as all sites are situated within relatively close proximity to each other. The influences of climate are general considered to be important at broader regional spatial scales. We also only explicitly explore the direct effects of climate, the indirect effects that occur through, for example, modifying hydrological conditions are incorporated into the hydrodynamic modelling of water levels. For example, air temperatures will affect rates of drawdown which, in turn, can influence species germinating from the seed bank (Nichol et al. 2003).

Variability in responses to hydrological regimes may also arise where plants are grouped together (e.g. functional groups) as individualistic responses to flooding and drying exhibited by plant taxa may confound relationships with flow (Dean et al. 2017, Moxham et al. 2019). However, temporary wetlands often harbor quite unique compositions, even those situated in relative close proximity, and hence plant functional classifications can allow for generalizations to sites with different plant compositions (Campbell et al. 2014). Furthermore, wetland floras tend to be highly diverse and variable and, it is not likely to be feasible to create species level models at broader spatial scales (Dean et al. 2016). Grouping species into response guilds facilitates generalizations and provides a common language in discussions between scientists and water managers (Merritt et al. 2010, Campbell et al. 2014) but need underpinning by robust and objective frameworks for determining guild membership.

The response variables used here reflect commonly specified ecological targets set for wetlands of high species richness and abundance for water responsive species (Brown et al. 2017, Campbell et al. In review). Both these targets, however, tend to be maximized after waters recede and minimized in the presence of surface water. Repeated and/or prolonged inundations typically reduce abundances and diversity (Casanova and Brock 2000) yet such conditions may support other important ecological values such as different wetland communities (native dry taxa) or low diversity communities of specific valued species (e.g. monocultures of reeds). There is a clear need to align vegetation objectives more closely to specific management goals or values (Campbell et al. In review) and recognize the inherently variable nature of semi-arid wetlands.

Finally, this study underlines the utility of existing long term monitoring datasets. Given the increasing sophisticated approaches that are available for dealing with the complexities of long term monitoring datasets (e.g. spatial and temporal autocorrelation, methodological and taxonomic inconsistencies), long-term monitoring datasets are under-utilized with emphasis on their limitation and not their capacity. Yet, particularly in variable/dynamic systems, short-term studies may not encompass the full range of environmental conditions likely to be encountered nor the rare or infrequent events that often govern the ecologies of these systems.

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Table 1. Summary of environmental predictors used in modelling. For codes time periods were designated as d3Mon, d1yr and d3yrs for the time periods 3 months, 4 months to 1.25 years and 1.25 – 3.25 years respectively.

Variable	Time frame (s)	Units	Data source	Mean and range
Hydrological Variables				
Inundated	At time of survey	TRUE/FALSE	Observational record	
Time since last inundated	Most recent event	days	Modelled data	
Proportion of days wet	3 months, 4 months – 1.25 year and 1.25 year to 3.25 years		Modelled data	
Conditional mean water depth	3 months, 4 months – 1.25 year and 1.25 year to 3.25 years	meters	Modelled data	
Frequency of rewetting. Note that if the site was continuously wet or continuously dry this will score 0.	1 year and 3 years		Modelled data	
Flood frequency	30 years		Modelled data	
Climate				
Maximum temperature	3 months and 1 year preceding sampling	^{o}C	(Nulkwyne Kiamal station, BOM 2019).	
Minimum temperature	3 months and 1 year preceding sampling	^{o}C	(Nulkwyne Kiamal station, BOM 2019).	
Mean temperature	3 months and 1 year preceding sampling	^{o}C	(Nulkwyne Kiamal station, BOM 2019).	
Total rainfall	3 months and 1 year preceding sampling	mm	Parks Victoria data	
Other non-flow variables				
Eastings	NA	meters		
Northing	NA	meters		

Table 2 Overview of candidate models considered for response metrics

Model	Description	Inference
Spatial	Spatial and spatiotemporal autocorrelative effects only (referred to as spatial effects for remainder of the table)	Performs best when there is no detectable influence of environmental predictors
Climate+Hydro	The additive effects of hydrological and climatic variables	Performs best in the absence of interactions and any spatiotemporal correlation
Climate	The additive effects of climatic variables only	Performs best when all variability can be attributed to climate variables and there are no interactions or spatiotemporal correlation
Hydro	The additive effects of hydrological variables only	Performs best when all variability can be attributed to hydrological variables and there are no interactions or spatiotemporal correlation
Climate+Hydro+ Spatial	The additive effects of hydrological and climatic variables and spatial terms	Performs best when variability is shared amongst environmental predictors and there is spatial variation that does not resemble important gradients of environmental predictors
Tree	Effects of hydrological and climatic variables using tree based learners	Performs best in the presence of strong interactions amongst predictors
Tree+Spatial	Effects of hydrological and climatic variables using tree based learners and including spatial autocorrelative terms	Performs best in the presence of strong interactions amongst predictors and spatiotemporal effects.

Table 3. Results of stable selection procedure on the best performing additive model as assessed on the out-of-bootstrap observations.

Response metric	Distribution	Best model	Pseudo R2	Variables and base learners selected more than 50% of the time
Native hydric species richness	Negative Binomial	Hydro	0.50 (0.47-0.51)	Time since last inundated (bbs, bols) 3 months conditional mean depth (bols) 3 months proportion of time wet (bols)
Native 'hydric wetland species abundance	Negative Binomial	Hydro	0.30 (0.27-0.32)	Time since last inundated (bbs) 3 months conditional mean depth (bols) 4 months-1.25 years proportion of time wet (bbs) 1.25-3.25 years conditional mean depth (bols)
Native 'xeric' wetland species abundance	Negative Binomial	Hydro	0.28 (0.23-0.32)	Time since last inundated (bbs, bols) 3 months conditional mean depth (bols) 3 months proportion of time wet (bols)

Table 4. Variable importance for native wetland plant species richness using boosted regression trees. Note that variable importance incorporates both additive as well as interactive effects.

Predictor	Variable importance
Time since last inundation	25
The conditional mean water depth in the 3 months preceding sampling	21
Proportion of time the site was wet in the 1.25 – 3.25 years preceding sampling	9.7
Flood frequency (over the 30 years preceding sampling)	8.0
Mean temperature in the 90 days prior to sampling	7.2
The conditional mean water depth in the 4 months – 1.25 years preceding sampling	5.1
The conditional mean water depth in the 1.25 – 3.25 years preceding sampling	4.7
Proportion of time the site was wet in the 4 months – 1.25 years preceding sampling	4.6
Accumulated rainfall in 1 year preceding sampling	3.7
Frequency of inundation in year preceding sampling	2.2

Table 5. Variable importance for native ‘hydric’ wetland plant abundance using boosted regression trees. Note that variable importance incorporates both additive as well as interactive effects.

Predictor	Variable importance
Time since last inundation	22
The conditional mean water depth in the 3 months preceding sampling	13
Proportion of time the site was wet in the 1.25 – 3.25 years preceding sampling	11
Flood frequency (over the 30 years preceding sampling)	9.1
Mean temperature in the 90 days prior to sampling	8.9
The conditional mean water depth in the 1.25 – 3.25 years preceding sampling	6.6
The conditional mean water depth in the 4 months – 1.25 years preceding sampling	6.4
Proportion of time the site was wet in the 4 months – 1.25 years preceding sampling	5.8
Accumulated rainfall in 1 year preceding sampling	3.78
Accumulated rainfall in 90 days preceding sampling	2.97

Table 6. Variable importance for native terrestrial plant abundance using boosted regression trees. Note that variable importance incorporates both additive as well as interactive effects.

Predictor	Variable importance
Time since last inundation	22
The proportion of time the site was wet in the 1.25 – 3.25 years preceding sampling	15
The proportion of time the site was wet in the 3 months preceding sampling	14
Mean temperature in the 90 days prior to sampling	13
The conditional mean water depth in the 3 months preceding sampling	11
The conditional mean water depth in the 1.25 – 3.25 years preceding sampling	6.6
Accumulated rainfall in 1 year preceding sampling	5.2
Flood frequency (over the 30 years preceding sampling)	3.4
The conditional mean water depth in the 4 months – 1.25 years preceding sampling	2.4
Minimum temperature in the 90 days prior to sampling	2.2

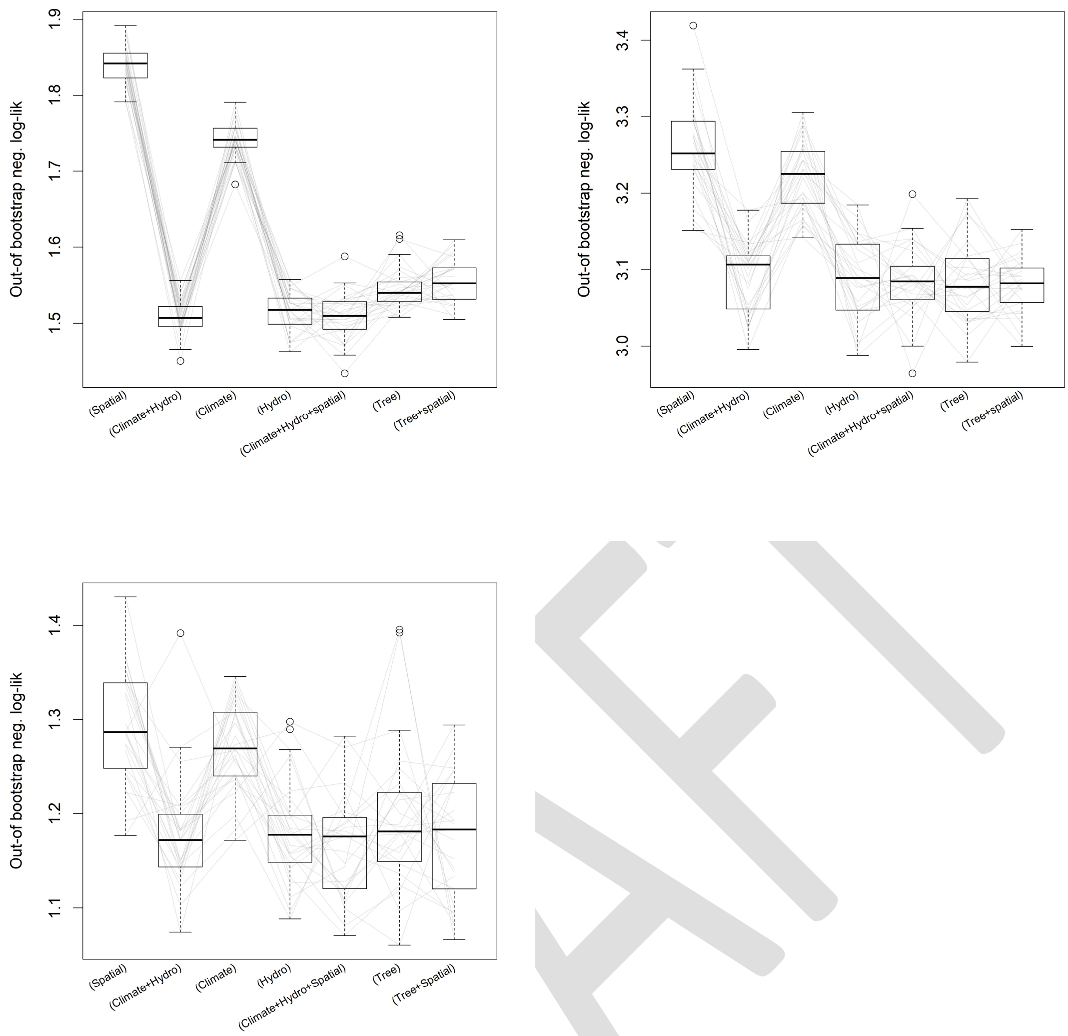

Figure 2. Candidate model evaluation of (a) native wetland species richness, (b) native wetland species abundances, and, (c) native terrestrial species abundances. The out-of-bootstrap distribution of the negative log-likelihood is given for models with different complexities and components. Lower values for the negative log-likelihood indicate better models. The differences between models for each response variable were formally assessed using multiplicity adjusted all-pairwise comparisons at the family-wise error α=0.05.

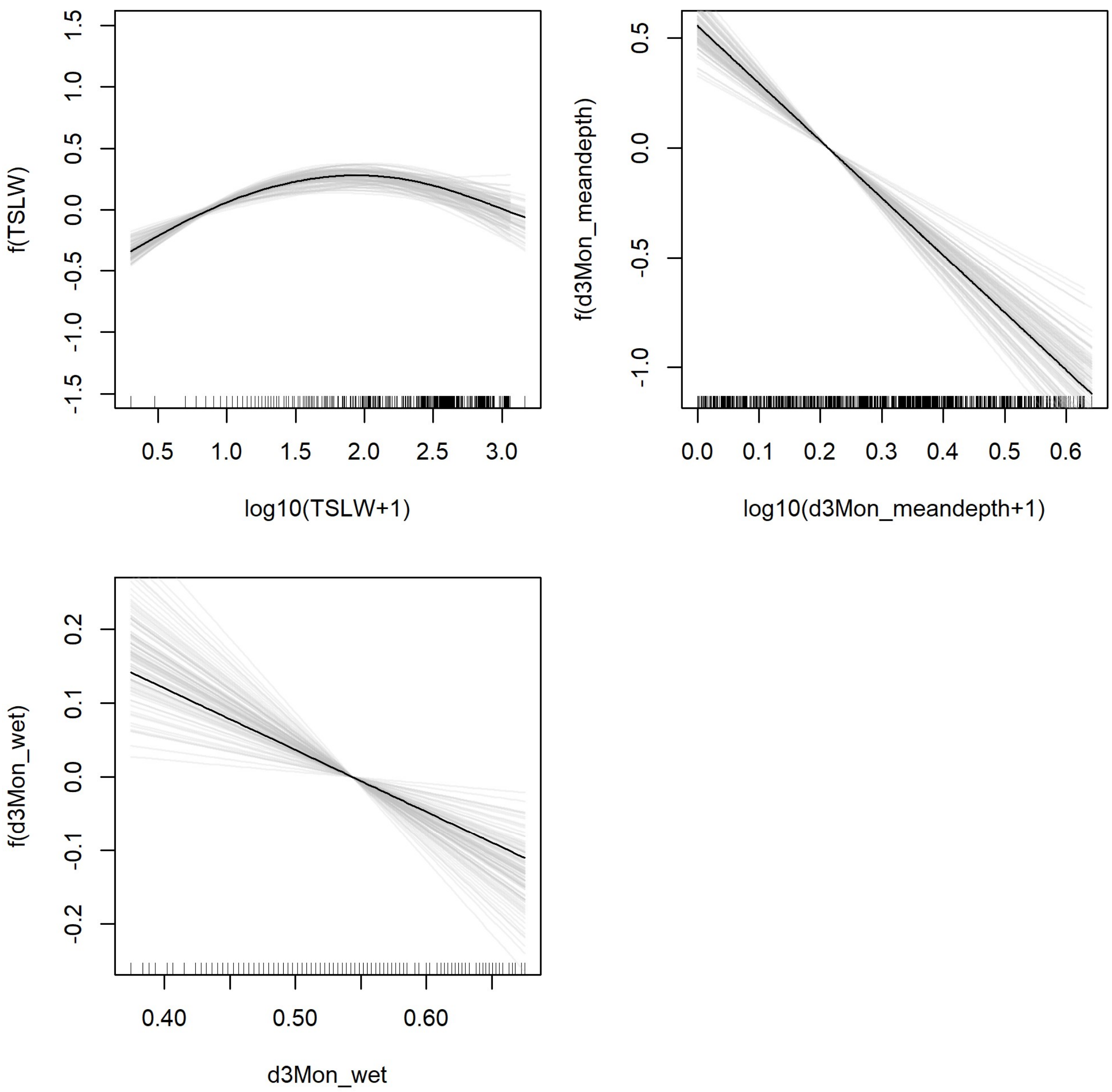

Figure 3. Estimated partial effects of key environmental variables on native wetland plant species richness in the hydro model as selected by the stability selection procedure. TSLW=Time since last inundated (days), d3Mon_mean depth (m) = the conditional mean water depth in the three months preceding sampling, d3Mon_wet= the proportion of time the site was wet in the three months preceding sampling. Rug lines at the bottom of the plot indicate observed sample values. Grey lines represent marginal functional estimates from 100 bootstrap samples of the full data set.

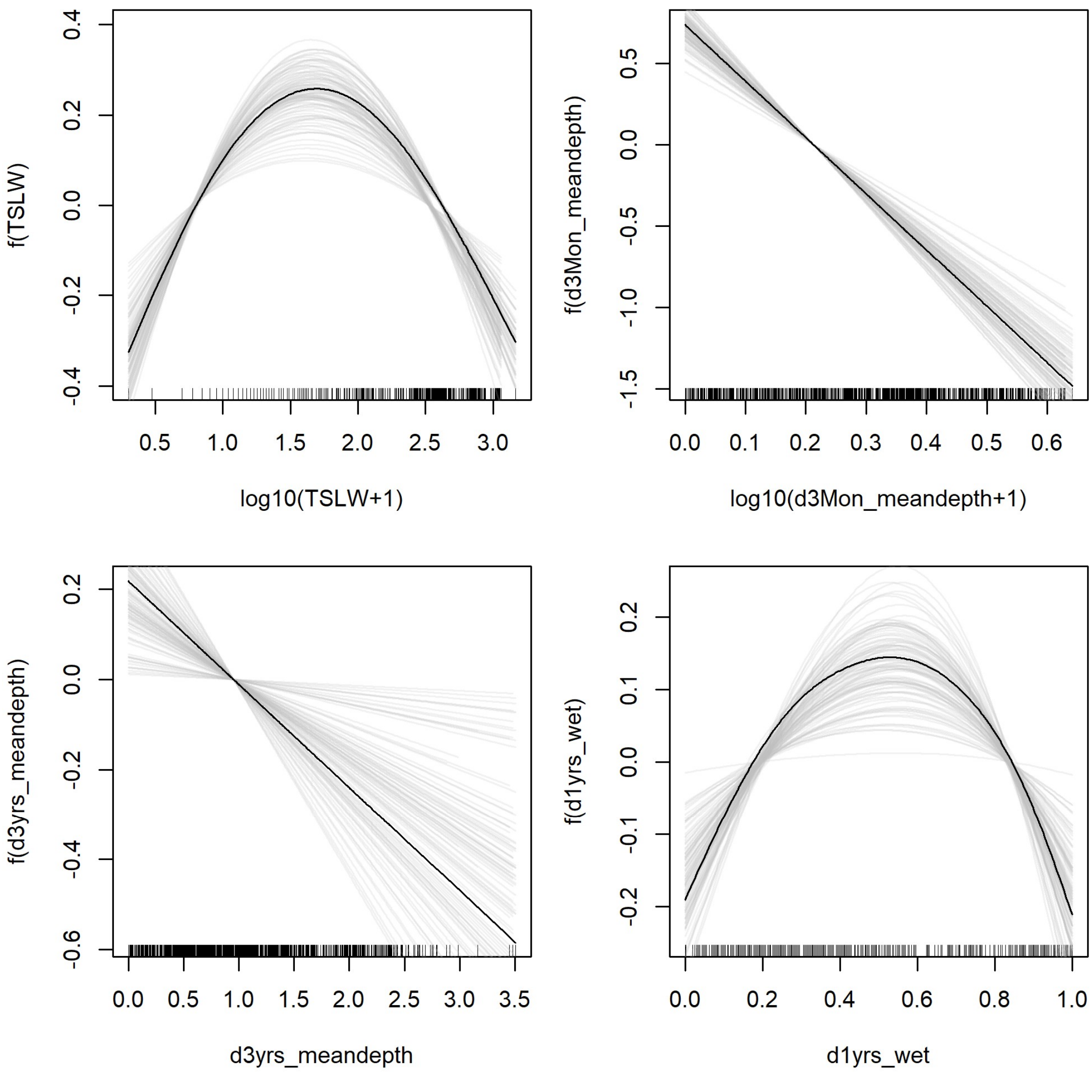

Figure 4. Estimated partial effects of environmental variables on native hydric wetland plant abundances in the hydro model as selected by the stability selection procedure. TSLW=Time since last inundated (days), d3Mon_mean depth (m) = the conditional mean water depth in the three months preceding sampling, d3yrs_meandepth= the conditional mean water depth in the 1.25-3.25 years preceding sampling, d1yrs_wet=proportion of time the site was wet in the 4 months – 1.25 years preceding sampling. Rug lines at the bottom of the plot indicate observed sample values. Grey lines represent marginal functional estimates from 100 bootstrap samples of the full data set.

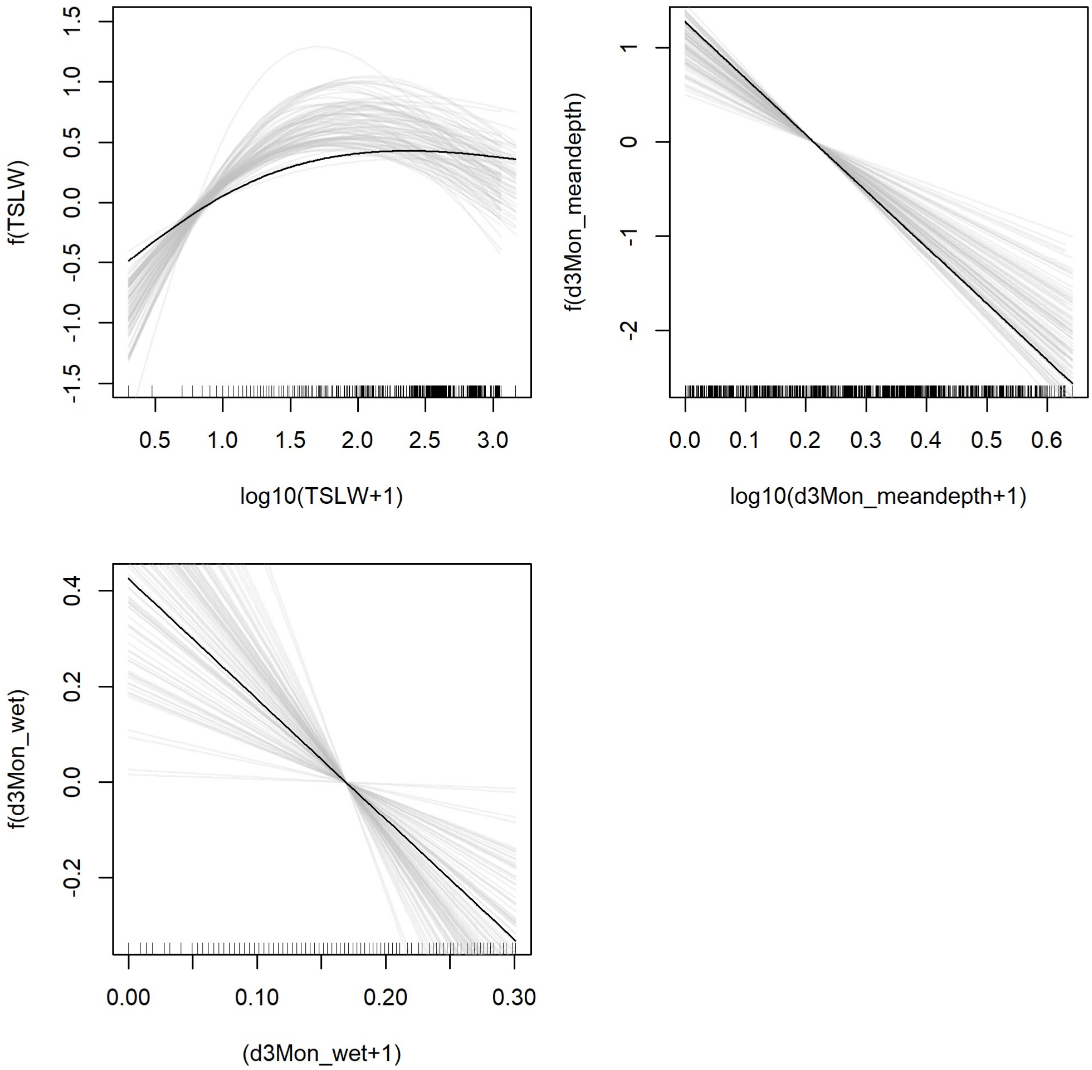

Figure 5. Estimated partial effects of environmental variables on native xeric wetland plant abundances in the hydro model as selected by the stability selection procedure. TSLW=Time since last inundated (days), d3Mon_mean depth (m) = the conditional mean water depth in the three months preceding sampling, d3Mon_wet = proportion of time the site was wet in the three months preceding sampling. Rug lines at the bottom of the plot indicate observed sample values. Grey lines represent marginal functional estimates from 100 bootstrap samples of the full data set.

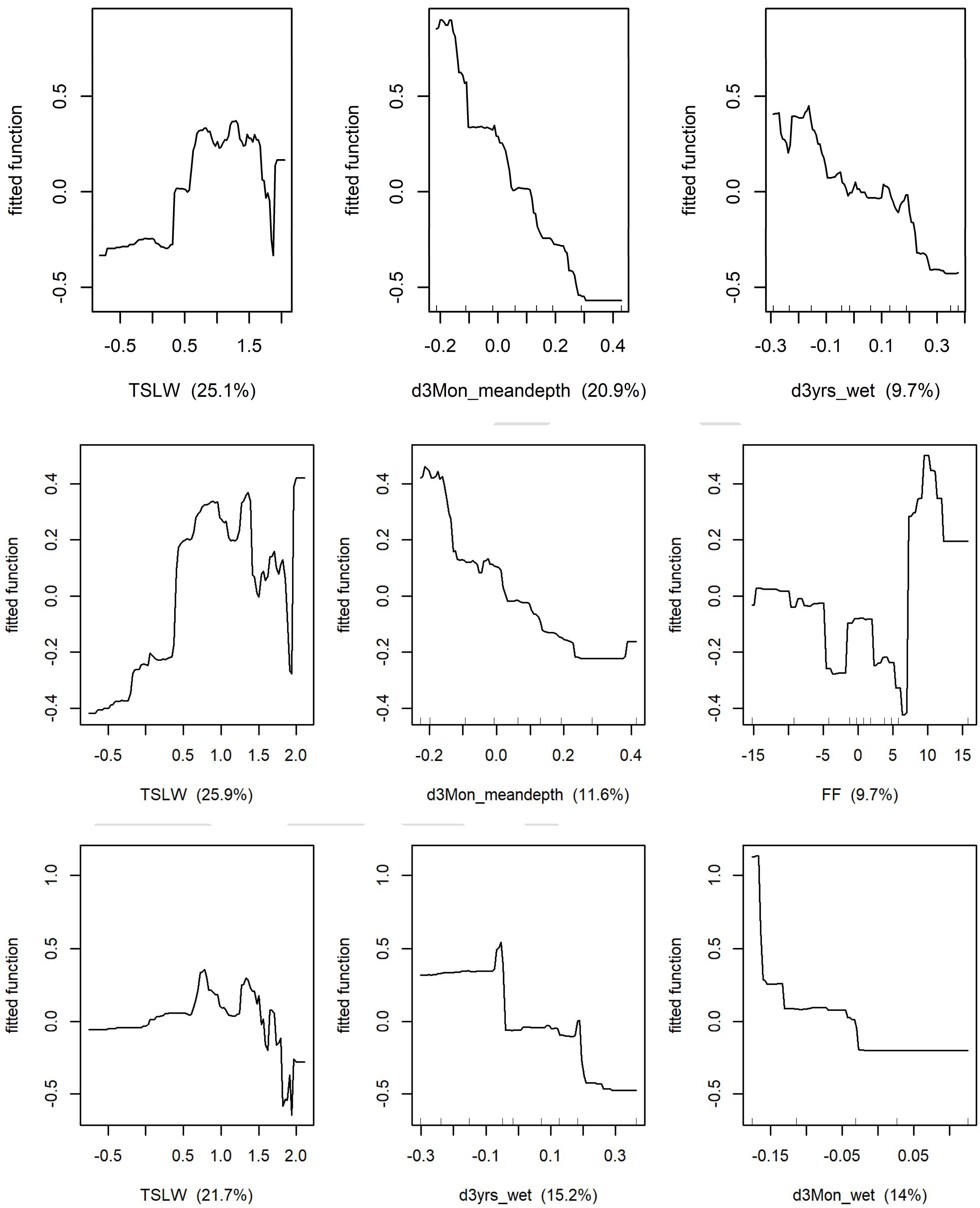

Figure 6. Estimated partial effects of the three most influential variables in the BRT models for wetland species richness (top row), abundance of 'hydric' wetland plants (middle row) and abundance of 'xeric' wetland plants (bottom row).

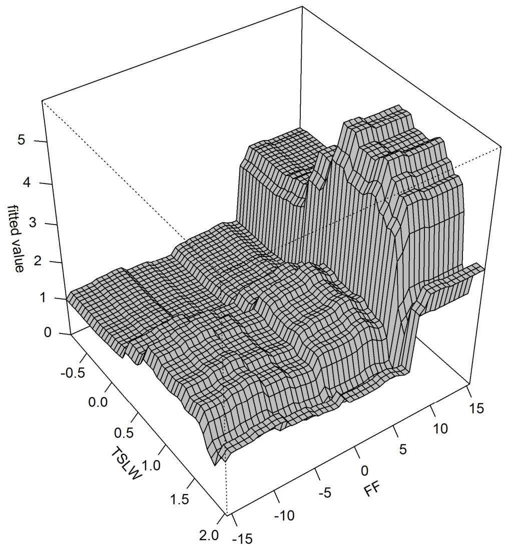

Figure 7. Three-dimensional partial dependence plots for the strongest interaction in the model for native wetland plant species richness. For an explanation of the variables and their units see Table 1.

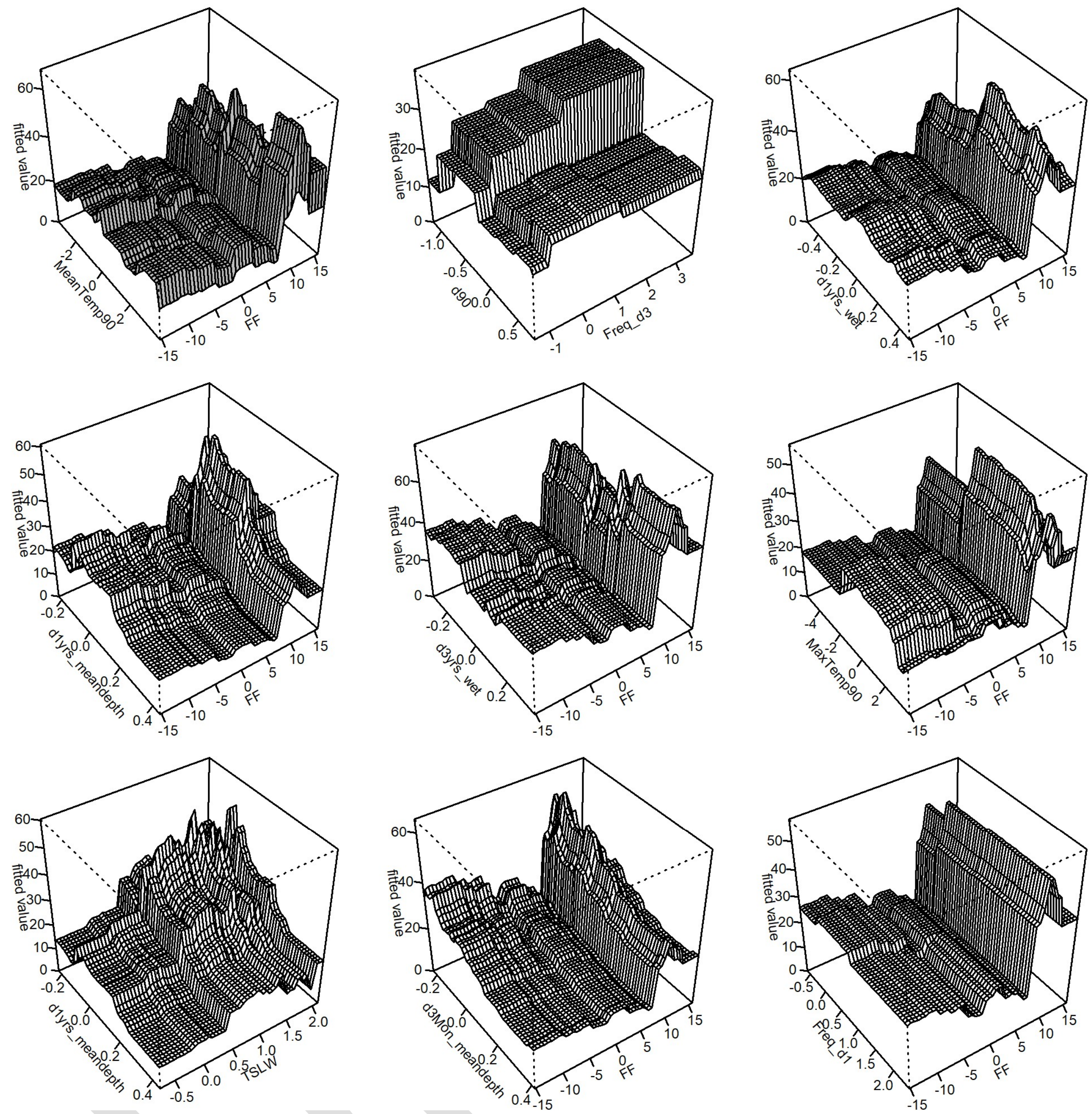

Figure 8. Three dimensional plots showing the important interactive effects in the boosted regression tree model of native 'hydric' wetland plant abundance. FF= flood frequency, TSLW=Time since last wet, d3Mon_wet = the proportion of time the site was wet in the three months preceding sampling, d3Mon_meandepth = the conditional mean water depth in the three months preceding sampling, d1yrs_meandepth= the conditional mean water depth in the 4 months – 1.25 years preceding sampling. d3yrs_wet= the proportion of time the site was wet in the 1.25 to 3.25 years preceding sampling, d3yrs_meandepth= the conditional mean water depth in the 1.25 to 3.25 years preceding sampling. Freq_d3 = frequency of rewetting in the three years preceding sampling. d90=accumulated rainfall in the three months preceding sampling. MaxTemp90 and MeanTemp90 = the maximum and mean daily temperatures in the 3 months preceding sampling respectively.

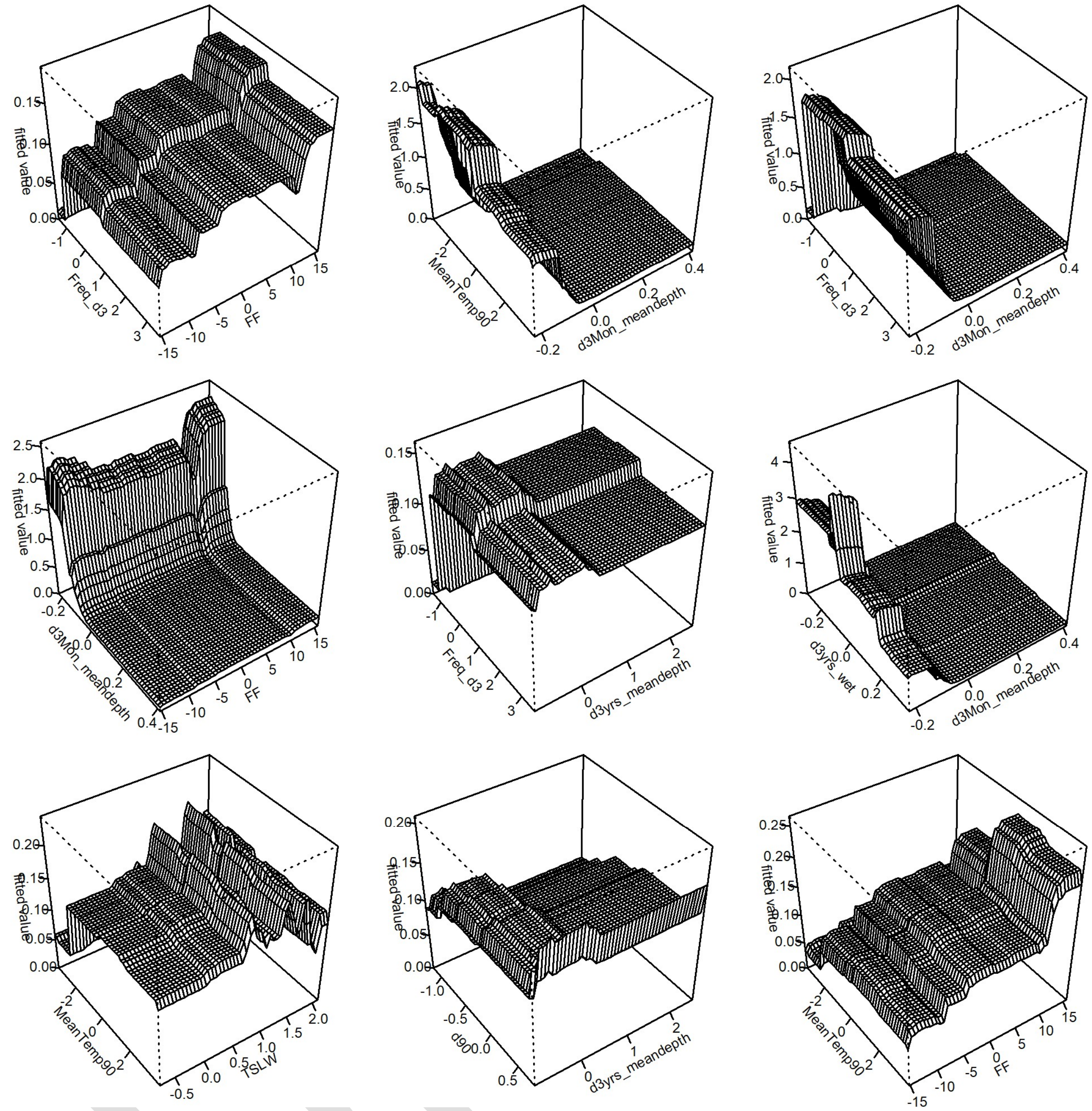

Figure 9. Three dimensional plots showing the important interactive effects in the boosted regression tree model of native ‘xeric’ wetland plant abundance. FF= flood frequency, TSLW=Time since last wet, d3Mon_meandepth = the conditional mean water depth in the three months preceding sampling, d3yrs_meandepth= the conditional mean water depth in the 1.25 to 3.25 years preceding sampling. d90=accumulated rainfall in the three months preceding sampling. Freq_d3 = frequency of rewetting in the three years preceding sampling. MaxTemp90, MeanTemp90 and MinTemp90 = the maximum, mean and minimum daily temperatures in the 3 months preceding sampling respectively.

Appendix A Details of wetland sites monitoring data. Values in brackets denote a change in the number of elevations monitored on one or more occasions. Map grid zone 54.

Wetland name	Easting	Northing	Years	No. replicate transects	No. elevations	Total n
Bitterang	626058	6163226	3	4	6	72
Bulla	624688	6153528	8	4	6	192
Boich	627053	6153223	8	3	3	72
Brockie	626263	6153544	8	3	5	120
Chalka Creek South	629209	6157750	8	4	4	128
Hattah	623255	6153010	8	4	7 (6)	216
Kramen	633594	6150527	5	4	5	100
Little Hattah	623174	6153822	8	4	3	96
Mournpall	623989	6158850	8	4	7 (5)	207
Chalka Creek North	627529	6162393	4	4	4	64
Nip Nip	628067	6153850	8	4	3	96
Yerang	625003	6158254	8	4	3	96

Appendix B Species list with functional group and exotic status designation.

Scientific name	Species code	Functional group	Exotic status
Abutilon theophrasti	Abu.theo	Tda	TRUE
Acacia brachybotrya	Aca.brac	Tdr	FALSE
Acacia sp.	Aca.spp.	Tdr	FALSE
Acacia stenophylla	Aca.sten	ATw	FALSE
Ajuga australis	Aju.aust	Tdr	FALSE
Alternanthera denticulata	Alt.dent	Tda	FALSE
Alternanthera nodiflora	Alt.nodi	Tda	FALSE
Alternanthera sp.	Alt.sp.	Tda	FALSE
Alternanthera sp. 1 (VIC)	Alt.sp.	Tda	FALSE
Ammannia multiflora	Amm.mult	ARp	FALSE
Aristida calycina var. calycina	Ari.caly	Tda	FALSE
Asphodelus fistulosus	Asp.fist	Tdr	TRUE
Asperula gemella	Asp.geme	Tda	FALSE
Asteraceae (exotic)	Asterace	Tdr	TRUE
Asteraceae	Asterace	Tdr	FALSE
Atriplex eardleyae	Atr.eard	Tdr	FALSE
Atriplex leptocarpa	Atr.lept	Tdr	FALSE
Atriplex lindleyi subsp inflata	Atr.lind	Tdr	FALSE
Atriplex pumilio	Atr.pumi	Tdr	FALSE
Atriplex semibaccata	Atr.semi	Tdr	FALSE
Atriplex sp.	Atr.sp.	Tdr	FALSE
Atriplex stipitata	Atr.stip	Tdr	FALSE
Atriplex suberecta	Atr.sube	Tdr	UNCERTAIN
Austrostipa drummondii	Aus.drum	Tdr	FALSE
Austrobryonia micrantha	Aus.micr	Tda	FALSE
Austrostipa scabra	Aus.scab	Tdr	FALSE
Austrostipa sp.	Aus.sp.	Tdr	FALSE
Azolla rubra	Azo.rubr	ARf	FALSE
Azolla sp.	Azo.sp.	ARf	FALSE
Blue-green algae	Blu.alga	S	FALSE
Boerhavia dominii	Boe.domi	Tdr	FALSE
Brachyscome ciliaris	Bra.cili	Tdr	FALSE
Brachyscome lineariloba	Bra.line	Tdr	FALSE
Brachyscome sp.	Bra.sp.	Tdr	FALSE
Brassicaceae	Bras.sp.	Tdr	FALSE
Brassica sp.	Bras.sp.	Tdr	TRUE
Brassica tournefortii	Bras.tour	Tdr	TRUE
Brassica x juncea	Bras.x.jun	Tdr	TRUE
Bromus sp.	Bro.sp.	Tdr	TRUE
Calotis cuneifolia	Cal.cune	Tdr	FALSE
Calotis erinacea	Cal.erin	Tdr	FALSE
Calotis hispidula	Cal.hisp	Tdr	FALSE
Calandrinia sp.	Cala.sp.	Tdr	FALSE

Callitriche sp.	Call.sp.	ATl	FALSE
Centipeda cunninghamii	Cen.cunn	ATl	FALSE
Centipeda minima	Cen.mini	ATl	FALSE
Centipeda sp.	Cent.sp.	ATl	FALSE
Centaurea sp.	Centaure	Tdr	TRUE
Centaurium sp.	Centauri	Tdr	TRUE
Characeae sp.	Cha.sp.	S	FALSE
Chenopodium desertorum subsp. desertorum	Che.dese	Tdr	FALSE
Chenopodium melanocarpum (NSW only)	Che.mela	Tdr	FALSE
Chenopodium sp.	Che.sp.	Tdr	FALSE
Chenopodiaceae	Chenopod	Tdr	FALSE
Chloris truncata	Chl.trun	Tdr	FALSE
Chondrilla juncea	Cho.junc	Tdr	TRUE
Convolvulus remotus	Con.remo	Tdr	FALSE
Convolvulus sp	Con.sp	Tdr	FALSE
Crassula sp.	Cras.sp.	Tdr	FALSE
Cucurbitaceae	Cucurbit	Tdr	FALSE
Cynodon dactylon	Cyn.dact	Tdr	UNCERTAIN
Cyperus gymnocaulos	Cyp.gymn	ATe	FALSE
Cyperus sp.	Cyp.sp.	ATe	FALSE
Cyperaceae	Cyperace	ATe	FALSE
Dissocarpus paradoxus	Dis.para	Tdr	FALSE
Dittrichia graveolens	Dit.grav	Tdr	TRUE
Dodonaea viscosa subsp angustissima	Dod.visc	Tdr	FALSE
Duma florulenta	Dum.flor	ATw	FALSE
Dysphania cristata	Dys.cris	Tdr	FALSE
Dysphania pumilio	Dys.pumi	Tdr	FALSE
Eclipta platyglossa subsp. platyglossa	Ecl.plat	Tda	FALSE
Einadia nutans	Ein.nuta	Tdr	FALSE
Elatine gratioloides	Ela.grat	ATl	FALSE
Eleocharis pusilla	Ele.pusi	ATe	FALSE
Eleocharis sp.	Ele.sp.	ATe	FALSE
Enchylaena tomentosa	Enc.tome	Tdr	FALSE
Enneapogon nigricans	Enn.nigr	Tdr	FALSE
Eragrostis australasica	Era.aust	ATe	FALSE
Eragrostis dielsii	Era.diel	Tdr	FALSE
Eragrostis lacunaria	Era.lacu	Tdr	FALSE
Erigeron bonariense	Eri.bona	Tdr	TRUE
Erigeron	Erigeron	Tdr	TRUE
Erodium crinitum	Ero.crin	Tdr	FALSE
Eucalyptus camaldulensis	Euc.cama	ATw	FALSE
Eucalyptus largiflorens	Euc.larg	ATw	FALSE
Euphorbia dallachyana	Eup.dall	Tdr	FALSE
Geococcus pusillus	Geo.pusi	Tda	FALSE
Glinus lotoides	Gli.loto	Tda	FALSE
Glinus oppositifolius	Gli.oppo	Tda	FALSE

Glossostigma elatinoides	Glo.elat	ARp	FALSE
Glossostigma sp.	Glo.sp.	ARp	FALSE
Glycyrrhiza acanthocarpa	Gly.acan	Tda	FALSE
Goodenia glauca	Goo.glau	Tda	FALSE
Gratiola pubescens	Gra.pube	Tda	FALSE
Gratiola pumilo	Gra.pumi	Tda	FALSE
Haloragis sp.	Halo.sp.	Tdr	FALSE
Heliotropium curassavicum	Hel.cura	Tda	TRUE
Heliotropium europaeum	Hel.euro	Tdr	TRUE
Helichrysum luteo-album	Hel.lute	Tdr	FALSE
Heliotropium sp.	Hel.sp.	Tdr	TRUE
Heliotropium supinum	Hel.supi	Tda	TRUE
Hypericum gramineum	Hyp.gram	Tdr	FALSE
Hypochaeris radicata	Hyp.radi	Tdr	TRUE
Isolepis australiensis	Iso.aust	ATe	FALSE
Isolepis hookeriana	Iso.hook	ATe	FALSE
Juncus bufonius	Jun.bufo	ATe	UNCERTAIN
Juncus sp.	Jun.sp.	ATe	FALSE
Juncus subsecundus	Jun.subs	ATe	FALSE
Lachnagrostis filiformis	Lac.fili	Tda	FALSE
Lactuca serriola	Lac.serr	Tdr	TRUE
Lachnagrostis sp.	Lac.sp.	Tda	FALSE
Lemna disperma	Lem.disp	ARf	FALSE
Lemna sp.	Lemn.sp.	S	FALSE
Leontodon taraxacoides subsp. taraxacoides	Leo.tara	Tdr	TRUE
Lepidium pseudohyssopifolium	Lep.pseu	Tdr	FALSE
Limosella australis	Lim.aust	ATl	FALSE
Limosella sp.	Lim.sp.	ARp	FALSE
Lotus cruentus	Lot.crue	Tdr	FALSE
Ludwigia peploides	Lud.pepl	ARp	FALSE
Lythrum hyssopifolia	Lyt.hyss	Tda	FALSE
Maireana brevifolia	Mai.brev	Tdr	FALSE
Maireana spp	Mair.sp.	Tdr	FALSE
Maireana sp.	Mair.sp.	Tdr	FALSE
Malva sp.	Mal.sp.	Tdr	FALSE
Malva weinmanniana	Mal.wein	Tdr	FALSE
Marsilea drummondii	Mar.drum	ARp	FALSE
Marsilea sp.	Mar.sp.	ARp	FALSE
Marrubium vulgare	Mar.vulg	Tdr	TRUE
Medicago minima	Med.mini	Tdr	TRUE
Medicago spp	Med.sp.	Tdr	TRUE
Medicago sp.	Med.sp.	Tdr	TRUE
Myriophyllum sp.	Myr.sp.	ARp	FALSE
Myriophyllum verrucosum	Myr.verr	ARp	FALSE
Olearia pimeleoides	Ole.pime	Tdr	FALSE
Osteocarpum salsuginosum	Ost.sals	Tdr	FALSE

Osteocarpum sp.	Ost.sp.	Tdr	FALSE
Ottelia ovalifolia subsp ovalifolia	Ott.oval	ARf	FALSE
Paspalum distichum	Pas.dist	ARp	TRUE
Pentameris airoides subsp. Airoides	Pen.airo	Tdr	TRUE
Pentaschistis airoides	Pen.airo	Tdr	TRUE
Persicaria decipiens	Per.deci	ATe	FALSE
Persicaria lapathifolia	Per.lapa	ATe	FALSE
Persicaria prostrata	Per.pros	ATl	FALSE
Phyllanthus lacunarius	Phy.lacu	Tda	FALSE
Podolepis capillaris	Pod.capi	Tdr	FALSE
Polygonum aviculare	Pol.avic	Tdr	TRUE
Polygonum plebeium	Pol.pleb	Tda	FALSE
Portulaca oleracea	Por.oler	Tdr	FALSE
Potamogeton sulcatus	Pot.sulc	ARf	FALSE
Pseudoraphis spinescens	Pse.spin	ARp	FALSE
Psilocaulon granulicaule	Psi.gran	Tdr	TRUE
Ranunculus pentandrus var. platycarpus	Ran.pent	Tda	FALSE
Reichardia tingitana	Rei.ting	Tdr	TRUE
Rhagodia sp.	Rha.sp.	Tdr	FALSE
Rhagodia spinescens	Rha.spin	Tdr	FALSE
Rhodanthe corymbiflora	Rho.cory	Tdr	FALSE
Rorippa palustris	Ror.palu	Tda	TRUE
Rorippa sp.	Rori.sp.	Tda	FALSE
Rumex brownii	Rum.brow	Tda	FALSE
Rumex crystallinus	Rum.crys	Tda	FALSE
Rumex tenax	Rum.tena	Tda	FALSE
Rumex sp.	Rume.sp.	Tda	FALSE
Rytidosperma sp.	Ryti.sp.	Tdr	FALSE
Salsola tragus	Sal.trag	Tdr	FALSE
Salvia verbenaca	Sal.verb	Tdr	TRUE
Schenkia australis	Sch.aust	Tdr	FALSE
Schismus barbatus	Sch.barb	Tdr	TRUE
Sclerolaena brachyptera	Scl.brac	Tdr	FALSE
Sclerolaena diacantha	Scl.diac	Tdr	FALSE
Sclerolaena divaricata	Scl.diva	Tdr	FALSE
Sclerolaena muricata	Scl.muri	Tdr	FALSE
Sclerolaena obliquicuspis	Scl.obli	Tdr	FALSE
Sclerolaena patenticuspis	Scl.pate	Tdr	FALSE
Sclerolaena stelligera	Scl.stel	Tdr	FALSE
Sclerolaena sp.	Scle.sp.	Tdr	FALSE
Senecio quadridentatus	Sen.quad	Tdr	FALSE
Senecio runcinifolius	Sen.runc	Tda	FALSE
Sida sp.	Sid.sp.	Tdr	FALSE
Solanum nigrum	Sol.nigr	Tdr	TRUE
Sonchus asper	Son.aspe	Tdr	TRUE
Sonchus oleraceus	Son.oler	Tdr	TRUE

Sonchus sp.	Sonc.sp.	Tdr	TRUE
Spergularia rubra	Spe.rubr	Tdr	TRUE
Sphaeromorphaea littoralis	Sph.litt	Tda	FALSE
Sporobolus mitchellii	Spo.mitc	Tda	FALSE
Stemodia florulenta	Ste.flor	Tda	FALSE
Stellaria media	Ste.medi	Tdr	TRUE
Swainsona microphylla	Swa.micr	Tdr	FALSE
Tetragonia moorei	Tet.moor	Tdr	FALSE
Teucrium racemosum	Teu.race	Tdr	FALSE
Trigonella suavissima	Tri.suav	Tda	FALSE
Vallisneria australis	Val.aust	S	FALSE
Verbena bonariensis	Ver.bona	Tdr	TRUE
Verbena officinalis	Ver.offi	Tdr	FALSE
Verbena sp.	Ver.sp.	Tdr	FALSE
Verbena supina	Ver.supi	Tda	TRUE
Vittadinia cuneata	Vit.cune	Tdr	FALSE
Vittadinia dissecta	Vit.diss	Tdr	FALSE
Vittadinia gracilis	Vit.grac	Tdr	FALSE
Vittadinia pterochaeta	Vit.pter	Tdr	FALSE
Vittadinia sulcata	Vit.sulc	Tdr	FALSE
Vittadinia sp.	Vitt.sp.	Tdr	FALSE
Wahlenbergia fluminalis	Wah.flum	Tda	FALSE
Xanthium strumarium	Xan.stru	Tda	TRUE
Zygophyllum sp.	Zygo.sp.	Tdr	FALSE

Appendix V2.2: Data Integration and Synthesis Research Activity Report

Authors (and institution): Cassandra James (James Cook University), Cherie Campbell (LTU), Sam Capon (Griffith University), Kay Morris (Arthur Rylah Institute), Jason Nicol (SARDI), Daryl Nielsen (CSIRO), Rachael Thomas (NSW OEH / UNSW), Susan Gehrig (MDFRC)

Document purpose: Summary document to capture the outputs and outcomes from the Data Integration and Synthesis component of the MDB EWKR Vegetation theme. This document complements and refers to other outputs rather than duplicates information.

• Research Questions

This component explored the utility of existing long-term data sets to address the broad research question '*what drives vegetation responses to watering actions?'* and well as *'how can we learn more from existing data?'*

Long-term monitoring of wetland and floodplain complexes provides an opportunity to investigate how vegetation responses relate to hydrological regimes and, in particular, to interrogate the influences of antecedent condition and historical legacies on these responses. Patterns in the composition and structure of wetland vegetation reflect both contemporary hydrological conditions and the legacy of past hydrological events, as well as a wide range of potential non-flow factors such as soil type and historical land management. These 'historical' factors may act to modify vegetation responses to contemporary management such as flow provisions resulting in unexpected outcomes. This work relates to predicting outcomes to environmental watering events and using those predictions to help plan or prioritise watering actions.

This component comprised a number of related phases: i) workshop and initial consideration of potential datasets and approaches; ii) collation and exploration of accessible data and iii) development of vegetation response models.

The aims associated with each of these components are:

- Workshop
  a) To discuss the potential for analysing large, combined datasets and to assess the breadth and availability of datasets
- Collation and exploration of accessible datasets
  a) To collate available datasets, and
  b) Based on the available data (both vegetation and environmental), to refine research questions and analytical approaches
- Development of vegetation response models
  a) To model vegetation responses based on refined research questions and approaches

• Methods

Phase 1: Workshop and initial consideration of potential datasets and approaches

A workshop was held in Canberra, 4th-5th November 2015, with approximately 30 participants and a further 10 who indicated their interest in staying informed. The workshop was about connecting vegetation ecologists, water managers, statisticians and modellers with a broad range of experiences and knowledge, as well as about discussing the potential for analysing large, combined datasets. An overview of the EWKR project was presented and provided context for why the Vegetation Theme is seeking data from collaborators. A series of thought-provoking presentations were given that led into group conversations. These conversations and break-out sessions resulted in robust discussions around priority questions from both science and management perspectives, potential datasets, challenges associated with accessing and managing datasets, as well as potential analysis approaches.

Phase 2: Collation and exploration of accessible data

Collation of datasets

Following the workshop we sought available datasets to undertake preliminary analysis and to assess the feasibility of potential approaches. We sourced vegetation data collected as part of The

Living Murray (TLM) Condition Monitoring program from Hattah Lakes, Lindsay-Mulcra-Wallpolla Islands, Chowilla Floodplain, Gunbower Forest, Koondrook-Pericoota Forest and Barmah Forest (Figure 1). The preliminary analysis and detailed response modelling, however, concentrated on wetland and floodplain understorey data from Hattah Lakes because of the ease of accessibility and familiarity with the dataset within the thematic program leadership group.

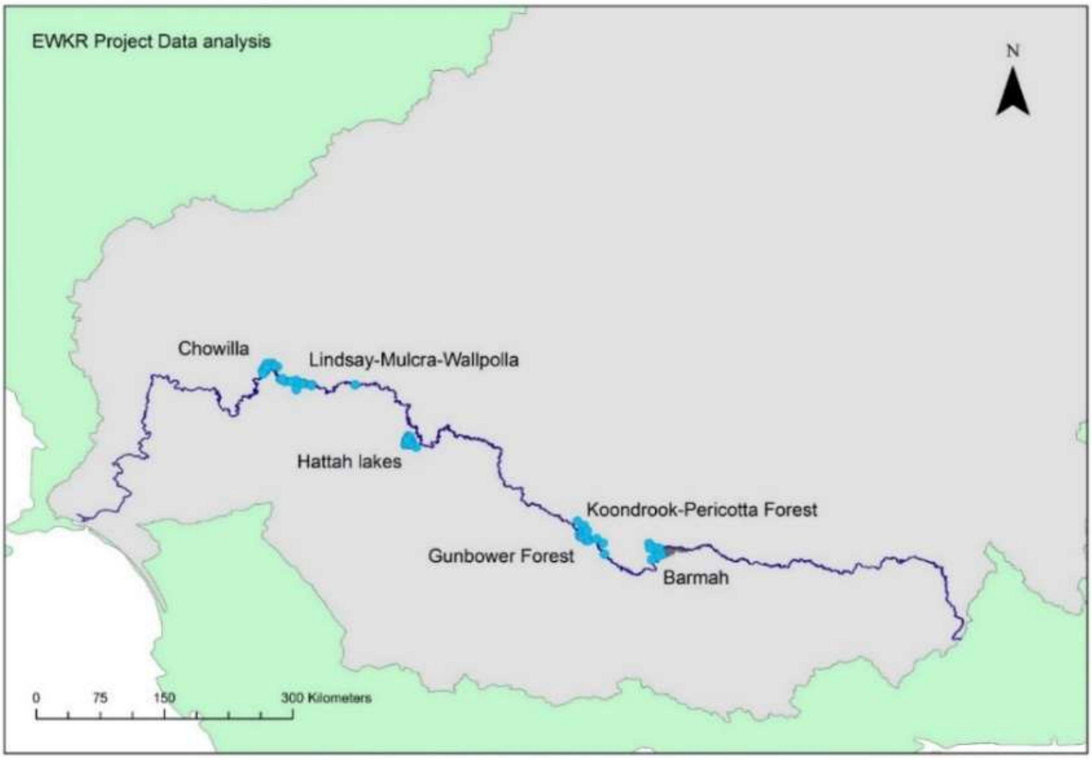

Figure 1: Location of sites from which wetland vegetation data sets were sourced.

Long-term datasets collected in a standardised manner through time are particularly useful for capturing the natural variability of ecological systems, detecting trends over time and responses to rare events. Monitoring of icon wetland complexes provides an opportunity to investigate patterns in wetland plant diversity in relation to wetting and drying regimes both within and between different complexes at broader spatial scales. In addition, for many icon sites considerable complementary environment data exists (particularly in the form of hydrological data and modelling) that can be used to explore ecology relationships with key predictors, particularly hydrology and climate variables.

Different data collection methods

The individual wetland understorey datasets used within the combined dataset were collected using different sampling methods. Hattah Lakes, Lindsay-Mulcra-Wallpolla Islands and Chowilla Floodplain all use a similar method based on (Nicol and Weedon 2006). This method consists of 1m x 15m quadrats positioned at a number of different elevations in each wetland. There are three to four replicate transects per wetland. The number of elevations varies per wetland depending on the wetland depth, however there is a minimum of three elevations. For these wetland complexes the majority of surveys are undertaken annually in summer. Sampling of understorey wetland data at Gunbower Forest and Koondrook-Pericoota Forest consists of two meter wide transects that span the wetland system. The length of the wetland transects varies from 46m to 300m, with data recorded in 'distinct vegetation zones' along each transect. The location and number of the vegetation zones varies between monitoring surveys in response to the recent water regime. Survey season varies with 1 – 2 surveys per year. Understorey wetland data in Barmah Forest is surveyed via two transects, within which 20m x 20m quadrats are placed in each vegetation zone of each transect (there are usually three zones identified per transect so six quadrats per wetland). Ten 1m square quadrats are placed randomly within each 20m x 20m quadrat. The data are averaged across the ten subsamples (mean data was provided). Data has been collected in all four seasons and is available from 1990 – 1994 and 2006 – 2016.

Data formatting and quality control

The TLM vegetation datasets are stored and formatted in a number of different ways. To permit analysis of multiple datasets together, all datasets had to be formatted in a consistent manner suitable for analysis and species names standardised. This was achieved by creating a single species master list which included all names as they appear in the various datasets. The list was then manually checked to correct obvious name issues (spelling errors/changes) and create unique species codes. The species named were verified using the R package Taxise (Chamberlain and Szocs, 2013) and the name attributed according to The International Plant Names Index (and checked against the Australian Plant Name Index). In the same master list, each species was assigned a plant functional group (PFG) and introduced status. Introduced status was assigned based on the Victorian Flora (VicFlora, 2016). For PFGs, Michelle Casanova provided a list against which records were cross-referenced with available published literature and reports. Conflicting PFG allocations were discussed within the leadership group, and expert opinion was used to assign the final classifications.

For each wetland complex a hierarchy of datasets was created:

Complex_site_transect_year_season

Complex_site_year_season

Complex_site_year

Complex_site

The following prefixes are used for each of the icon site datasets: Hattah Lakes = HAT, Lindsay-Mulcra-Wallpolla Islands = LMW, Chowilla Floodplain = CHOW, Gunbower Forest = GUN, Koondrook-Pericoota Forest = KP and Barmah Forest = BARM. Site refers to individual wetlands within each icon site.

A single combined dataset of all six icon sites was generated by aggregating the 'Complex_site' data frame for each icon site. This data was then converted to presence/absence of species for each icon site and wetland combination. The final aggregated dataset had 444 wetland species.

Data exploration

Exploring wetland diversity

Understanding how wetland plant diversity is distribution in time and space is essential for prioritizing management actions. For example, understanding to what extent the native wetland flora is represented in a subset of wetland sites may inform how limited resources are allocated. Is it necessary to conserve all the wetlands within or between complexes in order to protect wetland biodiversity or, can a subset of sites fulfill this role adequately? What are the natural hydrological (and other) processes responsible for maintaining diversity? Whilst these are relatively simple questions to ask, they are deceptively difficult to answer. At the broader spatial scales needed to monitor biodiversity for conservation management and planning, lack of standardized protocols for collecting biodiversity data is a serious hindrance (Chiarucci et al. 2011).

Beta diversity is central to our understanding of how regional diversity is distributed. Diversity can be partitioned into different subcomponents in order to understand how sub-communities contribute to local diversity (alpha), variation in composition between communities (beta) and to overall diversity at the regional scale (gamma). Beta diversity is a measure of distinctiveness (Colwell and Coddington, 1994) and it captures an important facet of diversity – its distribution in time and space and links local alpha diversity with the regional species pool (Koleff et al. 2003). Beta diversity itself can be the result of two different process: the replacement or turnover of species and the loss (or gain) of species (Carvalho et al. 2013). Where species losses or gains dominate, communities tend to become nested. Assessment of beta diversity and its components have obvious implications for conservation and management. For example, where nestedness is high, resources may be better focused on a smaller subset of the most diverse sites. Alternately, where turnover is high targeting multiple spatially distinct sites may be an appropriate strategy (Socolar et al. 2016).

Beta diversity is a very complex field generating a great deal of animated discussion in the literature (see Baselga 2012 and references referred to therein). There are essentially two approaches both of which were explored in this analysis:

- Similarity/Dissimilarity between samples (no knowledge of gamma required but species identities need to be known). Pairwise dissimilarity is considered less sensitive to differences in sampling design and sampling size (Marion et al. 2017).
- Classic assessments may be additive or multiplicative (Gamma-Alpha, Alpha/Gamma). The former allows beta to be reported in the same units but is sensitive to the size of the regional species pool. The latter reports beta as a proportion so beta is unitless (so the beta estimate is not an absolute measure). Both classic methods require relatively complete inventories for comparisons to be made across different sites with different sampling schemes but species identities don't need to be known.

Assessment of wetland inventory completeness

One of the most serious problems encountered when trying to combine datasets for the purposes of diversity comparisons is the issue of differences in sampling methodologies and sampling effort which can bias diversity measurements and affect site comparisons particularly where surveys are relatively incomplete. Greater effort (e.g. number of transects, larger survey area, more sampling events) will usually result in higher species richness and a greater chance of recording rare species regardless of other factors. However, if surveys are relatively complete and new samples are not adding to the species complement, the differences in sampling approaches will be less important and a combined wetland species analysis is less likely to be biased by the different survey methods and record lengths available.

To explore the completeness of the species inventories we generated species accumulation curves using the R package vegan (Oksanen et al. 2019). A species accumulation curve is a plot of the cumulative number of species identified with a specified area (and/or time) as a function of some measure of sampling effort. We also calculated a nonparametric estimator, the Chao 2 estimator (Chao 1987) which is based upon the number of species only occurring once or twice within each record. In this second approach, a numeric estimator of species richness (the 'true' number of species) is generated but the identity of the species is not taken into account.

nMDS analysis

Non-metric multidimensional scaling was performed on i) all species, ii) common species only, iii) PFGs and iv) native/exotic to assess similarities/dissimilarity in plant composition within icon sites and across icon sites. The analyses were undertaken on presence/absence data in two dimensions using Bray-Curtis dissimilarity. We subsequently explored multivariate dispersion using the 'betadisper' function of the R package vegan by calculating the average distance of group members to the group centroid for each icon site complex. This is a measure of beta diversity (Anderson et al. 2006) reflecting the variability in species composition amongst wetlands within an icon site complex. Finally, indicator species analysis was undertaken using the r package 'indicspecies' (De Caceres and Legendre 2009) to investigate the species that were driving the differences between the icon sites (Appendix 2, figures A2.4, A2.6 and A2.8).

Beta diversity

Analysis of beta diversity was based on the framework of Baselga and Orme (2012) and the R package 'betapart' using the Sørensen index. Total beta diversity (βsor) is proportioned into its species turnover (βtn) and nestedness (βnest) components. These components are not absolute measures but a measure of the proportion of dissimilarity that can be attributed to the different beta components. This analysis allows the assessment of patterns in beta diversity using multiple site dissimilarity measures. The beta analyses is sensitive to differences in the number of sample units (in this case different numbers of wetlands within icon sites) and hence we used inbuilt functions within the 'betapart' package to resample to eight sites.

In a subsequent analysis, for a single wetland complex (Hattah Lakes), we explored how beta diversity and its components varied in relation to time since last inundated (See Figure 2 and text for explanation). The data for the Hattah Lakes wetland complexes was subset into different periods since flooding (currently inundated, 1 month dry, 3 months dry, 6 months dry, 9 months dry, 12 months dry and greater than 1 year dry using the outputs from the hydrological model (discussed below). The data were resampled to 20 records because of the differences in record numbers for the different timeframes that can influence multi-site comparisons of beta diversity.

Availability of complementary environmental data

We sourced available hydrological and other data (e.g. local climate) that could be used to explore the broad research question '*what drives vegetation responses to watering actions?'*

The use of hydrological variables modelled at a very fine spatial resolution (individual 1m x 15m quadrats) was a pivotal and time-consuming component of this project. Additional details of the inundation model validation process are provided below. We also acquired local rainfall data collected from Hattah Lakes Information Centre, Parks Victoria and temperature data from the closest Bureau of Meterology station (Nulkwyne Kiamal BOM station no. 76043). Part of the former records had to be transcribed from paper prior to analysis. Purpose written r scripts were developed (available at: https://github.com/CassieJames/EWKR/) to calculate antecedent hydrological and climate metrics for each monitoring site and sampling date over a range of temporal scales.

Hattah Lakes Inundation model validation

Hydrological information for this study have been generated using the Murray Darling Basin Authority (MDBA) Bigmod model of Hattah Lakes (calibrated to the MDBA MIKE21 hydrodynamic model of Hattah Lakes and verified/refined using measured water level data since 2013). Pumping rates for environmental watering prior to 2013 were estimated from pumping records and volume estimates from modelling and satellite images. The model generated daily water depths for each monitored quadrat for the period between January 2005 and December 2016.

Model outputs for every quadrat and every sampling period (n=1459) were checked against inundation observations recorded during vegetation monitoring (see Figure 2 as an example). As there were inundation observations for every quadrat at different elevations within wetlands, we were able to highlight discrepancies. Where misalignment between model outputs and on-ground observations were identified, we investigated the cause and, when necessary, the model was adjusted accordingly. This involved collaboration with Andrew Keogh (MDBA) who developed the hydrodynamic model for Hattah Lakes, conversations with on-ground field staff (MDFRC) and cross-checking inundation information provided by the Mallee Catchment Management Authority. This enabled model refinement through the identification of mismatches and ensured that all pumping events, or alternatively, channel closures for works, were incorporated into the model.

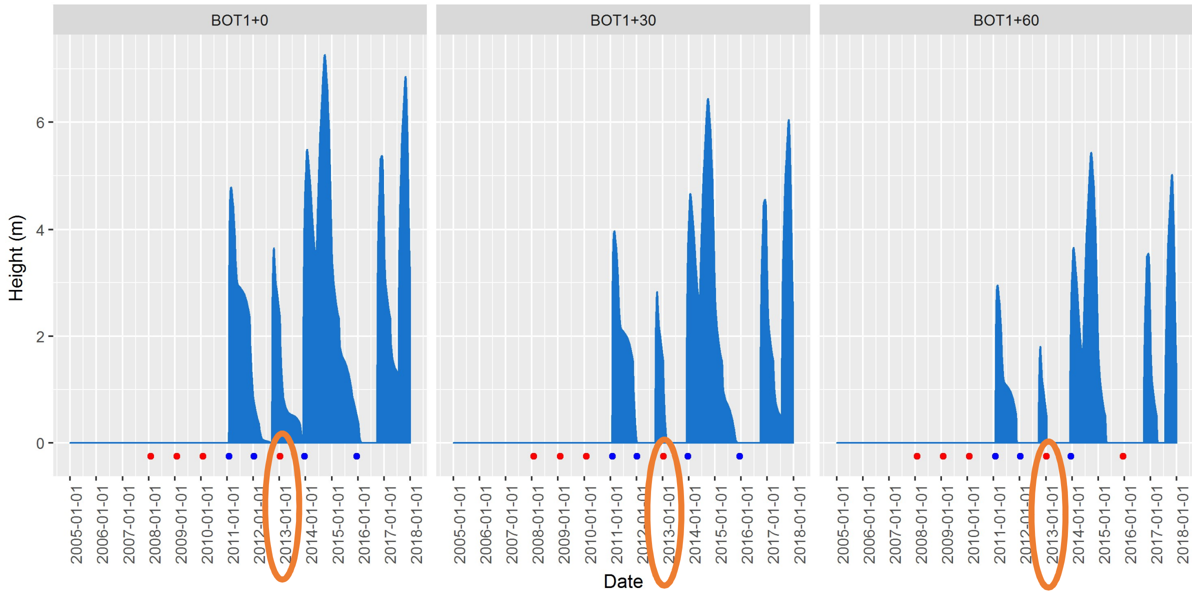

Figure 2: Example of the original misalignment between model outputs and on-ground observations that led to model refinement. (Blue dots = wet field observations, Red dots = dry field observations, orange circles highlight misalignment; BOT = Boich; T1 = transect 1; +0, +30, +60 represent quadrats at different elevations)

Refinement of research questions - diversity

Using the combined icon dataset (presence-absence) we explored the question: *How unique is your wetland? Determining diversity variation in time and space.*

Wetlands with variable hydrological regimes are dynamic ecosystems variously referred to in the literature as temporary, ephemeral, intermittent, but may also include permanent/semi-permanent wetlands where water levels fluctuate substantially. Hydrologically variable wetlands show high variation in plant assemblages both in time (contrast between wet and dry conditions for example) and in space (spatial variation in hydrology related to factors such as geomorphology, soil type, hydrological connectivity and distance from the river). Different stages of the wetting and drying cycle are fundamentally different environmental niches and accordingly support contrasting wetland and terrestrial plant communities (the latter considered here an integral component of the wetland flora – Deane et al. 2016). Dynamic habitats such as these therefore allow the coexistence of higher numbers of species within a single area/patch than would be supported in a more constant/stable environment (e.g. Katz et al. 2011). Consequently, we expect beta diversity to be higher overall for more hydrologically variable wetlands compared to their more permanent/hydrologically stable counterparts particularly where strongly contrasting habitat types occur (flooding and drought).

Beta diversity is also predicted to change over time in relation to wetland wetting and drying regimes and can be conceptually described depending on the connectivity stage of the hydrocycle (Figure 3). We predict that beta diversity would be lowest during periods of hydrological connectivity because flooding tends to homogenise communities. However, during drying and dry times beta diversity will be higher as other local environmental factors start to exert a greater influence on plant regeneration and growth. However, responses to beta diversity during drying will be contingent on the characteristics of the flora (i.e. beta diversity may be low if the community is dominated by a few good dispersers).

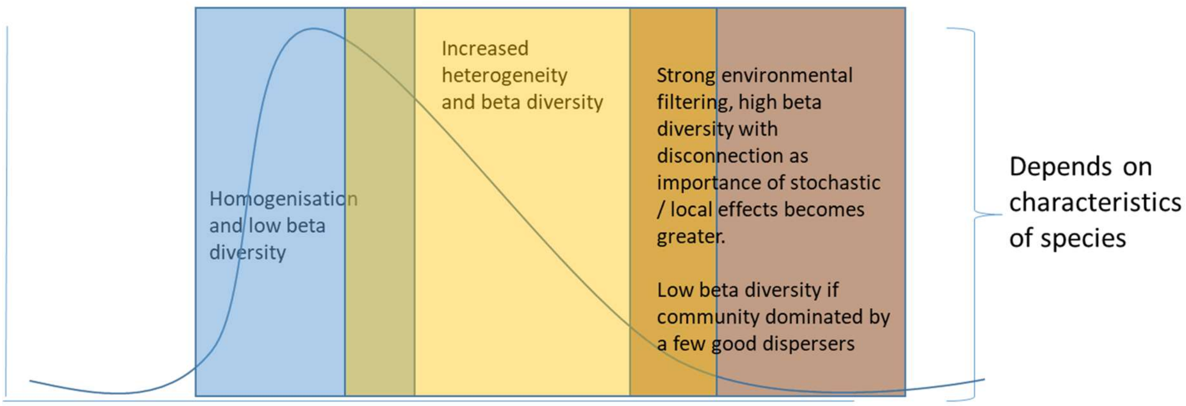

Figure 3: Conceptual representation of beta diversity depending on the connectivity stage of the hydrocycle

Refinement of research questions – flow relationships and legacy affects

Using the Hattah Lakes wetland monitoring dataset we explored the question: *Can we disentangle flow-vegetation relationships and legacy effects to inform environmental flows?* This research question formed the third phase of the project, development of vegetation response models and is discussed in greater detail below. We focussed on the Hattah Lakes data in addressing this research question because of the ready accessibility of the dataset and the familiarity of the thematic group team leaders with both the data and the icon site itself. This allowed issues with the data relating to taxonomy, sampling dates and locations etc to be resolved rapidly.

Phase 3: Development of vegetation response models

Because of the strong 'ecological memory' of wetlands, i.e. 'the capacity of past states or experiences to influence present or future responses of the community' (Padisak, 1992), understanding wetland vegetation responses to flows in the short-term requires consideration of antecedent conditions, both with respect to hydrology and other factors, e.g. climate, as well as recent modifiers, e.g. weather.

To develop vegetation response models, we explored the influence of hydrological and climatic conditions on the contrasting wet and dry floristic components of temporary semi-arid wetlands using understory plant species data collected from Hattah Lakes as part of The Living Murray program. We used the plant functional groups of Brock and Casanova (1997), to categorize species as wetland species and dryland species (dryland species = Tdr, wetland species = all other categories;

S, Arf, Arp, Atl, Ate, Tda). A temporal hierarchy of antecedent conditions was considered from relatively recent (3 months), short term (4 months to 12 months) and medium term (1 year to 3 years) to longer term (30 year flood frequency). Using boosted generalized additive models (GAMs) and boosted regression trees, we investigated the relative importance of hydrological and climate variables on four wetland vegetation response metrics: i) native wetland plant species richness; ii) native wetland plant species abundance; iii) native aquatic plant species occurrence; and iv) native dryland plant species abundance.

For detailed methods of the modelling approaches, refer to James et al. (draft). *Disentangling flow-vegetation relationships and legacy effects to inform environmental flows* (Appendix V2.1).

• Results

Phase 1: Workshop and initial consideration of potential datasets and approaches

The workshop aimed to connect vegetation ecologists, water managers, statisticians and modellers with a broad range of experiences and knowledge, as well as discuss the potential for analysing combined datasets over large spatial areas. An overview of the EWKR project was presented and provided context for why the Vegetation Theme was seeking data from collaborators. A series of thought-provoking presentations led into group conversations. These conversations and break-out sessions resulted in robust discussions around priority questions from both science and management perspectives, potential datasets, challenges associated with accessing and managing datasets, as well as potential analysis approaches.

There was agreement that combining and utilising existing datasets is a potentially powerful way of testing hypotheses or looking for patterns on large spatial (and possibly temporal) scales. It is also a recognition of the value of datasets and the extensive work undertaken by large numbers of people from a range of organisations and locations. It was a deliberate decision to engage collaborators early and this workshop was just the beginning of the process. The workshop highlighted the importance of having a strong theoretical basis underpinning our analysis and the need to refine data analysis questions.

Outputs from the workshop were circulated and these included:

1. Workshop Agenda Nov 2015
2. Workshop summary
3. Workshop notes
    a. Guiding principles (Developed by Dr Michael Reid, UNE, while at the workshop)
    b. Additional recruitment notes from one of the small group discussions
4. Workshop participation list
5. Metadata spreadsheet (list of > 150 potentially available vegetation datasets with contact, custodian and reference details)
6. Pdf copies of the nine presentations given during the workshop
    a. Cherie Campbell – Data workshop EWKR Veg overview
    b. Dr Jane Roberts – Australian vegetation ecology of wetlands, rivers and floodplains: outputs
    c. Dr Cassandra James – Analysing large datasets
    d. Dr Daryl Nielsen – Metadata summary
    e. Cherie Campbell – Rehash of Day 1
    f. Dr Bill Senior – QLD floodplain vegetation project: Watering requirements of floodplain vegetation asset species of the northern Murray-Darling Basin

g. Dr Shaun Cunningham – Applications of stand condition assessments
h. Drs Patrick Driver, Sharon Bowen and Simon Williams – A NSW perspective. Research opportunities under EWKR
i. Dr Angus Webb – Gaining predictive capacity: Terrestrial vegetation in river channels

Refer to Appendix V5.2 Vegetation Theme Outputs for details of the accessibility of the above information.

Phase 2: Collation and exploration of accessible data

Assessment of wetland inventory completeness

Accumulation curves were generated for presence/absence data from individual wetlands within each icon site (Appendix 1, figures A1:6). Curves for individual wetlands within icon sites are comparable because the measure of sampling is the same, but direct comparison of curves between icon sites cannot be made where survey methods and hence the measure of sampling effort is not the same.

Overall, the results suggested that none of the inventories were complete with most wetland accumulation curves showing a distinct incline and not reaching an asymptote as would be expected if the surveys were approaching completeness. The Chao 2 estimator suggested that on average 50 – 93% (mean 75%) of the estimated local richness was observed (by comparing the actually accumulated richness with the Chao 2 richness estimator). There were, however, clear differences between the wetland complexes. Surveys from Barmah forest, for example, were estimated to be relatively complete between 66 - 91% (mean 85%) with the majority of wetland inventories being >80% complete. This icon site has a longer monitoring record (1990-1994, 2007-2016) as well as undertaking surveys 4 times a year; hence, the relative completeness of these surveys compared with the other icon sites is not unexpected. This preliminary analysis underlines the difficulties in combining data collected using disparate sampling approaches and periods and, the need to interpret any combined analysis across the icon sites with caution.

Other sources of error such as taxonomic misidentification and geographic undersampling can also affect diversity indices (Schroeder and Jenkins 2018). We addressed the taxonomic issues via a rigorous process of species checking and rechecking and removing taxa not identified to species level unless the taxa could not be mistaken for another species already in the database (e.g. the genera was recorded at only one icon site). With respect to the issue of geographical undersampling, this occurs when insufficient sites are sampled and hence a site with rare species is excluded. The nature of the wetland sampling methodologies (multiple wetlands, elevations and replicates within a wetland or continuous transects that span the whole wetland) suggests that key hydrological/soil gradients are captured under the current methods and geographic undersampling is unlikely to be an issue.

Exploring wetland diversity

nMDS analysis

The combined full assemblage analysis of the icon wetland datasets suggests substantial differences in the floras of the different wetland complexes with the different wetland icon complexes occupying quite different locations within the nMDS space (Appendix 2, figure A2.1). There is also a clear separation in the nMDS space between those icon sites situated in the upstream sections of the westward-flowing Murray River (Barham Forest, Gunbower Forest and Koondrook-Pericoota Forest) with those situated downstream (Chowilla floodplain, Hattah Lakes and Lindsay-Mulcra-

Wallpolla Islands). However, methodological differences may also drive these patterns (particularly the east-west pattern). Notably however, even where methods are the same, the compositions don't overlap although they are proximal to each other in the ordination space. For example, Hattah Lakes and Lindsay-Mulcra-Wallpolla Islands use the same sampling protocols and have similar survey completeness 74% of the estimated local richness observed and are located distinct from each other on the nMDS.

Multivariate dispersion analysis also revealed an east-west pattern with icon sites located downstream (Chowilla floodplain, Lindsay-Mulcra-Wallpolla Islands and Hattah; distances to centroids 0.39, 0.36 and 0.31 respectively) having higher dispersion relative to those in the upstream locations (Koondrook-Pericoota Forest, Gunbower Forest and Barham Forest; distances to centroids (0.29, 0.23 and 0.22 respectively). Again, however, differences in sampling methodologies between these groups preclude any solid conclusions being drawn from this analysis.

The nMDS analyses were repeated using a robust reduced dataset where only common species were retained (Appendix 2, figure A2.2). Common species were defined as those recorded in each wetland site 3 or more times (pooling transects within wetlands for each time period). This resulted in a reduced species list of n=40. The nMDS plot of the common species only was generally similar to that found using the full dataset in there being a distinct separation in nMDS space between the upstream and downstream sites. The most notable feature of the common species nMDS was the complete overlap in the Hattah and Lindsay-Mulcra-Wallpolla and, Barmah Forest and Gunbower Forest, indicating a similar common species composition and that compositional dissimilarity in the full assemblage nMDS is being driven largely by rarer species. It is also noteworthy that the Barmah sites sit close to the Gunbower Forest and Koondrook-Pericoota Forest sites despite the greater intra year sampling and much longer species lists from Barmah.

Non-metric multidimensional scaling was also performed separately for key plant functional groups (Appendix 2, figures A2.3, A2.5 and A2.7). The wetland native categories included all species that are likely to respond to wet or damp conditions and so this category includes species classified as terrestrial damp species which germinate, grow and/or reproduce on saturated soils. Terrestrial native species were determined as those species classified as terrestrial dry preferring species using the groupings of Casanova and Brock (1997). The main pattern observed from this analysis was the greater compositional dissimilarity in wetland native plants (Appendix 2, figure A2.7) compared with the terrestrial native plants (Appendix 2, figure A2.3) which likely reflects a number of different factors. For example, the highly dispersed nature of the terrestrial flora means that these species are widely distributed across the region, versus the importance of the local hydrological regimes in governing wetland species composition through the suitability of the regeneration niche. This finding contrasts with the notion that the larger species pool for terrestrial species would decrease similarity between sites (Deane et al. 2016; Chambers et al. 2008).

The nMDS performed on the exotic terrestrial species only (Appendix 2, figure A2.5) revealed a much greater degree of overlap in the ordination space for the icon sites relative to the native wetland species ordination. Few terrestrial exotic species were identified through the indicator species analysis unique to a single wetland in the case of the more western sites (Hattah, Lindsay-Mulcra-Wallpolla Islands and Chowilla Floodplain). High overlap was also observed for Gunbower Forest and Koondrook-Pericoota Forest, which given the proximity of these icon sites, suggests a common pool of terrestrial exotic species. These results align with other studies on the River Murray in identifying the dominance of terrestrial exotic species and is likely to reflect the increases in opportunities for introductions of terrestrial species relative to wetland taxa through intentional introductions for agriculture and horticulture into the surrounding landscape and their subsequent spread into rivers and wetlands (Catford et al. 2011).

The extensive indicator species list for the terrestrial exotic species identified for Barmah Forest is notable. This may be due to the greater intra-annual sampling frequency with, for example, regular winter sampling increasing the detection of winter or early spring annual species.

Beta analysis

The multi site beta diversity analysis of the wetlands suggests generally higher beta diversity amongst wetlands located downstream on the River Murray (Appendix 3, figure A3.1) relative to those located upstream. This may reflect natural gradients towards increased aridity and hydrological variability but may also reflect anthropogenic changes to hydrological regimes and alterations to the variability of inundation patterns. However, methodological differences and undersampling bias may also be driving some of these patterns and beta diversity based on presence/absence data is sensitive to these issues (Beck et al. 2013). Overall, species turnover constituted a great proportion of the total beta diversity relative to nestedness indicating that even within wetland complexes, individual wetlands are relatively floristically unique rather than representing subsets of each other.

A second beta analysis focused on the Hattah Lakes dataset and we sort to explore patterns within Hattah Lakes with respect to time since last inundation (Appendix 3, figure A3.2) following the conceptualisation of how beta diversity may change over a hydrological cycle (Figure 3). The analysis did not find any support for this generalisation of changes in beta diversity suggesting that total beta diversity remained relatively similar for the different time periods and was dominated by species turnover.

Phase 3: Development of vegetation response models

The results of the modelling approach are detailed in James et al. (draft). *Disentangling flow-vegetation relationships and legacy effects to inform environmental flows* (Appendix V2.1). For details of the dataset and response model, including availability, refer to Appendix V5.1 Vegetation Theme Data and Model Inventory.

• Discussion / applications

Phase 1: Workshop and initial consideration of potential datasets and approaches

The workshop identified a large number of potential vegetation datasets (acknowledging this will be an incomplete list) and a general willingness to see these datasets used in other ways and, where possible, compared. There also appears to be additional data potentially available as raw data sheets (that has yet to be entered electronically).

The outcomes of this workshop highlight the importance of data management and the potential for a central repository, or at least a central list of potentially available datasets. It also identified the limitations (both time and financial and, differences in sampling methodologies) that constrain the potential value of collected data. There was a strong sense that collected data is under-utilised and under-analysed, mostly due to financial constraints (project funding finishes and researchers need to move onto the next paid job) or time.

Phase 2: Collation and exploration of accessible data

Analysis of the combined wetland dataset reveals substantial differences in the wetland floras of the different wetland complexes and high species turnover between wetland complexes. Although this is subject to the caution that differences in sampling methodologies may drive some of these differences, the result is consistent with other assessments undertaken on understory community composition (e.g. Campbell and Nielsen 2014, Capon and Campbell 2017). This does pose the question: why are the floras different (even amongst the dry/terrestrial species assemblages) given these sites are located within the same river catchment and connected along a river system that does not vary substantially in latitude or altitude – both of which are known to drive broad patterns in vegetation due to the biophysical constraints imposed by the climate and dispersal constraints. Climatic gradients, however, do exist along the River Murray from east to west particularly in terms of rainfall and temperatures. However, many of the species within the combined data set have relatively broad distributions and are largely composed of cosmopolitan species. Exploring distributions of the species through, for example, the 'Atlas of Living Australia website at http://www.ala.org.au' demonstrates that many of these species occur throughout the Murray catchment and hence the climatic gradient is unlikely to be the only factor driving species assemblages.

There is incredible variation in local wetland plant communities in space and time. Our analysis has also demonstrated the importance of considering both the wet and dry components of these ecosystems as both contribute to the overall wetland plant diversity and uniqueness of the sites. Such a finding is supported by other recent research (e.g. Deane et al. 2016). For temporary wetlands, the dry native flora is an important component of the diversity and has many associated ecological values related to both the dry phases (e.g. habitat and food sources for terrestrial insects and terrestrial phases of insect life histories) and wet phases (e.g. organic matter and nutrient inputs).

As ecologists and water managers, we try to tease out the causes of this variation and understand its drivers to inform wetland management. We can start to consider the drivers of diversity in a number of ways. For example, location appears to be a very strong predictor of local wetland vegetation composition. We are not yet able to define the specific location attributes driving differences in community composition but it's likely to be a combination of factors such as habitat, local pressures such as grazing pressure and invasive species, differences in short, medium and long-term flow regimes, and climate (temperature, rainfall). Hence, the story is complicated and unique management histories (no two locations will have been managed in exactly the same way over time) make generalizations difficult but not impossible.

There may also be considerable scope in exploring the drivers of species absence (what is missing may tell us as much as what is present). This is, of course, the idea of "dark diversity" (see Partel et al. 2011). Having identified what species are 'missing' from wetland complex datasets the first question to ask is 'are they really missing or just missing from the databases?' This can be addressed by discussion with icon site managers and on ground field staff as well as other potential sources of species records (e.g. other datasets). Is the species composition a function of the timing of sampling and/or sampling strategy differences? If species are missing (rather than simply missing from the dataset), are there (sets of) characteristics of the missing species that might explain their absence? In this way, we may be able to identify particular traits/habitat preferences that are common amongst the species that are absent. This approach may be used to complement approaches based on observed species in understanding the drivers of wetland biodiversity (i.e. are there particular hydrological regimes or components of these regimes that result in wetland species being absent)?

Another potential explanation for the variability in species composition across the wetlands is the role of chance/stochastic events. If this is the case we would expect the species 'missing' to either be fairly random with respect to their characteristics or to have specific characteristics that might result in dispersal limitation either in time (for example, short lived propagules that are not drought resistant) or space (requiring particular hydrological conditions for dispersal to occur).

River regulation and drought conditions and, the length of time different sites have been hydrologically disconnected are also likely to have contributed to observed variation in species composition. The legacy of past dry (and wet) conditions resulting from regulation, drought conditions and environmental watering will influence current assemblages. Recolonizations depend on dispersal opportunities being in synchrony with suitable habitat availability. For species not present, in-situ propagule banks, managing wetland complexes as a whole and giving consideration to the timing of events and environmental watering events in other wetlands and complexes in order to facilitate recolonization will be important.

<u>Phase 3: Development of vegetation response models</u>

For a full discussion of the results of the modelling approach refer to James et al. (draft). *Disentangling flow-vegetation relationships and legacy effects to inform environmental flows* (Appendix V2.1).

Phase 3 of this research component sought to disentangle some of these flow-vegetation relationships. Response model outcomes provide additional evidence for the key drivers and timeframes for non-woody vegetation responses. This, in turn, helps to explain current vegetation conditions with the potential to predict responses to future regimes. Recent (last three months) and short to medium-term (last three years) regimes have the strongest influence on non-woody wetland vegetation richness and abundance while longer term regimes appear to have a greater influence through their interactive effects with more recent conditions. Time-since-last inundation (the strongest predictor of current vegetation state) has a non-linear (hump-shaped) relationship with abundance. Wetland plant abundance increases as water recedes (as time-since-last inundation increases) but as soil moisture decreases with increasing time since inundated, abundance then decreases. For data modelled from Hattah Lakes, abundance was maximised when plots were dry approximately 50% of the time. The results support the need to maintain wet-dry regimes in semi-arid wetland systems.

Wetland plant abundances were influenced by metrics across the recent to medium term temporal scales examined whereas the dry native species community appeared to be most strongly affected by the recent flood conditions (TSLW) and conditional mean water depth in the recent 3 months. The negative relationship between recent water depth and dry preferring species makes sound ecological sense as the presence of any surface water in the preceding 3 months prior to sampling dictated that for most sites conditions were not likely to be sufficiently dry to promote germination and growth of xeric species.

While the model has been developed using understory data from wetland habitats at Hattah Lakes, there is considerable potential to test the transferability of the relationships identified here with other datasets and for other wetland sites. These may include data from other habitats at Hattah Lakes (e.g. floodplain understory data), from other locations using the same sampling methods (e.g. Lindsay-Mulcra-Wallpolla Islands and Chowilla Floodplain), or other location based or combined data sets (e.g. TLM icon sites, LTIM, EWKR field data). There is also the capacity to test other defined vegetation responses, e.g. response metrics based on classifications such as life-form, life-history or functional group that may inform management

Finally, the process undertaken to validate the Hattah Lakes inundation model highlights the potential value of field observations within TLM condition monitoring data to aid validation of inundation models at other icon sites (if the process hasn't already occurred). It also provides a rigorous, independent test of the high degree of accuracy of the inundation model at relatively fine spatial scales (1m x 15m quadrats at different elevations within wetlands).

Learnings related to the analysis of existing data

This component has provided a number of learnings related to the analysis of existing data. In particular, we determined that existing datasets provide a valuable sources of information and the quality of the databases provided from the icon sites was of a very high standard (in terms of associated metadata and the ease with which the individual databases could be interpreted).

There is however a need for i) available and easily accessible complementary data, such as hydrology and mapping of inundation patterns, ii) good data management processes to enable access to data in comparable formats, and iii) analytical expertise and accepted methods for the analysis of data from different sources (with different survey methods and sampling effort). It is also worth noting that future projects seeking to analyse existing data would benefit from factoring in the considerable amount of time required to source and clean data, transform and collate data (from potentially quite different original formats), consistently align metrics (e.g. plant species names, units, trait classifications) and quality check data. We acknowledge that the time taken in undertaking these processes was considerably underestimated in this project yet the value in creating robust datasets to subsequent analysis cannot be stated highly enough.

• Conclusions / further work

For this component of the EWKR program we focussed on a subset of analytical approaches in order to explore the available datasets. However, there is substantial scope to explore the integrated datasets further and analytical methods that are better equipped to deal with some of the challenges of integrating and analysing large monitoring datasets.

We focused on beta diversity (β) because of its capacity to describe changes in species composition across the landscape and over time and explored changes in the composition of species in space between and within wetlands. This analysis is preliminary because of the issues related to the differences in sampling methodologies that could drive some of the patterns observed. Further robust analysis (for example analysis of only those sites where the surveys are deemed relatively complete) may help address these issues. There is also considerable scope to use other approaches such as species distribution modelling to explore beta diversity. These approaches can extrapolate the localized site observations (as well as drawing on records from other data sources such as the Australian Living Atlas). These approaches need supporting environmental layers at suitable spatial resolutions (particularly hydrological but also climate and soils) with which to build useful distribution models (see James et al. 2017 for an example from other freshwater biotic groups).

Finally, the vegetation response models have been developed for one habitat type (wetlands) at one location (Hattah Lakes). Initial work in this component, particularly through the workshop held in November 2015, identified a large number of potential data sets and identified a strong willingness from data custodians to see this data further utilised. Further research could test the models transferability to other locations and to other response metrics, and hence determine the transferability of predicted outcomes and key drivers between different locations and situations:

- Where data is available define, develop and test different vegetation response metrics to incorporate structural and process responses or responses at difference levels of ecological organisation (e.g. seedling recruitment, strata, communities)
- Explore the development of environmental metrics (currently hydrological and climate) relevant to different spatial scales
- Explore the inclusion of additional environmental metrics (e.g. soil type, soil moisture, canopy cover/condition)
- Test the response model in different habitat types and different locations.

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Appendix 1: Assessment of wetland inventory completeness

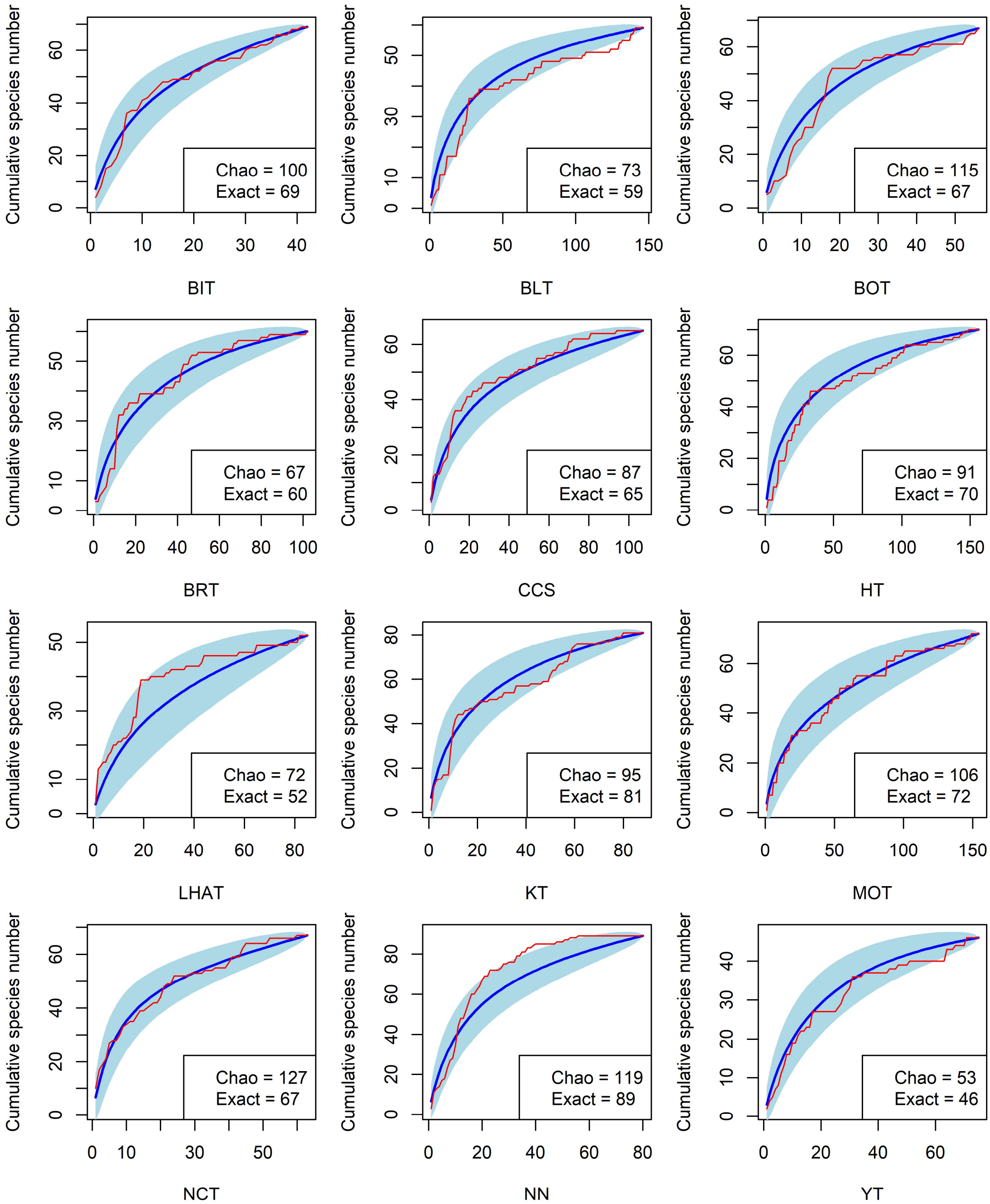

Figure A1.1 Species accumulation curves for Hattah lakes wetlands (on x axis are numbers of 1m x 15 m transects sampled cumulatively across multiple years). Red line is actual species accumulation, blue line is the smoothed (resampled) species accumulation and the shaded area is the 95% confidence interval. The Chao2 estimate provides a non-parametric estimate of the 'true' species richness based on the numbers of single and double occurrence of species. BIT = Bitterang, BLT = Bulla, BOT = Boich, BRT = Brockie, CCS = Chalka Creek South, HT = Hattah, KT = Kramen, LHAT = Little Hattah, MOT = Mournpall, NCT = North Chalka Creek, NN = Nip Nip, YT = Yerang

	Transect No.	Elevation No.	2008	2009	2010	2011	2012	2013	2014	2016
HAT_BIT	4	6	0	0	0	0	0	24	6	12
HAT_BLT	4	6	24	23	24	21	9	13	19	13
HAT_BOT	3	3	9	9	9	0	4	9	7	9
HAT_BRT	3	5	15	15	15	15	5	11	14	12
HAT_CCS	4	4	13	12	15	10	12	15	8	12
HAT_HT	4	7	26	27	27	27	12	15	9	13
HAT_KT	4	5	0	0	0	8	20	20	20	20
HAT_LHAT	4	3	12	12	4	12	4	12	12	12
HAT_MOT	4	7	26	26	26	26	12	16	9	13
HAT_NCT	4	4	0	0	0	0	15	16	16	16
HAT_NN	4	3	12	12	12	9	8	12	3	12
HAT_YT	4	3	11	12	10	12	4	12	12	2

Table A1.1. Hattah Lakes data summary. The number for each wetland in each year indicates the number of quadrats containing plant species data (the same number of quadrats are surveyed each year, however quadrats may contain no species due to factors such as water cover / depth, dense leaf litter, extremely dry conditions etc.). Transect No. refers to the number of transects establish and Elevation No. refers to the number of elevations along each transect with a surveyed quadrat (e.g. 4 transects x 7 elevations = 28 quadrats surveyed annually). NB the majority of surveys were undertaken in summer with some in early autumn. Kramen (KT) was first surveyed in 2011, Northern Chalka (NCT) in 2012 and Bitterang (BIT) in 2013. BIT = Bitterang, BLT = Bulla, BOT = Boich, BRT = Brockie, CCS = Chalka Creek South, HT = Hattah, KT = Kramen, LHAT = Little Hattah, MOT = Mournpall, NCT = North Chalka Creek, NN = Nip Nip, YT = Yerang

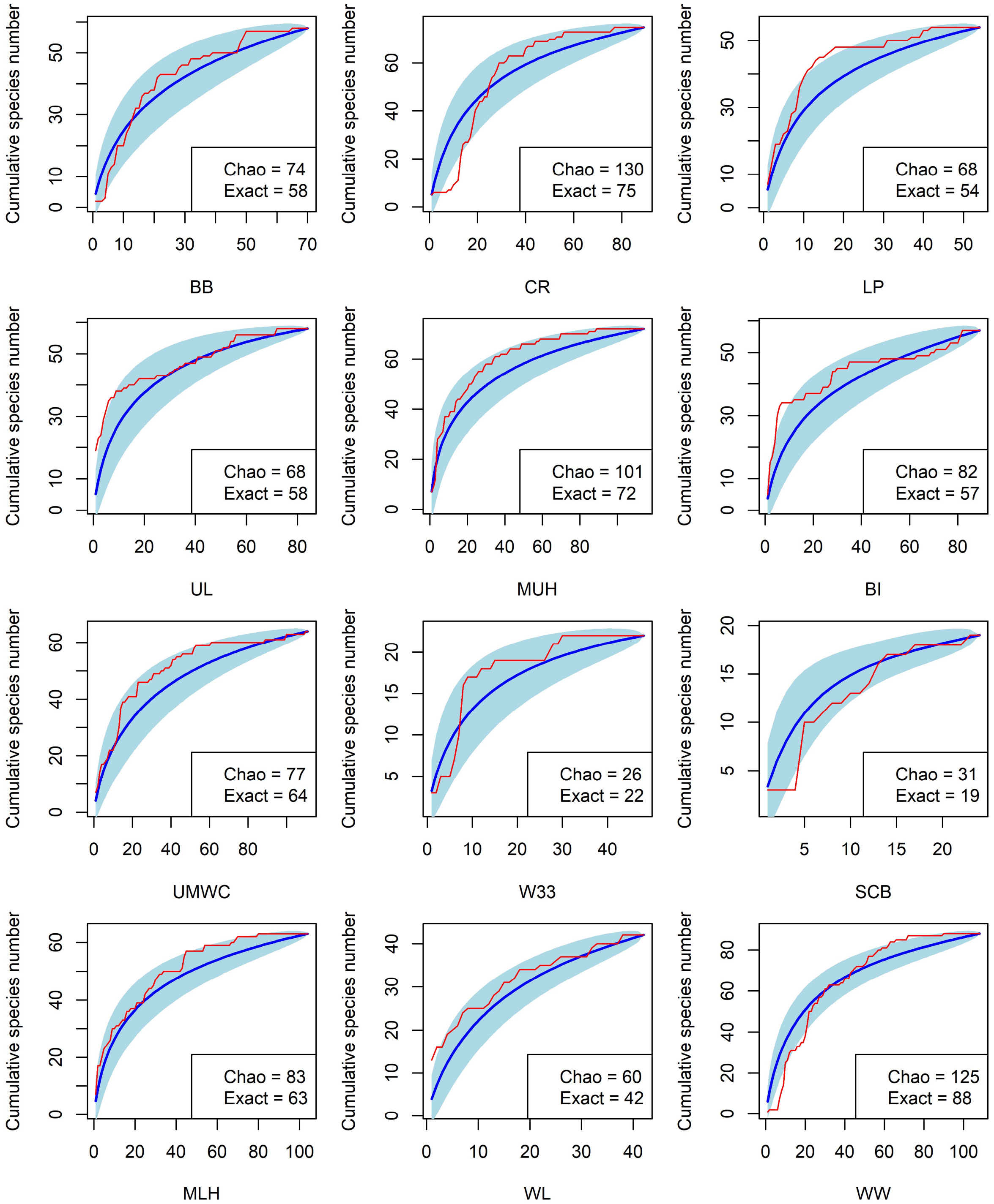

Figure A1.2 Species accumulation curves for Lindsay-Mulcra-Wallpolla wetlands (on x axis are numbers of 1m x 15 m transects sampled cumulatively across multiple years). Red line is actual species accumulation, blue line is the smoothed (resampled) species accumulation and the shaded area is the 95% confidence interval. The Chao2 estimate provides a non-parametric estimate of the 'true' species richness based on the numbers of single and double occurrence of species. BB = Bilgoes Billabong, CR = Crankhandle, LP = Lilyponds, UL = Upper Lindsay, MUH = Mulcra Upper Horseshoe, BI = Bottom Island, UMWC = Upper Mullaroo Wetland Complex, W33 = Wetland 33, SCB = Scotties Billabong, MLH = Mulcra Lower Horseshoe, WL = Websters Lagoon, WW = Walla Walla

	Transect No.	Elevation No.	2008	2009	2010	2011	2012	2013	2014	2016
LMW_BB	4	3	10	10	10	11	11	6	5	6
LMW_CR	4	3	11	12	12	12	12	12	9	7
LMW_LP	4	3	12	0	3	3	2	3	11	12
LMW_UL	4	3	9	9	10	12	8	8	12	12
LMW_MUH	4	4	14	16	16	16	12	9	10	16
LMW_BI	4	3	11	12	10	12	10	9	11	11
LMW_UMWC	4	4	15	16	15	0	16	14	16	16
LMW_W33	4	3	12	12	12	0	0	0	0	12
LMW_SCB	4	?	4	4	4	4	0	0	4	4
LMW_MLH	4	6	22	15	16	24	2	0	1	24
LMW_WL	4	3	8	11	11	0	0	0	2	6
LMW_WW	4	6	0	0	23	10	16	16	23	19

Table A1.2. Lindsay-Mulcra-Wallpolla Islands data summary. The number for each wetland in each year indicates the number of quadrats containing plant species data (the same number of quadrats are surveyed each year, however quadrats may contain no species due to factors such as water cover / depth, dense leaf litter, extremely dry conditions etc.). Transect No. refers to the number of transects establish and Elevation No. refers to the number of elevations along each transect with a surveyed quadrat (e.g. 4 transects x 4 elevations = 16 quadrats surveyed annually). NB the majority of surveys were undertaken in summer with some in early autumn. Walla Walla (WW) was first surveyed in 2010. Scotties Billabong was originally set up as a floodplain site with four quadrats and doesn't follow the same transect/elevation design as the other wetlands. BB = Bilgoes Billabong, CR = Crankhandle, LP = Lilyponds, UL = Upper Lindsay, MUH = Mulcra Upper Horseshoe, BI = Bottom Island, UMWC = Upper Mullaroo Wetland Complex, W33 = Wetland 33, SCB = Scotties Billabong, MLH = Mulcra Lower Horseshoe, WL = Websters Lagoon, WW = Walla Walla

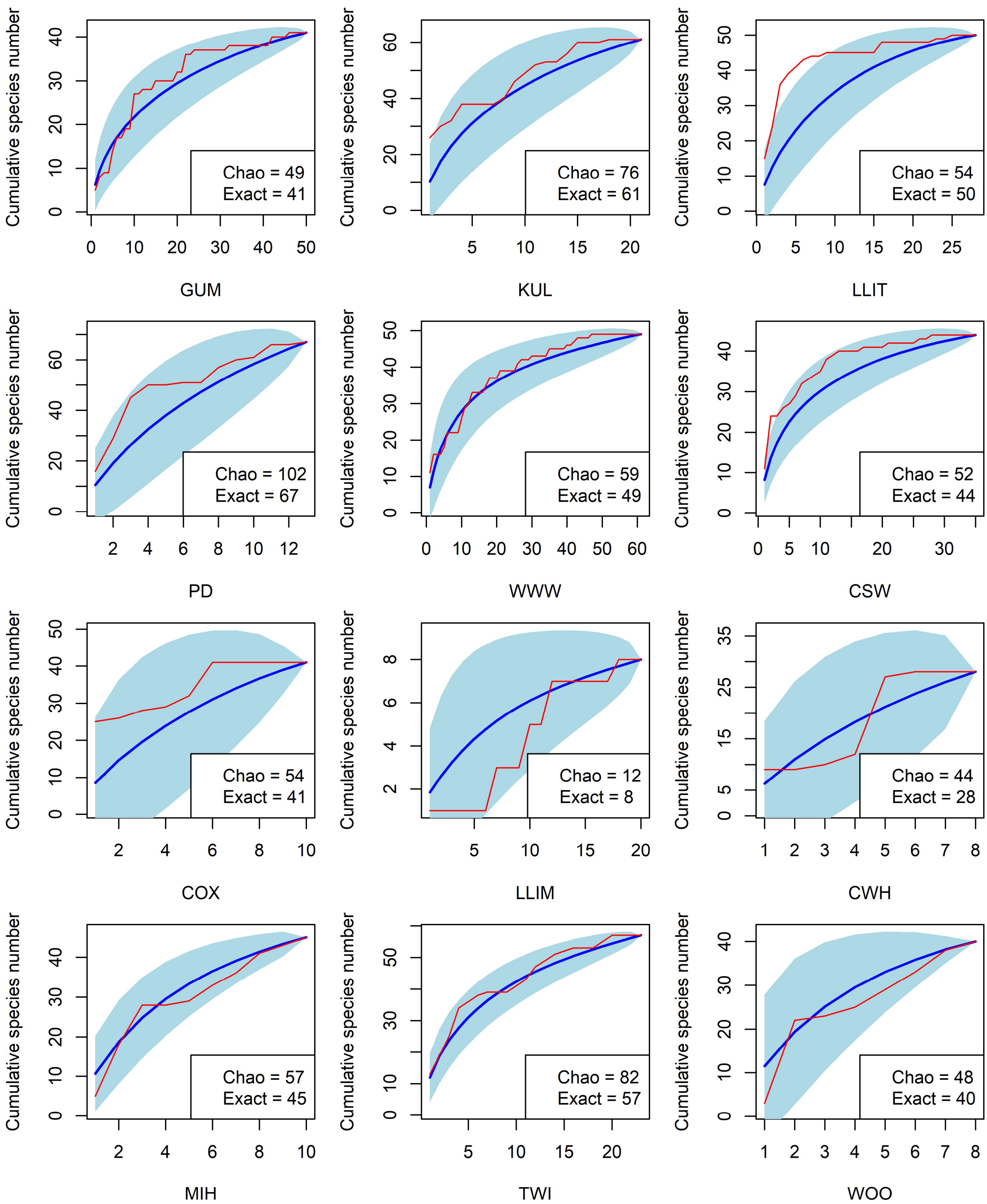

Figure A1.3 Species accumulation curves for Chowilla wetlands (on x axis are numbers of 1m x 15m transects sampled cumulatively across multiple years). Red line is actual species accumulation, blue line is the smoothed (resampled) species accumulation and the shaded area is the 95% confidence interval. The Chao2 estimate provides a non-parametric estimate of the 'true' species richness based on the numbers of single and double occurrence of species.

	Total No.	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	SU	AU	WI	SP
CHOW_GUM	50	0	0	5	5	5	5	5	5	5	5	0	5	5	40	5	5	0
CHOW_KUL	21	0	3	2	0	6	0	0	0	0	4	4	0	2	13	0	2	6
CHOW_LLIT	28	0	4	0	0	4	4	0	0	4	4	0	4	4	16	4	6	2
CHOW_PD	13	0	3	1	0	1	3	0	0	1	2	1	0	1	8	0	3	2
CHOW_WWW	61	0	17	2	0	8	8	0	0	2	6	0	1	17	41	1	10	9
CHOW_CSW	35	0	0	0	0	0	0	7	0	0	7	7	7	7	21	14	0	0
CHOW_COX	10	0	2	0	0	0	0	0	0	0	2	2	2	2	6	2	0	2
CHOW_LLIM	20	1	4	0	0	0	0	3	0	3	3	3	0	3	16	0	3	1
CHOW_MON	5	0	1	0	0	0	0	0	0	0	1	1	1	1	3	1	0	1
CHOW_CWH	8	0	0	2	0	2	0	0	0	0	0	2	0	2	5	0	2	1
CHOW_MIH	10	0	2	0	0	2	2	0	0	0	0	2	0	2	6	0	2	2
CHOW_TWI	23	0	3	3	0	3	3	2	0	0	0	3	3	3	14	3	4	2
CHOW_CIL	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0
CHOW_BBW	2	0	1	0	0	0	0	0	0	0	0	0	1	0	0	1	0	1
CHOW_WOO	8	1	1	0	0	4	0	0	0	0	0	0	0	2	5	0	0	3

Table A1.3. Chowilla Floodplain data summary. The number for each wetland in each year indicates the number of surveyed transects (with three replicate quadrats). The Total No. is the cumulative number of transects (with three replicate quadrats) surveyed across all years.

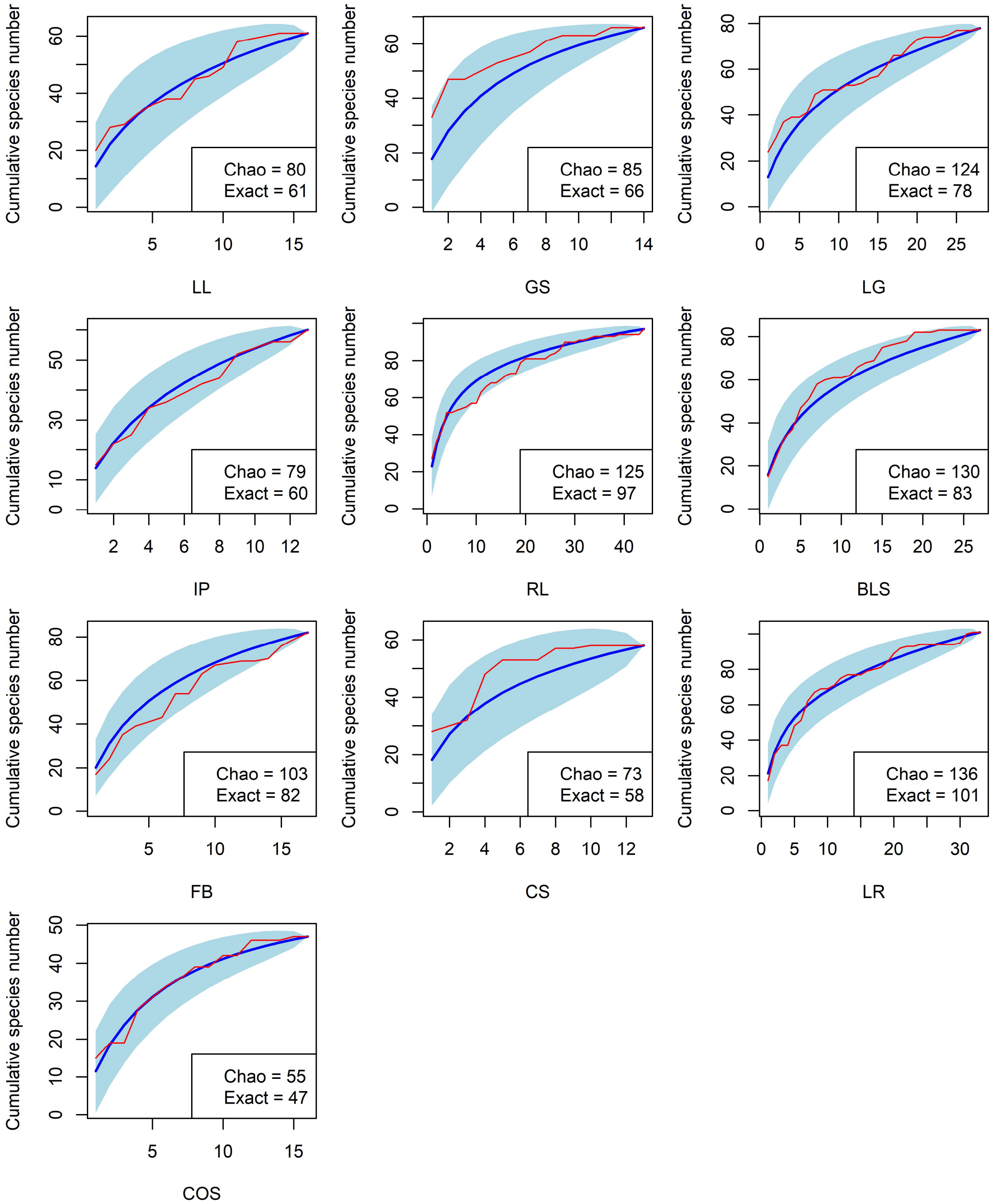

Figure A1.4 Species accumulation curves for Gunbower Forest wetlands (on x axis are cumulative numbers of transects across multiple years; sampling effort is a single transect spanning the entire wetland, the length of the transect varies within a wetland across different years/seasons and between wetlands). Red line is actual species accumulation, blue line is the smoothed (resampled) species accumulation and the shaded area is the 95% confidence interval. The Chao2 estimate provides a non-parametric estimate of the 'true' species richness based on the numbers of single and double occurrence of species.

	Total No.	Transect	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	SU	AU	SP
GUN_LL	16	1	1	2	1	2	1	1	1	0	2	1	2	2	3	9	4
GUN_GS	14	1	1	1	0	2	1	1	1	1	1	1	2	2	2	10	2
GUN_LG	28	2	2	2	0	4	2	2	2	2	2	2	4	4	4	20	4
GUN_IP	13	1	1	1	0	2	1	1	1	0	1	1	2	2	2	9	2
GUN_RL	44	3	3	3	0	6	3	3	3	3	3	3	8	6	8	30	6
GUN_BLS	28	2	2	2	0	4	2	2	2	1	2	2	4	4	4	20	4
GUN_FB	17	1	1	2	1	2	1	1	1	1	2	1	2	2	3	10	4
GUN_CS	14	1	1	1	0	2	1	1	1	0	1	1	2	2	2	10	2
GUN_LR	33	2	2	3	1	4	2	2	2	2	3	2	6	4	7	20	6
GUN_COS	16	1	0	2	1	2	1	1	1	1	2	1	2	2	3	9	4

Table A1.4. Gunbower Forest data summary. The number for each wetland in each year indicates the number of surveyed transects (though may reflect two separate surveys of the one transect within the year). The Total No. is the cumulative number of transects surveyed across all years. Transect is the number of transects within the wetland.

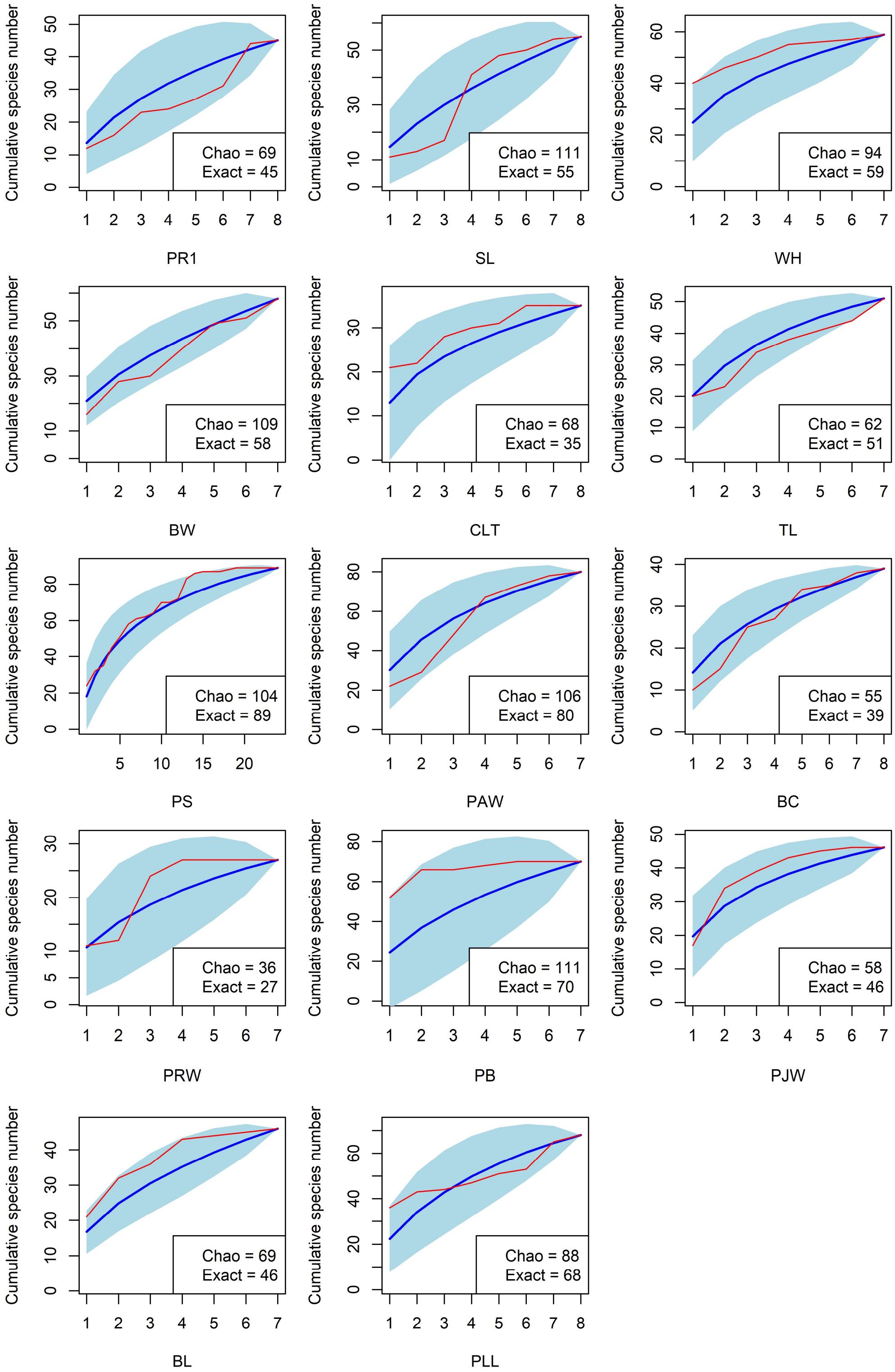

Figure A1.5 Species accumulation curves for Koondrook-Perricoota Forest wetlands (on x axis are numbers of transects). Red line is actual species accumulation, blue line is the smoothed (resampled) species accumulation and the shaded area is the 95% confidence interval. The Chao2 estimate provides a non-parametric estimate of the 'true' species richness based on the numbers of single and double occurrence of species.

	Total No.	Transect No.	2010	2011	2013	2014	2015	2016	SU	AU	SP
KP_PR	8	1	1	1	1	2	2	1	0	6	2
KP_SL	8	1	1	1	1	2	2	1	0	6	2
KP_WH	7	1	0	1	1	2	2	1	0	5	2
KP_BW	7	1	0	1	1	2	2	1	0	5	2
KP_CLT	9	3	0	1	1	2	2	2	0	7	2
KP_TL	7	1	0	1	1	2	2	1	0	5	2
KP_PS	24	3	3	3	3	6	3	6	3	18	3
KP_PAW	7	1	0	1	1	2	2	1	0	5	2
KP_BC	8	1	1	1	1	2	2	1	0	6	2
KP_PRW	7	1	0	1	1	2	2	1	0	5	2
KP_PB	7	1	0	1	1	2	2	1	0	5	2
KP_PJW	7	1	0	1	1	2	2	1	0	5	2
KP_BL	7	1	0	1	1	2	2	1	0	5	2
KP_PLL	8	1	1	1	1	2	2	1	0	6	2

Table A1.5. Koondrook-Pericoota Forest data summary. The number for each wetland in each year indicates the number of surveyed transects (though may reflect two separate surveys of the one transect within the year). The Total No. is the cumulative number of transects surveyed across all years. Transect No. is the number of transects within the wetland.

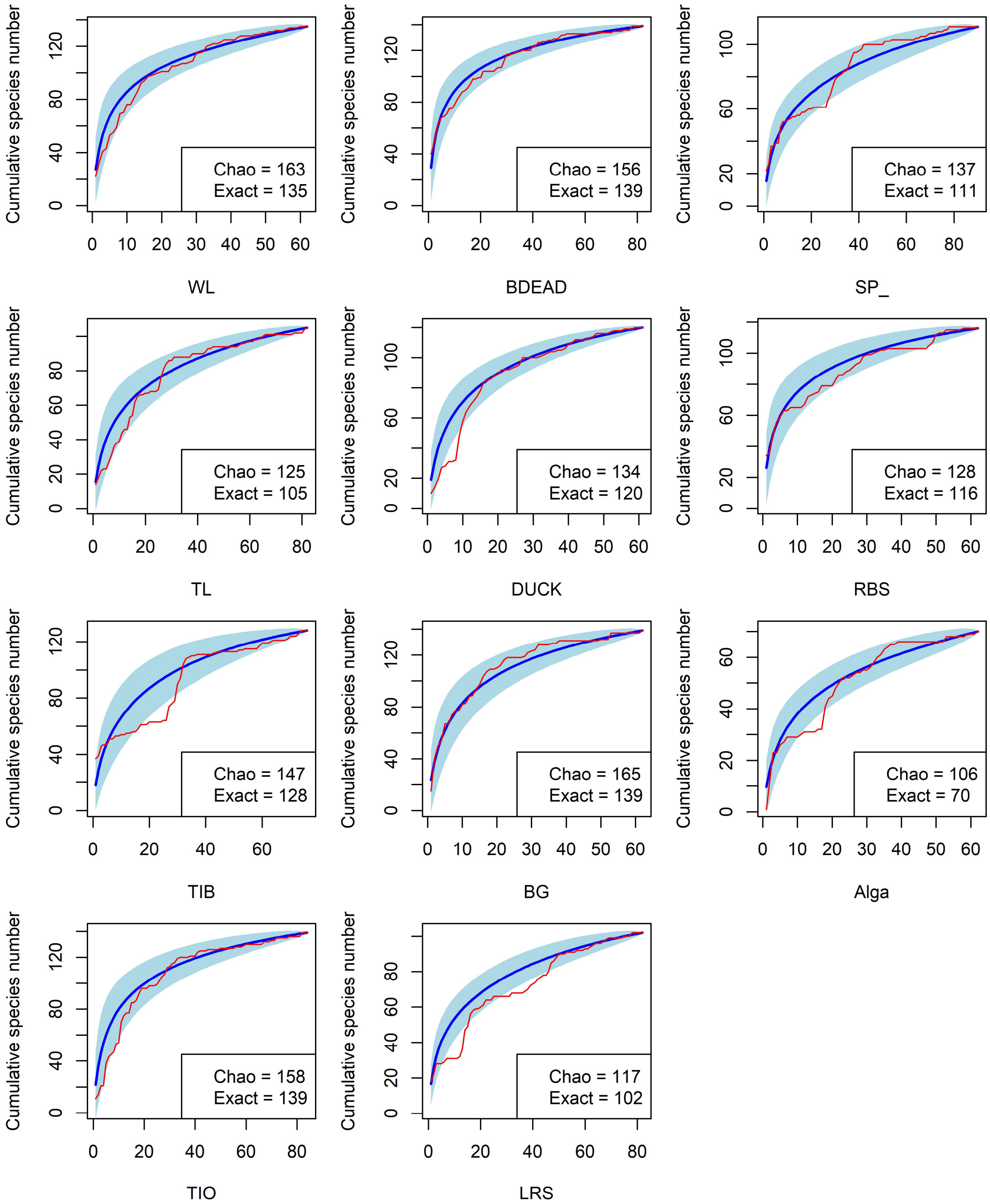

Figure A1.6 Species accumulation curves for Barmah Forest wetlands (on x axis are numbers of aggregated quadrats – three 20m x 20m quadrats). Red line is actual species accumulation, blue line is the smoothed (resampled) species accumulation and the shaded area is the 95% confidence interval. The Chao2 estimate provides a non-parametric estimate of the 'true' species richness based on the numbers of single and double occurrence of species.

	Total No.	Transect No.	1990	1991	1992	1993	1994	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	SU	AU	WI	SP
Barm_WL	62	2	0	0	0	0	0	0	4	8	6	8	8	8	4	0	4	8	16	16	16	14
Barm_BDEAD	84	2	0	0	2	2	2	4	6	8	6	8	8	8	8	4	4	8	22	22	22	18
Barm_SP_	92	2	4	2	4	2	2	4	6	8	6	8	8	8	8	4	4	8	24	24	22	92
Barm_TL	84	2	0	0	2	2	2	4	6	8	6	8	8	8	8	4	4	8	20	22	22	20
Barm_DUCK	62	2	0	0	0	0	0	0	4	7	6	8	8	8	4	0	4	8	16	16	16	14
Barm_RBS	62	2	0	0	0	0	0	0	4	8	6	8	8	8	4	0	4	8	16	16	16	14
Barm_TIB	78	2	0	0	0	0	0	4	6	8	6	8	8	8	8	4	4	8	20	20	20	18
Barm_BG	62	2	0	0	0	0	0	0	4	8	6	8	8	8	4	0	4	8	16	16	16	14
Barm_Alga	62	2	0	0	0	0	0	0	4	8	6	8	8	8	4	0	4	8	16	16	16	14
Barm_TIO	86	2	0	0	4	2	2	4	6	8	6	8	8	8	8	4	4	8	22	22	22	20
Barm_LRS	84	2	0	0	2	2	2	4	6	8	6	8	8	8	8	4	4	8	20	22	22	20

Table A1.6. Barmah Forest data summary. The number for each wetland in each year indicates the number of aggregated quadrats (three 20m x 20m quadrats). The Total No. is the cumulative number of aggregated quadrats surveyed across all years. Transect No. is the number of aggregated quadrats within the wetland.

Appendix 2: Community composition and indicator species

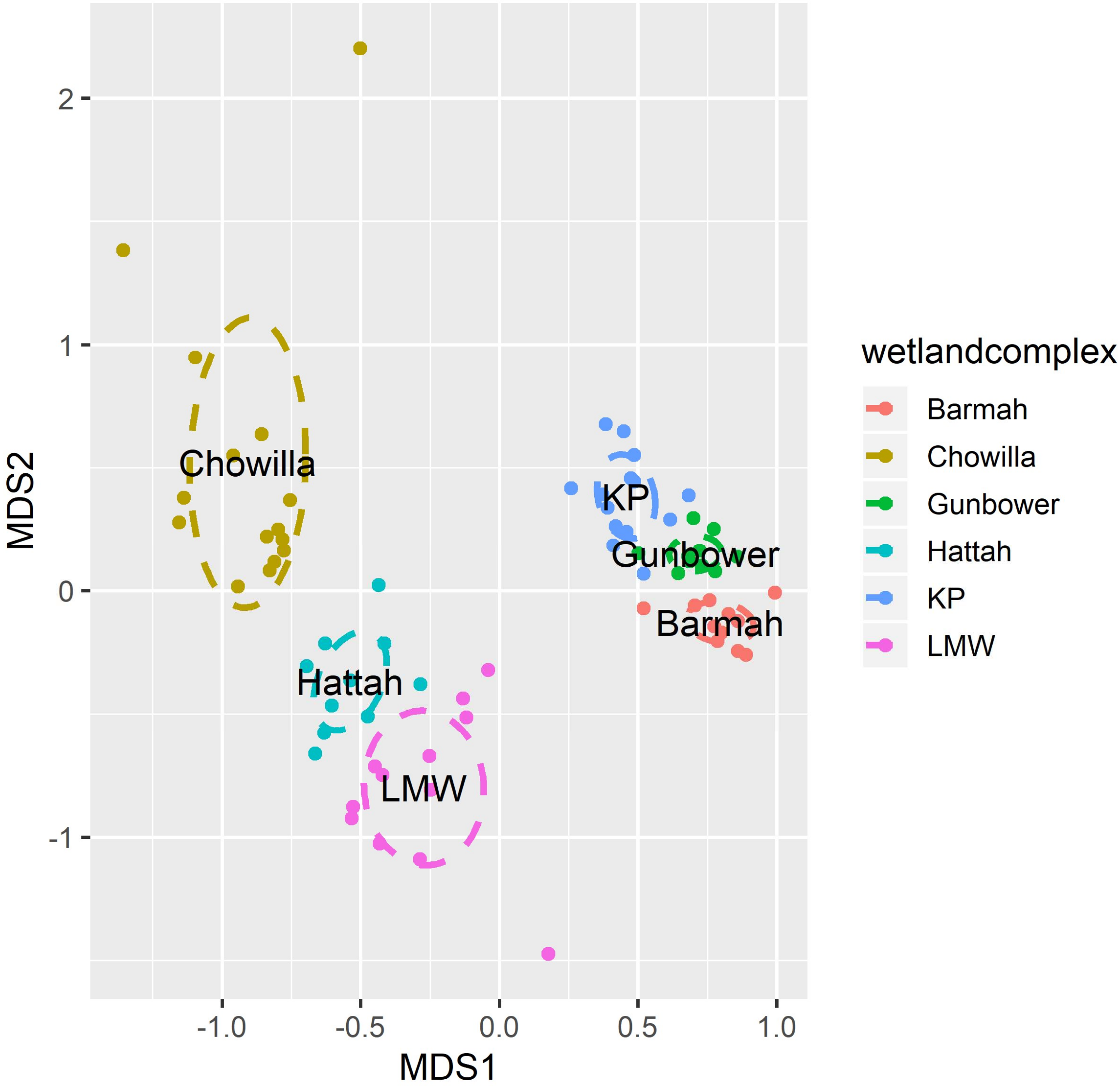

Figure A2.1 nMDS of presence/absence data for wetlands based on full species assemblage. Data combined across years. nMDS undertaken in two dimensions showing axes 1 and 2, stress = 0.15. Confidence ellipse shown for the covariance matrix.

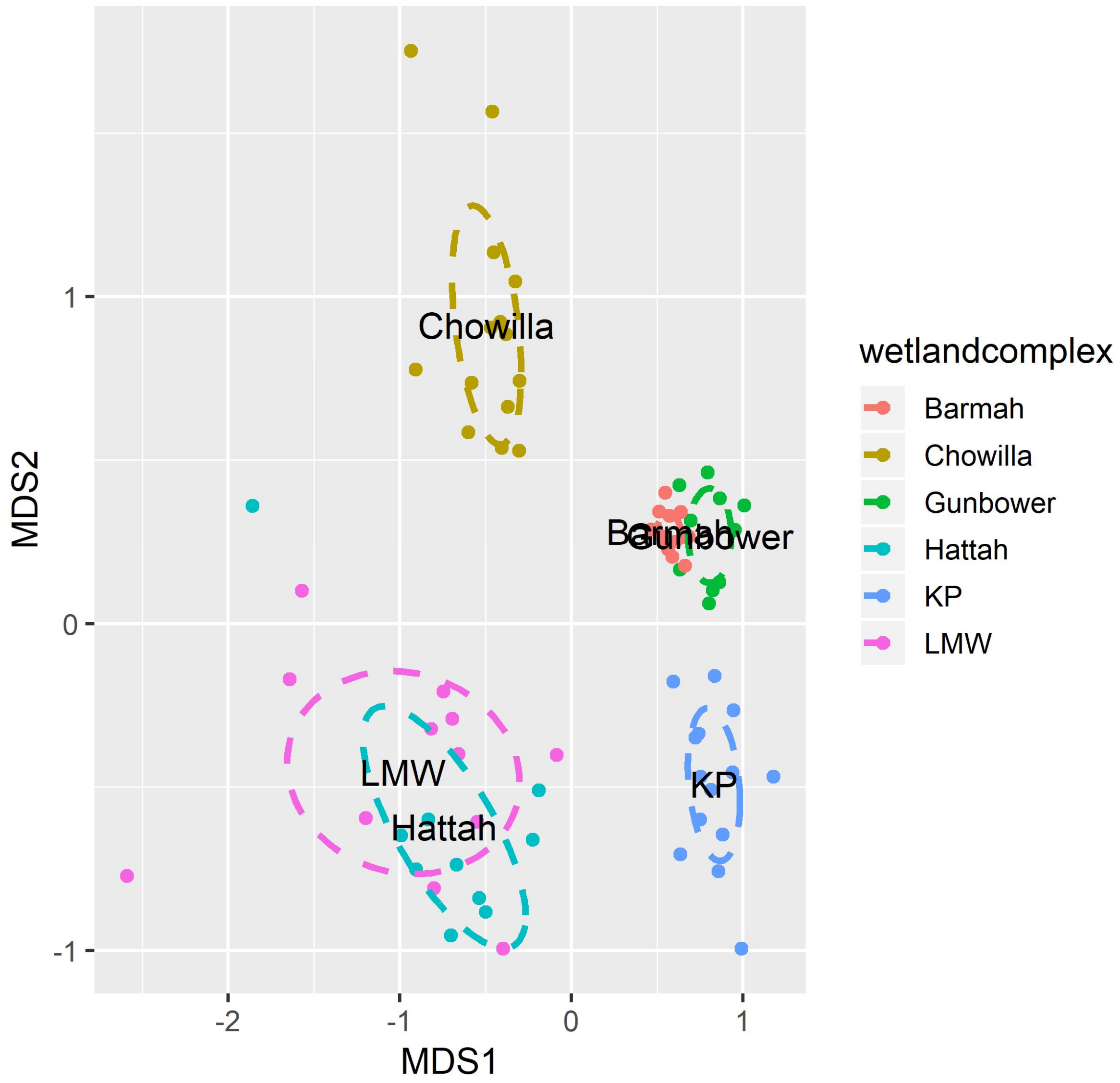

Figure A2.2 nMDS of presence/absence data for wetlands based on common species assemblage (species that occurred >2 in separate surveys. Data combined across years. nMDS undertaken in two dimensions showing axes 1 and 2, stress = 0.11. Confidence ellipse shown for the covariance matrix.

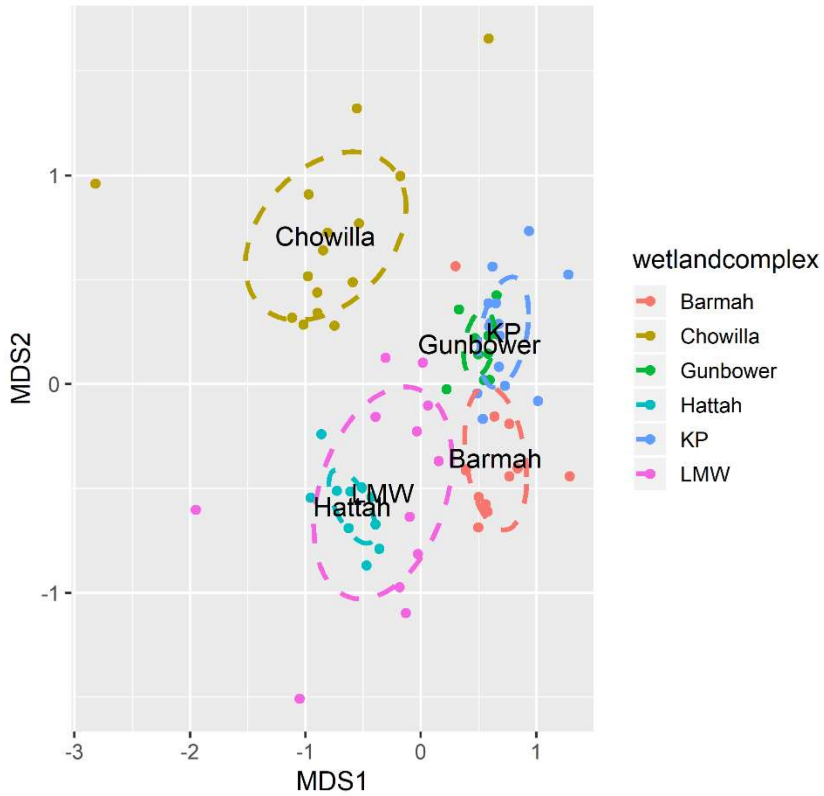

Figure A2.3 nMDS of presence/absence data for terrestrial native species only. Data combined across years. nMDS undertaken in two dimensions showing axes 1 and 2, stress = 0.16. Confidence ellipse shown for the covariance matrix.

Terrestrial natives only

Barmah indicators:

Vittadinia cuneata
Amaranthus macrocarpus
Rytidosperma setaceum
Rytidosperma duttonianu
Boerhavia dominii
Alternanthera nana
Vittadinia cervicularis
Acaena novae-zelandiae
Cassinia arcuata
Elymus scaber

Chowilla indicators:

Tetragonia tetragonoides
Disphyma crassifolium
Sclerolaena brachyptera
Mollugo cerviana
Osteocarpum acropterum
Plantago turrifera

Hattah indicators:

Dodonaea viscosa subsp angustissima
Vittadinia dissecta
Eragrostis dielsii
Schenkia australis
Austrostipa scabra
Ajuga australis
Atriplex pumilio
Hypericum gramineum
Lotus cruentus
Malva weinmanniana
Olearia pimeleoides
Zygophyllum sp.
Atriplex stipitata
Brachyscome ciliaris
Convolvulus remotus
Dysphania cristata
Swainsona microphylla

LMW indicators:

Atriplex lindleyi subsp inflata
Maireana decalvans
Maireana pentagona
Myoporum parvifolium
Sclerolaena calcarata
Sclerolaena tricuspis

Figure A2.4. Key indicator species for the different wetland complexes, describing the differences between terrestrial native species composition.

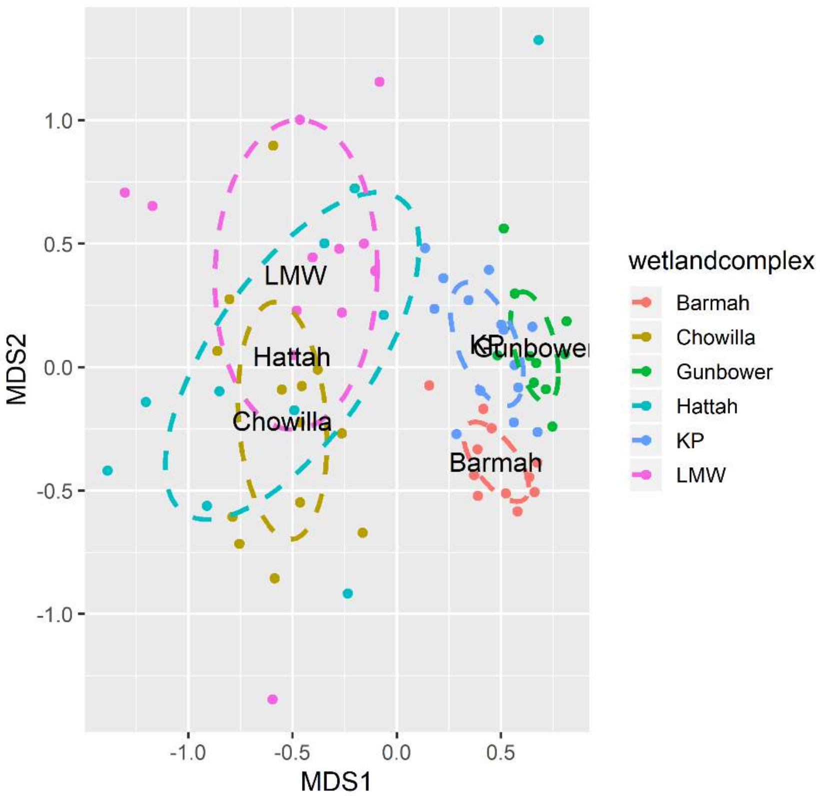

Figure A2.5 nMDS of presence/absence data for terrestrial exotic species only. Data combined across years. nMDS undertaken in two dimensions showing axes 1 and 2, stress = 0.20. Confidence ellipse shown for the covariance matrix.

Terrestrial exotics only

Barmah indicators:

Lolium perenne
Cerastium glomeratum
Trifolium arvense var. arvense
Vulpia bromoides
Sonchus asper
Spergularia rubra
Leontodon taraxacoides subsp. Taraxacoides
Arctotheca calendula
Cucumis myriocarpus
Urtica urens
Bromus madritensis
Pentaschistis airoides
Amaranthus albus
Chenopodium murale
Fumaria muralis
Phalaris minor
Bromus hordeaceus subsp. Hordeaceus
Kickxia elatine
Bromus rubens
Bromus catharticus
Xanthium spinosum
Rosa rubiginosa
Alopecurus geniculatus
Hypericum perforatum
Vulpia muralis

Chowilla indicators:

Mesembryanthemum crystallinum
Hordeum vulgare
Centaurea calcitrapa
Nothoscordum gracile

Gunbower indicators:

Physalis hederifolia

Hattah indicators:

Chondrilla juncea
Asphodelus fistulosus
Salvia verbenaca

LMW indicators:

Mesembryanthemum nodiflorum

Figure A2.6. Key indicator species for the different wetland complexes, describing the differences between terrestrial exotic species composition.

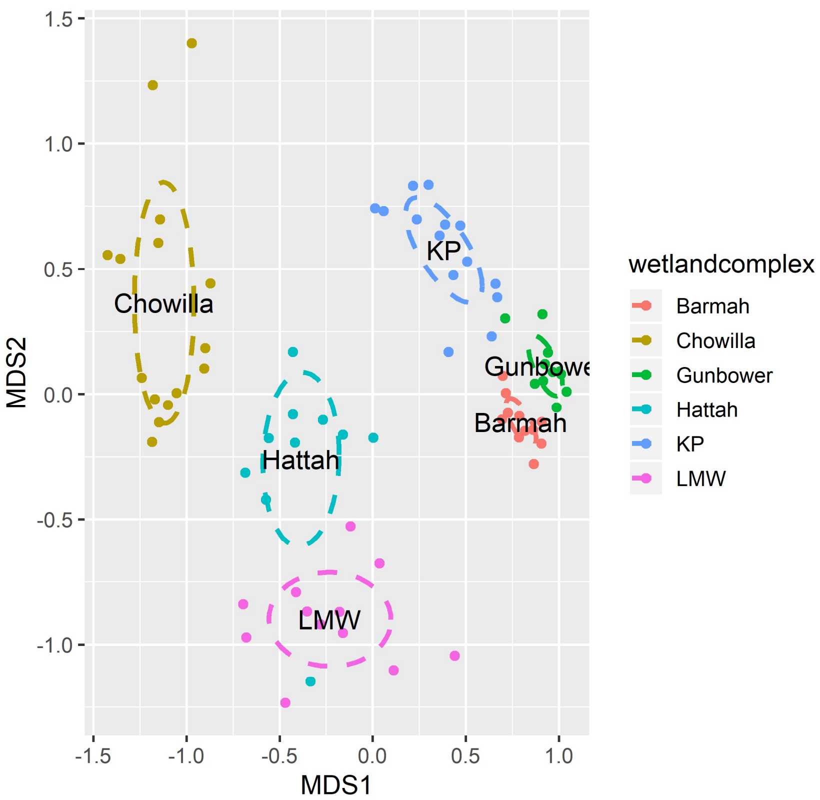

Figure A2.7 nMDS of presence/absence data for wetland native species only. Data combined across years. nMDS undertaken in two dimensions showing axes 1 and 2, stress = 0.17. Confidence ellipse shown for the covariance matrix.

Wetland natives only

Barmah indicators:

Ranunculus inundatus
Myriophyllum variifolium
Eleocharis pusilla
Crassula colorata
Typha orientalis
Persicaria hydropiper
Phragmites australis
Juncus holoschoenus
Lobelia concolor
Myriophyllum simulans

Chowilla indicators:

Thyridia repens
Isolepis hookeriana
Tecticornia pergranulata

Gunbower indicators:

Marsilea hirsuta
Myriophyllum caput-medusae
Typha domingensis
Juncus usitatus
Centipeda nidiformis
Juncus australis
Juncus pallidus
Pilularia novae-hollandiae
Ottelia ovalifolia subsp ovalifolia
Najas tenuifolia
Potamogeton ochreatus
Ceratophyllum demersum

LMW indicators:

Ammannia multiflora
Chenopodium nitrariaceum
Cyperus pygmaeus

KP indicators:

Juncus subsecundus

Figure A2.8. Key indicator species for the different wetland complexes, describing the differences between wetland native species composition. NB no nMDS or indicator analysis is shown for wetland exotic species as there were too few species recorded in the dataset for analysis.

Appendix 3: Beta diversity analysis

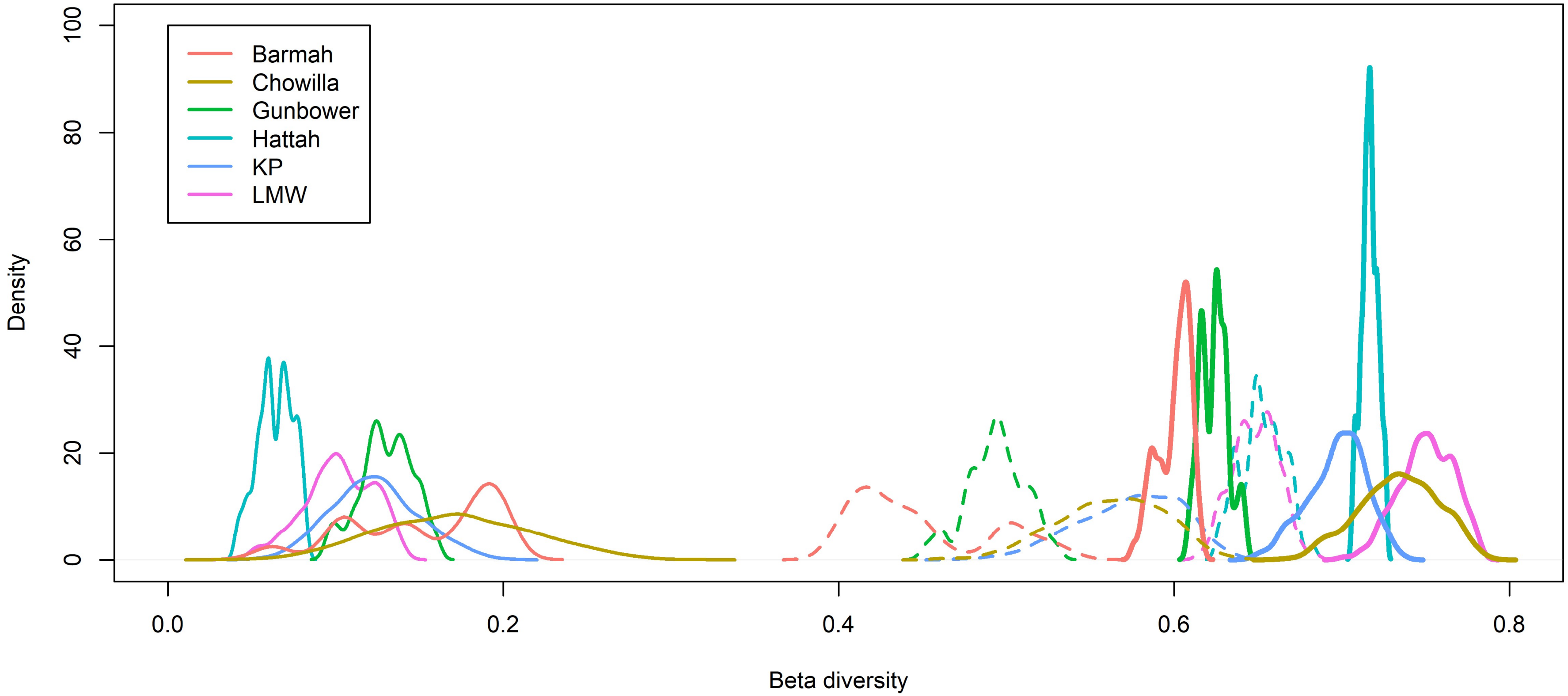

Figure A3.1. Comparing beta diversity and its components (turnover and nestedness) across wetlands within complexes. Data is presence / absence aggregated to wetland with resampling to eight sites because of different numbers of sites per complex. Thick solid lines are total dissimilarity based on Sorensen's index; dashed lines are turnover component; thin solid lines are nestedness (after Baselga and Orme, 2012).

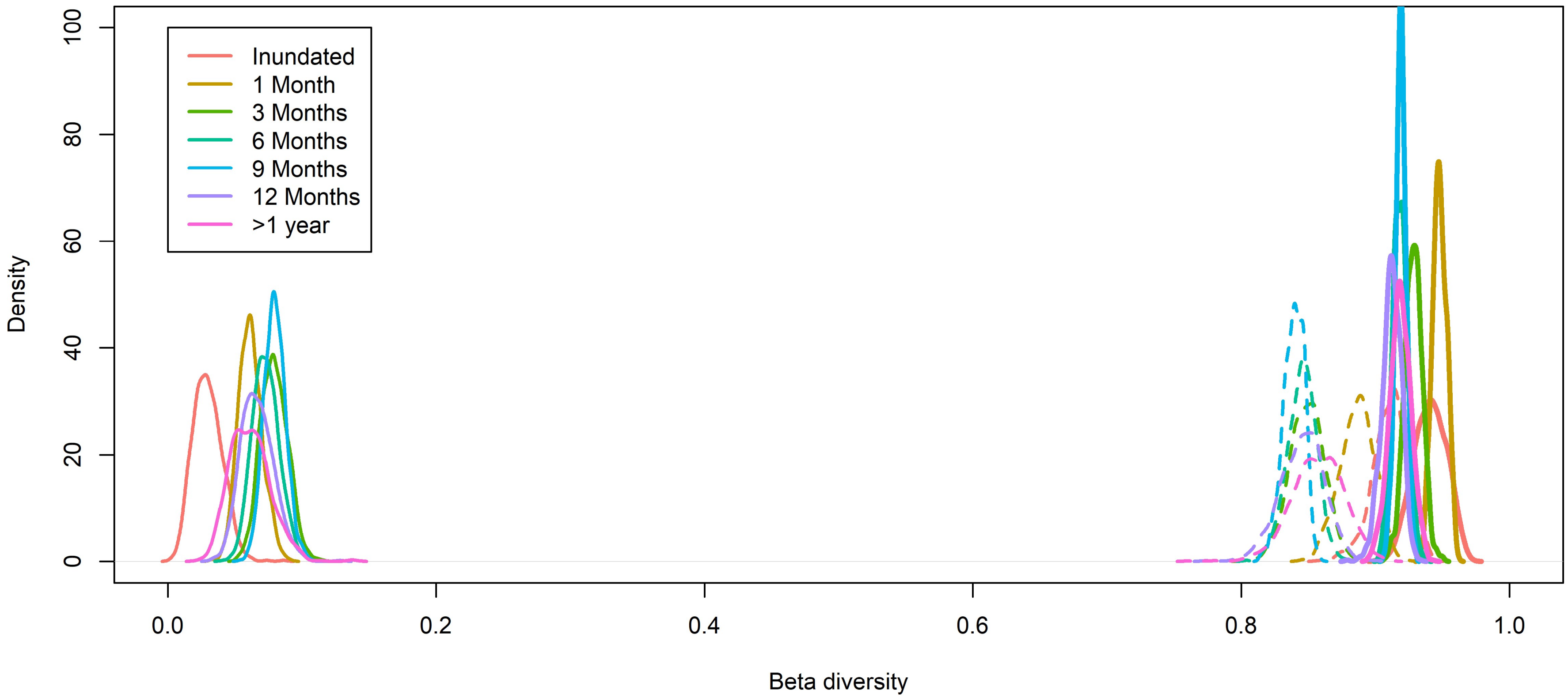

Figure A3.2. Comparing beta diversity and its components (turnover and nestedness) within Hattah Lakes for different periods after flooding. Data is presence / absence aggregated to wetland with resampling to 20 observations because of different numbers of records falling within each time frame. Thick solid lines are total dissimilarity based on Sorensen's index; dashed lines are turnover component; thin solid lines are nestedness (after Baselga and Orme, 2012).

Field Assessment Experimental Design report

Authors (and institution): Cherie Campbell (MDFRC), Susan Gehrig (MDFRC), Rachael Thomas (NSW OEH/UNSW), Samantha Capon (Griffith University), Cassandra James (JCU), Kay Morris (Arthur Rylah Institute), Jason Nicol (SARDI), Daryl Nielsen (MDFRC/CSIRO), David Wood (MDFRC), Fiona Freestone (MDFRC), Allen McIlwee (NSW OEH).

Citation: Campbell et al. (2019). Field Assessment Experimental Design report. Technical report produced for the Commonwealth Environmental Water Office for the Murray-Darling Basin Environmental Water Knowledge Research (MDB EWKR) project. Produced by the MDB EWKR Vegetation Theme.

Document purpose: Internal living document for the MDB EWKR Vegetation theme to aid the consistent design and implementation of field and germination methods.

1 Introduction

The fieldwork component of the Vegetation Theme will involve a program of work across the life of MDB EWKR, with fieldwork planning to be completed in 2015-16 and early 2016-17, fieldwork and data collection undertaken in 2016-17 and 2017-18, and analysis and reporting in 2017-18 and 2018-19.

Field site assessments are proposed for four locations across the Basin. Vegetation responses are likely to vary between different regions of the Basin, such as between the north and south, potentially driven by differences in climate. Field site assessments at different locations will allow broad comparisons of the variability in vegetation responses to advance the understanding of how both flow and non-flow drivers influence vegetation responses. The field-based assessment will also create opportunities to develop links with the other MDB EWKR research themes, for example by potentially assessing the response and condition of vegetation communities that are important waterbird or fish habitats or by estimating biomass accrual rates. One of the strengths of a field-based approach is that it enables research into 'real-life' responses to environmental watering events and associated drivers and stressors. Field site assessments record the actual response to watering events with the influence of a myriad of interacting variables, such as climate, soil type, geomorphology, grazing pressure, soil and groundwater salinity, access to groundwater, competition, disturbance by animals, shading, and disease etc.

For more information about the MDB EWKR research program, for the Vegetation Theme, all others themes and the program as a whole, please refer to Annual and Multi-Year Research Plans (MDFRC 2016a; b).

1.1 Document purpose

This document is primarily a field methods manual. The audience for this document includes the Vegetation Theme Leadership Group, additional personnel involved in the on-ground field assessments, Department of Environment and Energy, and relevant site managers at each of the four locations.

2 Objectives

2.1 Research questions

- How do extant understorey communities differ between structural class, flooding regime and location in relation to hydrologic conditions?
- How do seedbanks (the potential for vegetation response) vary in relation to structural class, flooding regime and location?

3 Methodology

3.1 MDB EWKR research locations

Vegetation surveys will occur at four locations across the Murray–Darling Basin (Figure 3-1):

- Lower Murray (LM)
- Mid Murray (MM)
- Macquarie Marshes (MQ)
- Narran Lakes (NL)

These locations were selected as priority research sites during the early planning phases for the MDB EWKR project. For details of the selection process for these four locations please refer to *Selection of Priority Research Questions and Research Sites* (Burns and Gawne 2014).

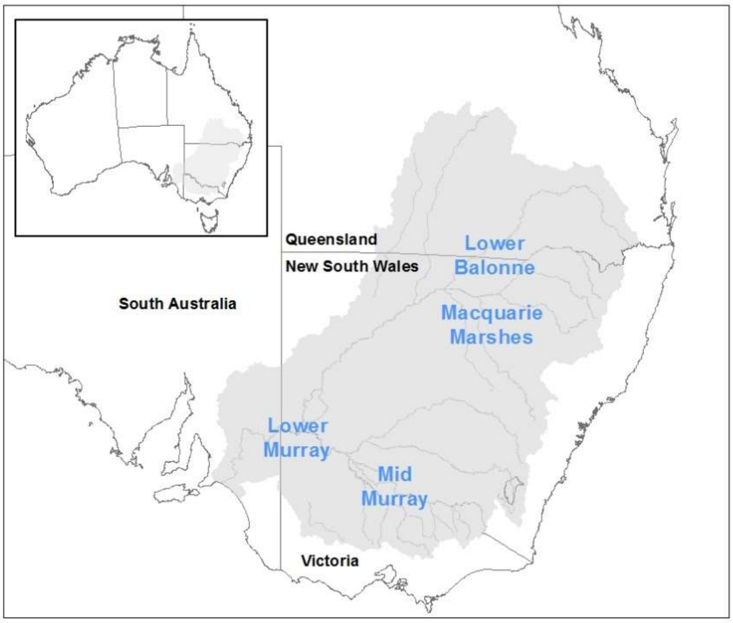

Figure 3-1: Map showing four site locations where vegetation surveys will occur within the Murray-Darling Basin (NB. for vegetation field surveys the Lower Balonne refers specifically to Narran Lakes).

3.2 Desktop site selection

A desktop site selection process occurred for each of the four locations. Initial site selection for the field assessment was undertaken using various spatial layers (e.g. vegetation layers and flood inundation maps) in ArcGIS (Geographic Information System). Site selection was stratified based on flood return frequency and vegetation structure.

Once a list of potential sites was developed, this information was discussed with relevant local managers and the list was refined to the final selection based on a number of pre-defined criteria, e.g. accessibility, alignment with historical survey sites or other projects etc.

3.2.1 Flood return frequency

Lower Murray and Mid Murray locations

Flow data was accessed from various gauging stations along the Murray River corresponding with the regions of interest (e.g. Lower Murray: Chowilla Floodplain, Lindsay-Mulcra-Wallpolla Islands (LMW) and south-west NSW floodplain downstream of the Darling River confluence and Mid Murray: Barmah-Millewa floodplain). Peak annual flow was extracted from daily flow data collected between 1988 and 2010 for flow across the Victorian–South Australian border (Chowilla; data provided by Jason Nicol, SARDI), Lock 9 (LMW and south-west NSW; data provided by Andrew Keogh, MDBA) and at Tocumwal (Barmah–Millewa; data downloaded from MDBA website, 30/6/2016). To calculate flood recurrence interval, peak annual flow was ranked according to magnitude (such that the highest peak flow volume = 1, second highest peak annual flow = 2 and so on) (Fuller 1914, cited by McConnell and Abel 2015). Recurrence interval (*RI*) was calculated using number of years (*n*) and relative ranking (*m*) such that;

$$RI = (n + 1)/m$$

Peak annual flow and recurrence interval (on a LOG scale) were plotted and a logarithmic line of best fit added. The formula resulting from the line of best fit was then used to calculate flow at a required return interval. For ease the resulting flow for LMW, SW NSW and Chowilla was rounded to the nearest 1 000 $ML.day^{-1}$. For Barmah- Millewa the < 1.5 and 1.5 – 3 year flows were adjusted to the nearest 5 000 $ML.day^{-1}$ to match the scale of the most up-to-date flood mapping for the region. The 3 – 5 year flow was rounded to the nearest 1 000 $ML.day^{-1}$. Flood return frequencies of interest and associated flow values for each region are shown in Table 3-1. Where no flow value is offered (e.g. < 1.5 years; Chowilla) it was deemed that no practical spatial areas existed (i.e. annual flows at Chowilla are generally in-channel and do not influence the vegetation communities of interest).

Table 3-1 Flood return frequency and associated flow values for each area: Lindsay–Mulcra–Wallpolla, Chowilla and Barmah–Millewa. N/A = not applicable (i.e. flood return frequency is not applicable to, or practical, at that location).

	Flood return frequency	**Flow ($ML.day^{-1}$)**		
		LMW and SW NSW	**Chowilla**	**Barmah–Millewa**
1	< 1.5 years (near annual)	N/A	N/A	< 25 000
2	1.5 – 3 years	18 000 – 48 000	22 001 – 51 000	25 001 – 65 000
3	3 – 5 years	48 001 – 70 000	51 001 – 73 000	65 001 – 99 000
4	5 – 10 years	70 001 – 101 000	73 001 – 102 000	N/A

In ArcGIS, flow information contained in River Murray Flood Inundation Mapping (RiMFIM) (Overton et al. 2006; Sims et al. 2015) layers applicable to the regions; Chowilla (Zone 16 and 17), LMW and SW NSW (Zones 13 – 16) and Barmah (Zone 3) and Millewa (EW02) were used to determine areas corresponding to flood return frequency. Additionally, more recent flood mapping (up to flows of 65 000 $ML.day^{-1}$), undertaken by the MDBA for Barmah-Millewa, was incorporated (data provided by Andrew Keogh, MDBA). This was undertaken by highlighting the flows corresponding to the flood return frequency and exporting this data into a new shapefile.

Macquarie Marshes and Narran Lakes locations

We used inundation frequency maps to determine the average return interval of floods across the floodplains of the Macquarie Marshes and Narran Lakes. Independent inundation events were initially determined from river flow peaks (Macquarie: DS Marebone Weir July 1988 to June 2013; Narran: Wilby Wilby January 1988 to December 2012). Inundation maps classified from Landsat satellite imagery (Thomas et al. 2015) from the specified periods and then allocated to each event. Inundation maps were then aggregated with pixels recoded to a value of one to create inundation event maps: 30 in total for the Macquarie Marshes and 16 for the Narran Lakes (Thomas et al In review; Thomas et al. 2016). The pixel values for all inundation event maps were then summed through time using Erdas Imagine (ERDAS 2015). To evaluate the likely number of years between floods we divide the total number of observation years (25 for the Macquarie and 26 for the Narran) by the number of time with inundation (count). These were then allocated to the flood frequency category.

Table 3-2 Inundation frequency (counts) and estimated return interval based on inundation event mapping for the Macquarie Marshes and Narran Lakes.

	Macquarie Marshes Flood return frequency	Number of years between floods	Inundation event count range
1	Annual	0.83-0.96	26-30
2	1 – 3 years	1-2.78	10-25
3	3 – 5 years	3-4.17	6-9
4	5 – 10 years	5-8.3	3-5
	Narran Lakes		
1	1.5-2*	1.63-1.86	14-16
2	2 – 3 years	2-2.89	9-13
3	3 – 5 years	3.25-4.33	6-8
4	5 – 10 years	5.2-8.67	3-5

*This category was separated from the 1-3 years because it covers the open water lakes of the Narran system: Narran Lake, Clear Lake, Back Lake and Long Arm

3.2.2 Vegetation classification

Using the best available vegetation based GIS layer files (Table 3-3), vegetation was categorised into three broad structural vegetation categories: Inland shrubland, Inland woodland and Non-woody wetland. These decisions were based on information contained within each layer file or in supporting documentation. Vegetation classes located high on the floodplain (e.g. sandhills) were excluded, as were farmland and Lake Victoria (in the Lower Murray). Chenopod and terrestrial grasslands and woodlands vegetation classes were also excluded (where these are mapped as a vegetation type, not where this vegetation has encroached into areas, such as wetland beds). Attached in Appendix 1 (Tables A – G) is the list of original vegetation categories for each region and the corresponding new category.

Table 3-3 GIS vegetation layer used to group vegetation type into three broad categories

Region	GIS layer
Lindsay–Mulcra–Wallpolla and Barmah (Victoria)	Native vegetation - Modelled 2005 Ecological Vegetation Class (with bioregional conservation status) (DEPI 2008)
Chowilla	Vegetation mapping data and wetland data for Chowilla floodplain (provided by J. Nicol, SARDI 2016). **Note:** wetland data was merged with vegetation data and it was assumed that all wetlands were 'Non-woody wetlands'.
South-west New South Wales	MurrayDarlingM305_Struct_E_917 (NSW OEH 2010a)
Millewa	Deniliquin NVMP VISmap 874 (NSW OEH 2010b)
Macquarie Marshes	2013 Macquarie Marshes and floodplain vegetation map (Bowen and Fontaine 2014)
Narran Lakes	Vegetation of the Barwon-Darling and Condamine-Balonne floodplain systems of New South Wales: Mapping and survey of plant community types (Eco Logical Australia 2015)

Vegetation classes were categorised in ArcGIS using the 'select by attribute' function to select the set of vegetation classes making up each of the new categories. The highlighted items were exported and renamed and matching vegetation structural category (*i.e.* non-woody wetland) and regions (*i.e.* Barmah and Millewa) were merged.

3.2.3 Combined flood return frequency and vegetation strata

Site selection was stratified based on flood return frequency and vegetation structure.

Individual strata were created by the intersection of each structural vegetation category and flood return frequency for each location (Table 3-4). In turn, each vegetation structural category was clipped within each flood return frequency resulting in new strata. Each new shapefile was edited and a new attribute was inserted describing the vegetation/flood return strata for identification. All shapefiles for each location were then merged into a single layer before the 'Dissolve layer' tool was used to create a single feature (combining all similar polygons into a single polygon) for each strata.

Table 3-4 Combinations of vegetation structural categories and flood return frequencies for Lower Murray, Mid Murray, Macquarie Marshes and Narran Lakes Locations.

Vegetation structural Category	Flood Return Frequency: *Lower Murray*	*Mid Murray*	*Macquarie Marshes*	*Narran Lakes*	Strata Nomenclature
Inland Woodland	N/A	<1.5 years (near annual)	<1 (annual)	<1.5 years (near annual)	IW-CAT1
	1.5 – 3 years	1.5 – 3 years	1-3 years	1.5-3 years	IW-CAT2
	3 – 5 years	3 – 5 years	3 – 5 years	3 – 5 years	IW-CAT3
	5 – 10 years	N/A	5 – 10 years	5 – 10 years	IW-CAT4
Inland Shrubland	N/A	N/A	N/A	<1.5 years (near annual)	IS-CAT1
	1.5 – 3 years	N/A	1-3 years	1.5-3 years	IS-CAT2
	3 – 5 years	N/A	3 – 5 years	3 – 5 years	IS-CAT3
	5 - 10 years	N/A	5 – 10 years	5 – 10 years	IS-CAT4
Non-Woody Wetland	N/A	<1.5 years (near annual)	<1 (annual)	<1.5 years (near annual)	NWW-CAT1
	1.5 – 3 years	1.5 – 3 years	1-3 years	1.5-3 years	NWW-CAT2
	3 – 5 years	N/A	3 – 5 years	3 – 5 years	NWW-CAT3
	5 – 10 years	N/A	5 – 10 years	5 – 10 years	NWW-CAT4

3.2.4 Potential site selection

In ArcGIS the 'Create random points' tool was used to select 25 random points for each strata. During the process 'linear unit' was set to 100 m which acts as a buffer such that no two random points are located closer than 100 m. The resulting points were edited and labelled with the appropriate flood return frequency and vegetation structure category. See Figures 3-2 to 3-5 for each of the four locations.

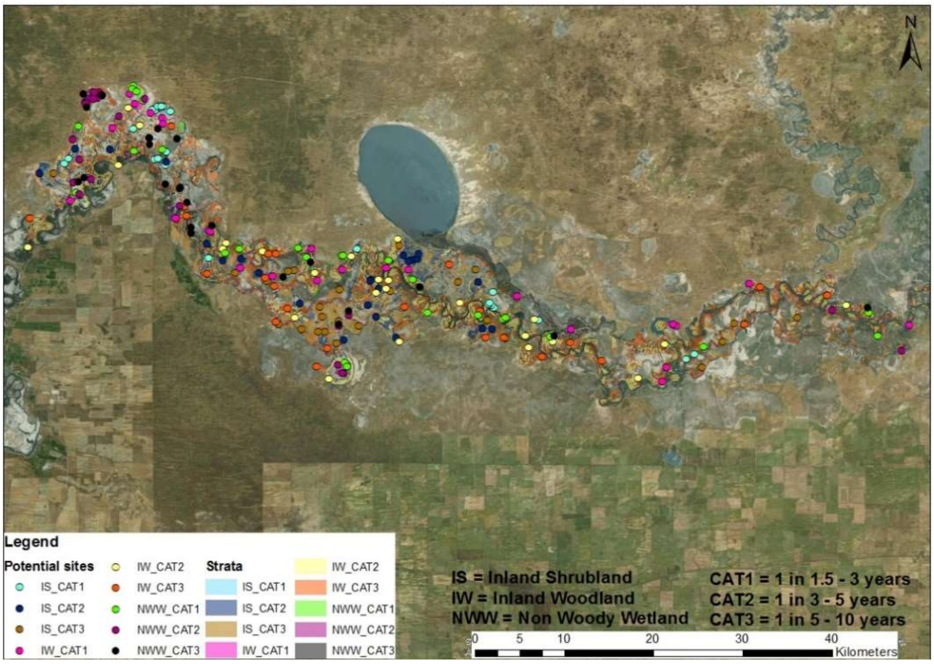

Figure 3-2: Randomly selected *potential* field sites within the Lower Murray (NB in the final site selection colours and labels have been modified slightly for consistency between locations)

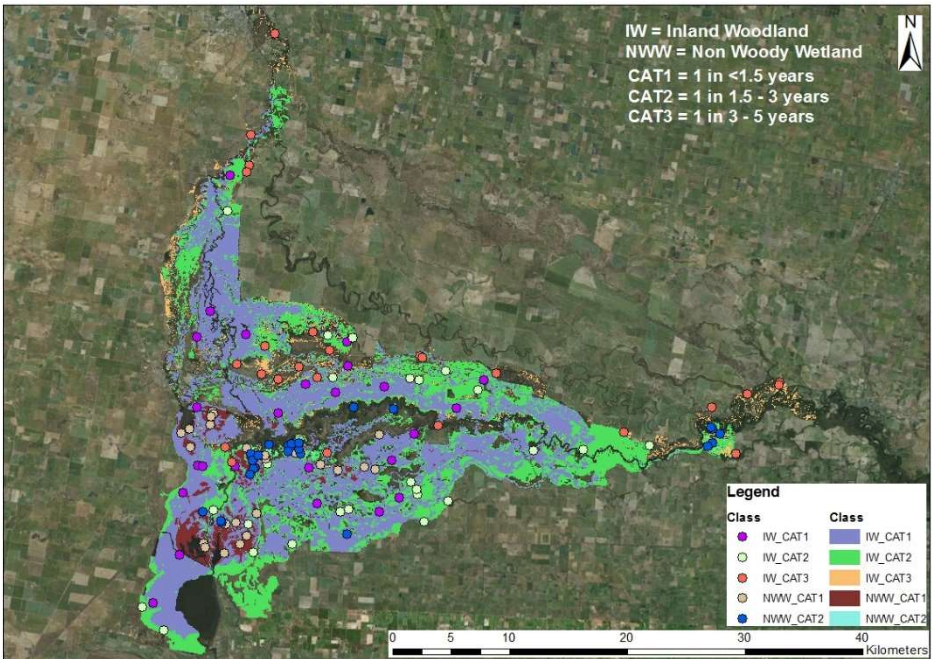

Figure 3-3: Randomly selected *potential* field sites within the Mid Murray (NB in the final site selection colours and labels have been modified slightly for consistency between locations)

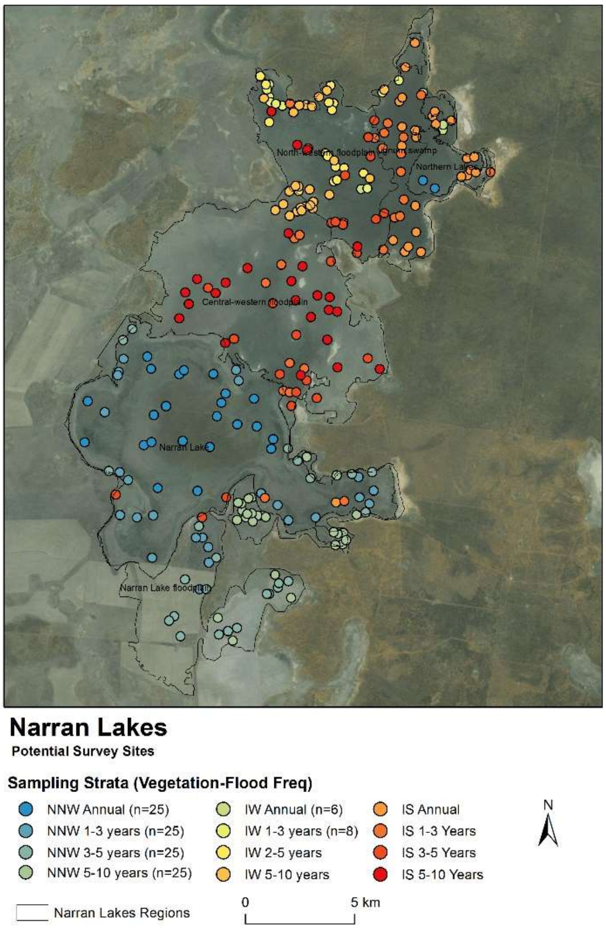

Figure 3-4 : Randomly selected *potential* field sites within the Narran Lakes

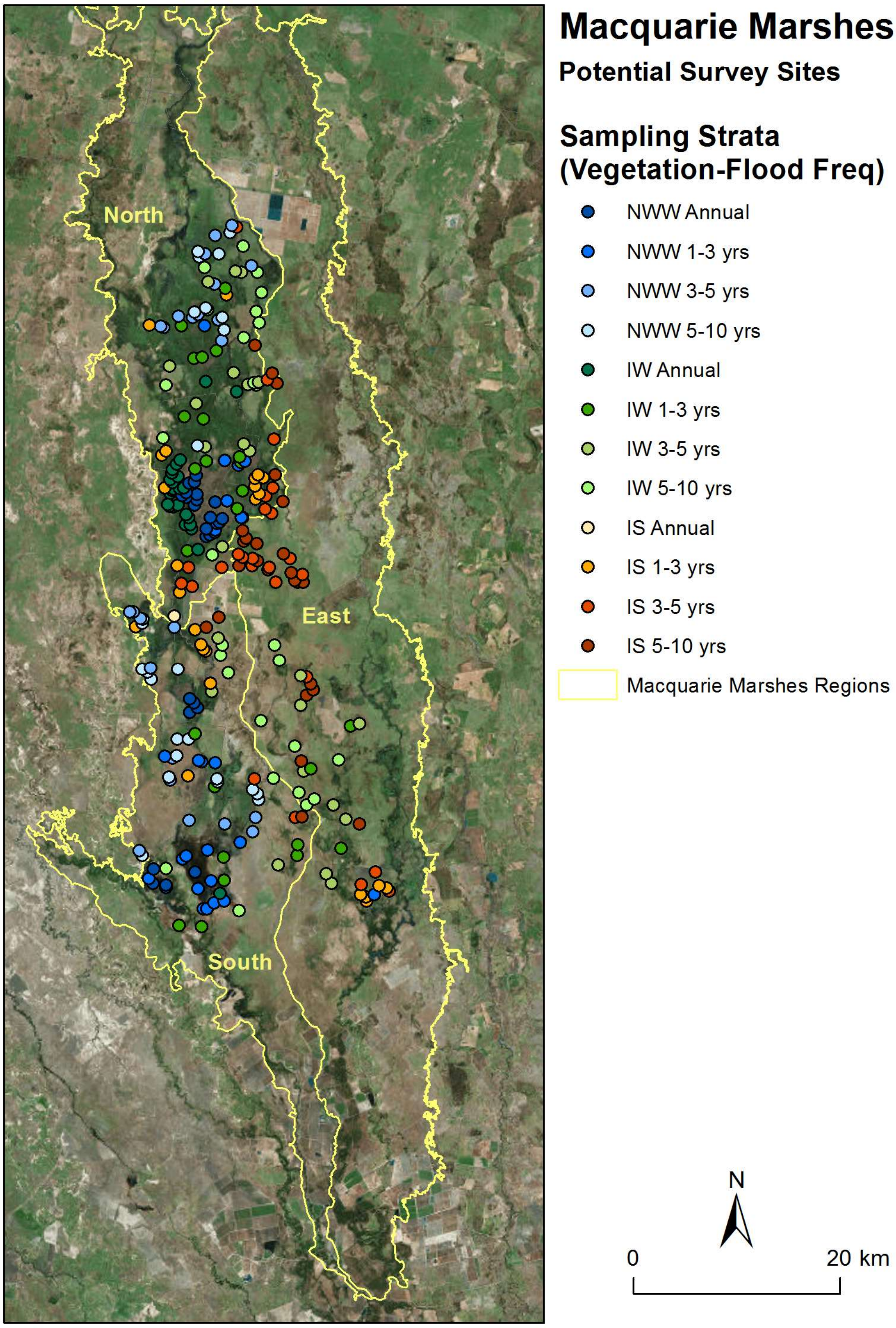

Figure 3-5 : Randomly selected *potential* field sites within the Macquarie Marshes regions (North, South and East).

3.2.5 Final site selection

A maximum of five sites per strata per location were selected. Final site selection was determined following consultation and expert input from local managers (Table 3-5) and consideration of the following criteria:

- the likelihood of inundation with managed flows (including weir pool manipulations)
- previous inundation with managed flows (including weir pool manipulations)
- availability of supporting data (existing monitoring or complementary data)
- known waterbird breeding sites
- access / landholder consent
- pixel area (very small areas are likely to be unrepresentative and potentially inaccurate)
- and any known inaccuracies with the spatial mapping (i.e. misalignment between vegetation categories and/or flood return frequency)

Consultation with local managers occurred on a number of occasions (between August and December 2016). A list of people consulted is included in Table 3-5.

Table 3-5: List of people and organisations consulted during the final site selection phase.

Lower Murray Mildura/Buronga 4th, 12th, 25th Aug, 26th Oct 2016	Mid Murray Shepparton / Deniliquin 14th, 15th Sept 2016	Macquarie Marshes (20th Dec 2016)	Narran Lakes
Andrew Greenfield (Mallee CMA) Emma Healy (Mallee CMA) Iain Ellis (NSW Fisheries) Jane White (Mallee CMA) Mark Henderson (NSW OEH) Sascha Healy (NSW OEH) Scott Jaensch (NSW Water) Jan Whittle (DEWNR) Jason Nicol (SARDI) Todd Wallace (Adelaide Uni) Alison Stokes (DEWNR) Susan Gehrig (MDFRC) David Wood (MDFRC) Cherie Campbell (MDFRC)	Keith Ward (Goulburn Broken CMA) Lisa Duncan (Goulburn Broken CMA) Rick Webster (Murray Wetlands Working Group) Paul Childs (NSW OEH) Alison Borrell (NSW Parks) Cherie Campbell (MDFRC)	Tim Hosking (NSW OEH) Sharon Bowen (NSW OEH) Stephanie Suter (NSW OEH) Paul Keyte (NSW OEH)	

Information on proposed sites (e.g. site name, coordinate information, vegetation structure category, flood return frequency and close-up maps of individual sites) was provided to field teams. Figure 3-6 and Figure 3-7 provides an example of the spread of potential sites within the Lower Murray and Mid Murray. Based on discussions with the regional environmental water managers of the Macquarie Marshes we decided to maintain the full suite of potential points and not select the final survey sites until we were out in the field. We also did an assessment of existing OEH environmental flow vegetation monitoring sites to determine the distribution within the strata classes. Due to the unpredictable nature of localised conditions which may have constrained access to points selected prior to the field trip we took a more flexible approach to site selection based on field conditions. Where established OEH environmental flow monitoring sites fell close to our potential site locations we included the site in our selection.

Details of final site selection will be provided in the reporting for this field site assessment and germination component.

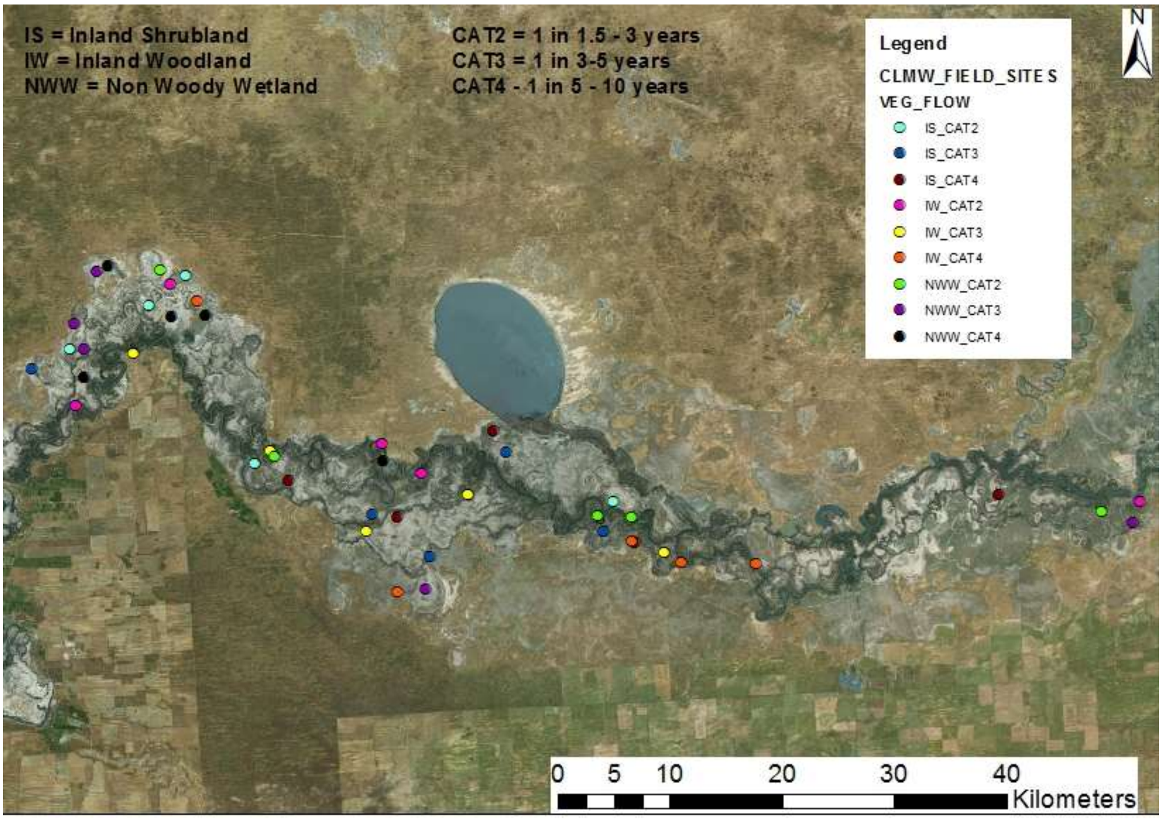

Figure 3-6: Location of proposed field sites within the Lower Murray

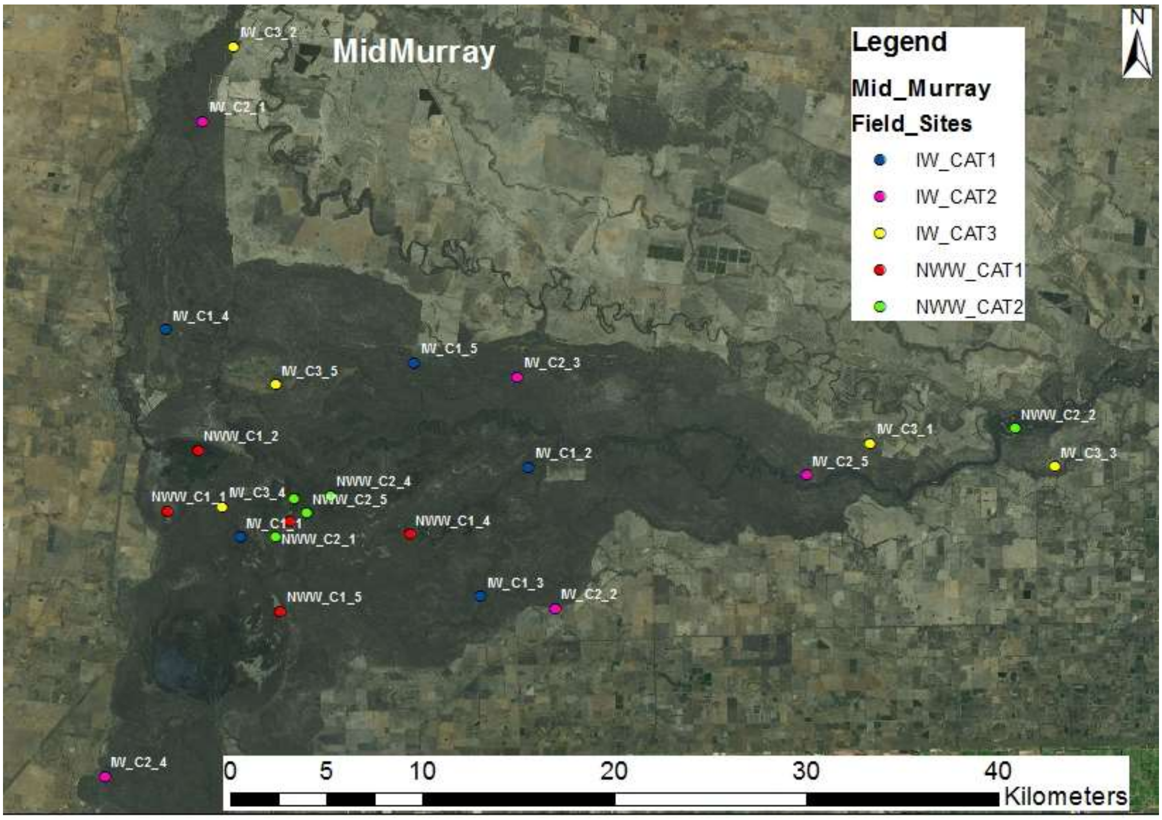

Figure 3-7: Location of proposed field sites within the Mid Murray

3.3 Field survey methods

3.3.1 Addressing objectives

The metrics to be collected will enable assessment of vegetation responses, at each site, each location, and across the four locations within the Basin, in relation to:

- Compositional responses
  - Species richness
  - Species composition
  - Functional/guild representation and diversity
  - Non-native species
- Structural responses
  - Cover of various structural forms (e.g. groundcover, shrub and canopy cover)
  - Evenness and dominance
- Process responses
  - Tree seedling recruitment
  - Lignum condition and reproduction (flowering/fruiting)
  - Seed bank germination
  - Biomass accumulation (estimated via structural metrics)
  - Bare ground
  - Litter accumulation

Factors potentially influencing these responses that can be investigated from the metrics collected and the stratified design include:

- Broad vegetation structure class (e.g. non-woody wetland, inland shrubland, inland woodland)
- Average flood return frequency
- Site specific vegetation structure (cover of various strata)
- Inundation / soil moisture at time of survey

Depending on the availability of desktop data, the following factors are also likely to be investigated:

- Rainfall (BOM/microclimate loggers)
- Temperature (BOM/microclimate loggers)
- Recent and long-term inundation history (e.g. frequency of events, time-since-last inundation)
- Land tenure / management

Where possible, environmental variables will be recorded in the field, such as:

- Microtopographical heterogeneity
- Evidence of disturbance (e.g. grazing pressure)
- Soil characteristics

3.3.2 Timing of surveys

Two field surveys are to be undertaken at each site. The first in autumn 2017 and the second in autumn 2018. Providing sites can be safely accessed (e.g. in periods of high flow access may be restricted), surveys should be undertaken regardless of whether the site is wet or dry.

3.3.3 Summary of data to be collected

A brief summary of data to be collected at each site is provided. Specific details of collection methods for each aspect are provided below;

1. Vegetation structure (e.g. point intercept of species (understorey and canopy) presence and height, classified into strata post collection: substrate composition (e.g. leaf litter, bare ground, lichen crust, coarse woody debris))
2. Species richness (native/non-native)
3. Lignum condition (for sites where lignum is the dominant strata)
4. Soil seedbank samples
5. Photo point images
6. Hemispherical photos
7. Site summary
    - Hydrological information
    - Tree recruitment (e.g. recruitment through presence of seedlings, reproductive status through presence of flowers/fruit)
    - Site characteristics

3.3.4 Equipment required

Minimum list of equipment required to undertake vegetation surveys:

- Copy of this protocol
- Data sheets (printed on water proof paper and/or field computer)
    - Point-Intercept transect data sheets (Appendix 2; Appendix 3)
    - Species list data sheets (Appendix 4; Appendix 5)
    - Lignum condition data sheets (Appendix 6; Appendix 7)
    - Site summary data sheets (Appendix 9; Appendix 10)
- Site maps including;
    - Site waypoint coordinates
    - Vegetation/flooding category
    - Landholder contact details (where necessary)
- Hand held GPS and spare batteries;
    - To find location of each site (general waypoint points provided, see above)
    - To record waypoint coordinates of quadrat corner pegs and hemispherical photo location
- Compass
    - For site set up (e.g. quadrat corner pegs and recording photo point direction)
- 100 m surveyor measuring tape
    - To run around perimeter of each quadrat
- Additional tapes (2 – 3 extra tapes)
    - To set up Point-Intercept transects within quadrats (can be 30 – 50 m tapes)
- Bicycle flags
    - May be useful to mark the start and end of each point intercept line in dense vegetation
- 2 m staff with laser pointers (see Figure 3-10).
    - For Point-Intercept transect surveys
- Wooden stakes (or equivalent) - 5 per site
    - To set-up 4 x quadrat corner pegs and 1 x centre for hemispherical photos
- Mallet

- Spray paint
    - To spray wooden pegs/hemispherical photo pegs
- Digital camera (> 5 megapixels) and spare batteries
    - See section 3.3.6 for explanation of camera set-up for photo points
    - See section 3.3.6 for explanation of camera set-up for hemiview photos
- Fisheye lens or adaptor with full 180 degree field of view
    - See section 3.3.6 for explanation of camera set-up for hemiview photos
- Tripod
- Photo point reference booklet
    - to be developed after the first survey to line up photo points in subsequent surveys
- Sample bags and tags for collecting plant specimens for ID
    - See section 3.3.6 for explanation of plant ID protocols

Soil seedbank samples

- Trowel with 5cm increment marked
- Ziplock bags and permanent markers for collecting soil and labelling
- Plastic tubs (or equivalent) for storing soil

3.3.5 Site establishment and surveys

Site establishment should be undertaken as part of the first round of surveys (i.e. the site will be surveyed as it is being established). For each site;

1. Find pre-determined site location using waypoints and maps provided;
    a) as far as possible, confirm that the vegetation in the area is consistent with the intended design (e.g. does the vegetation match the broad vegetation category?)
    b) if the quadrat is not within the correct vegetation community, reposition the quadrat to get the best representation of the desired vegetation community. Check maps to ensure any repositioning will still be within the correct flood return frequency. Record any details (and justification) for this on the **site summary** data sheet
    c) Where possible, align the 20 × 20 m quadrats with one quadrat side along the water's edge and/or flow front (Figure 3-8)

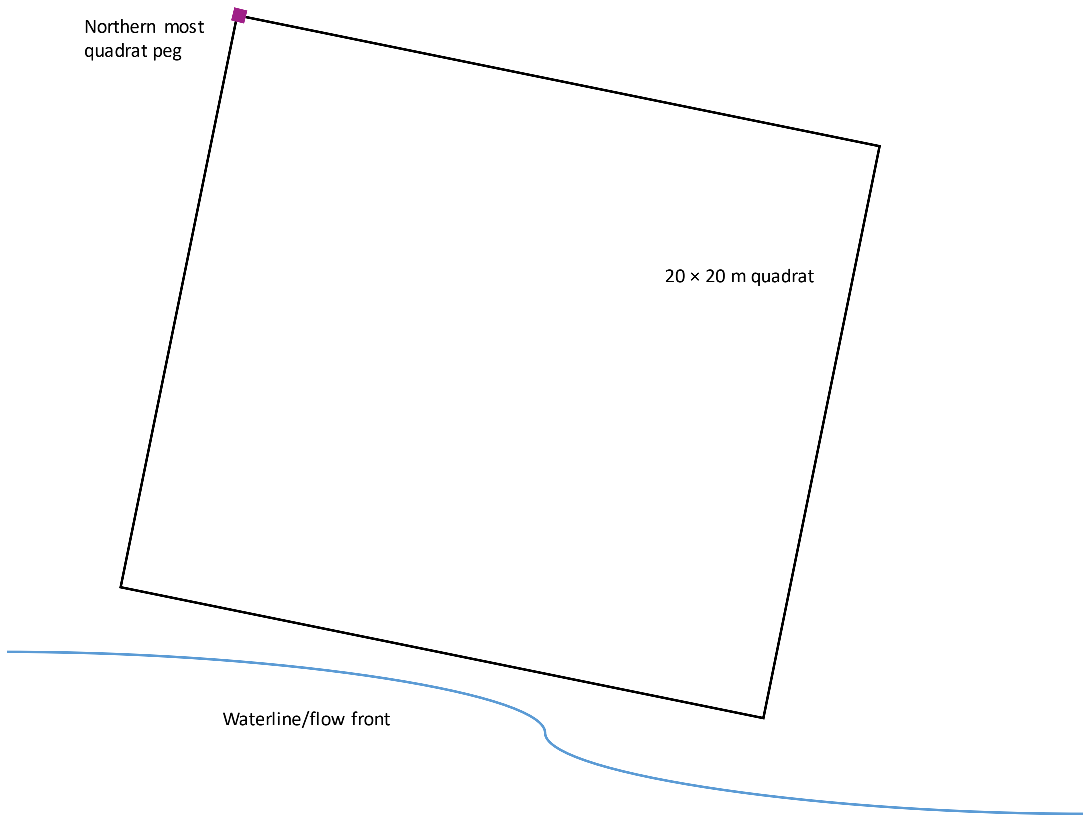

Figure 3-8: Schematic diagram indicating alignment of quadrat perpendicular to water's edge and/or flow front

2. Using the 100 m tape and compass - mark out the quadrat (20 m x 20 m square)
3. Using the mallet, hammer in 4 wooden pegs to mark each corner
4. Choose the northern most corner peg as the Photopoint position (paint pink) and the other 3 wooden stakes (paint yellow)
5. Using a mallet, hammer in one peg at the centre of the quadrat as the Hemispherical Photopoint position (also paint pink)
    - even if there is no canopy – still take hemiview photo
6. Using a hand held GPS, take a waypoint at each of the five pegs.
    - Record waypoint coordinates (indicate if datum GDA 94 or other) numbering the quadrat corner pegs 1 to 4 (with 1 being the most northern peg, which will then become the site photo point) and the centre peg as the hemispherical photo point.

3.3.6 Data collection methods and definitions

Complete the following steps in any order. Surveying of Lignum condition is only applicable to sites where Lignum is the dominant strata (e.g. Inland Shrubland vegetation categories and potentially some Inland Woodland or Non Woody Wetland categories). Lignum condition assessments are not required if lignum is a minor component of wetland or woodland communities. Where practical, all components of the survey should be completed on the same day.

1. Vegetation structure

A total of five point-intercept transects across the quadrat will be used to assess the cover of individual plant species, total vegetation cover, cover of substrate types (e.g. bare soil, leaf litter, lichen crust, rocks, coarse woody debris and/or the presence of water) and the height of lower, mid and upper storey vegetation strata.

Plot set-up

Where possible, align the 20 × 20 m quadrats with one quadrat side along the water's edge and/or flow front (see Figure 3-8). Transects will then run perpendicular to the water's edge (and/or flow front) and be spaced 5 m apart (i.e. 0, 5, 10, 15 and 20 m), with point-intercepts recorded every 2 m along the transects to provide a total of 55 points per quadrat (Figure 3-9).

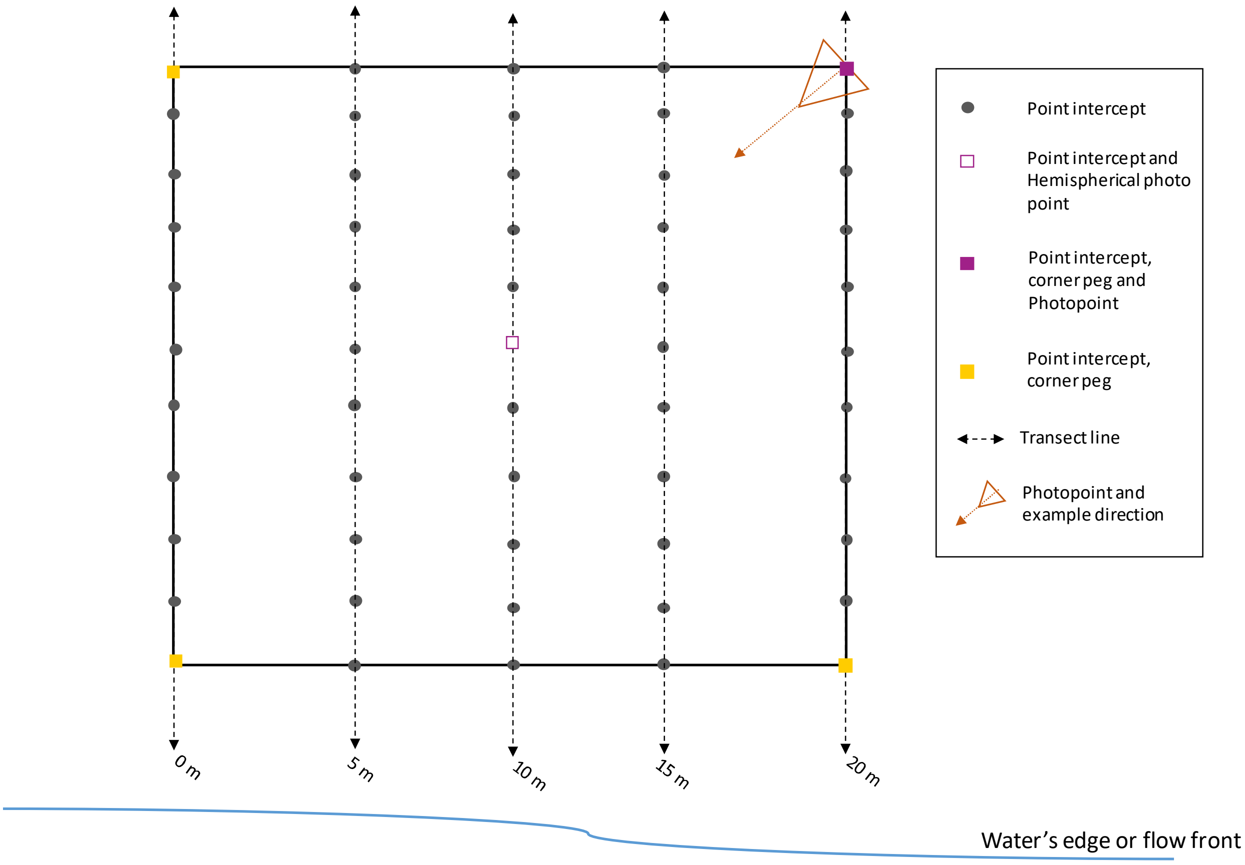

Figure 3-9: Schematic diagram illustrating the layout of the point-intercept transects across the 20 × 20 m quadrat. Transects are aligned perpendicular to the water's edge and/or flow front and spaced 5 m apart (0, 5, 10, 15 and 20 m). Point intercepts recorded every 2 m along transects to provide a total of 55 points per quadrat.

For each transect lay out a 30 – 50 m tape between the start and end-points (or corner pegs).

Ensure tape is:

- orientated to align with the grid
- straight, and
- on the ground (where possible) and not draped over shrubs
- in very dense, tall vegetation the use of flags, a compass bearing and a short-distance of tape may be more practical

To record substrate and vegetation cover a 2 m staff will be used (Figure 3-10) that includes:

- 10 cm graduations marked on it
- A laser pointer positioned at 1 m high (pointing downwards)
- Densitometer positioned at eyelevel
    - Note: laser pointer positioned at eye level and pointing upwards may also be used if a densitometer is not available (or preferred)

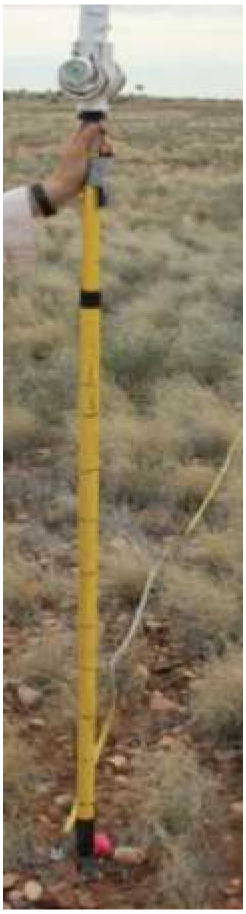

Figure 3-10: Example of 2 m staff used for point-intercept transects, with 10 cm graduations, a laser pen pointing downwards and a densitometer at eye level to record canopy cover (photo from TERN 2012)

Method

1. Using the staff start at the 0 m mark of the first transect , ensure the staff is vertical and the laser pointer is pointing downwards and parallel to the direction of transect.

2. Press the button of the laser pointer to determine the substrate type at the point of contact with the laser beam and/or any vegetation that intersects with the laser beam (i.e. below 1 m).

3. Record substrate type. Substrate categories are listed on the field data sheets (Appendix 2 and 3), and include:
    - bare (bare soil)
    - rock
    - lichen crust
    - coarse woody debris (detached wood >10 diameter at the intercept point)
    - water (record depth, cm)
    - leaf litter (record depth, cm)
    - thatch (record depth, cm) (added in 2018 survey season to distinguish between leaf litter and dense, decaying vegetation such as mounds of sedges or thick grass)
    - man-made structure

*when recording the substrate type, if the beam intersects with vegetation, move aside to determine what the beam intersects with at the ground/substrate level and record relevant category.

**when recording the substrate type and the beam intersects the substrate types: leaf litter or water, please record the depth of either to nearest cm.

4. If the laser beam intersects with a plant, record the uppermost height at the point of intersection (estimated to the nearest 5 cm from within the 10 cm graduations marked on the staff), along with the species name.

*If identification is uncertain, record a field name and collect a specimen (see section below on Species Richness).

5. Record all plants (species and uppermost height) that the staff touches between the laser pointer and the 2 m staff).

6. To record cover of the upper storey of vegetation, look through the densitometer to determine whether any portion of the tree or large shrub crown intersects with the vertical line of sight through the densitometer (or 2nd laser pointer, pointing upwards, if using).

   - If foliage or branches are sighted in the cross hairs of the densitometer, record each species name and provide an estimate of the height of the species at the uppermost intercept.
   - If no part of the foliage or branches are sighted in the cross hairs, but the vertical line is still within the canopy boundary, record as **in-canopy sky** (Figure 3-11). <u>Note:</u> when the tree is dead, in-canopy sky is not recorded.
   - Where the vertical line projects onto bare sky, that is not within a canopy, then nothing is recorded for the upper stratum (or simply record as 'sky').

7. Continue recording the same information (as points 3 to 7) at each 2 m interval along each of the five transects, laying out the tape for each transect. This will provide a total of 55 points for each plot.

Point	Canopy Intercepted?
0	nil-outside canopy
1	canopy hit
2	canopy hit
3	canopy hit
4	nil
5	canopy hit
6	"in-canopy sky"
7	canopy hit
8	canopy hit
9	Nil-outside canopy
10	Nil-outside canopy
11	Nil-outside canopy
12	canopy hit
13	"in-canopy sky"
14	canopy hit
15	nil-outside canopy
16	nil-outside canopy
17	nil-outside canopy
18	nil-outside canopy
19	nil-outside canopy

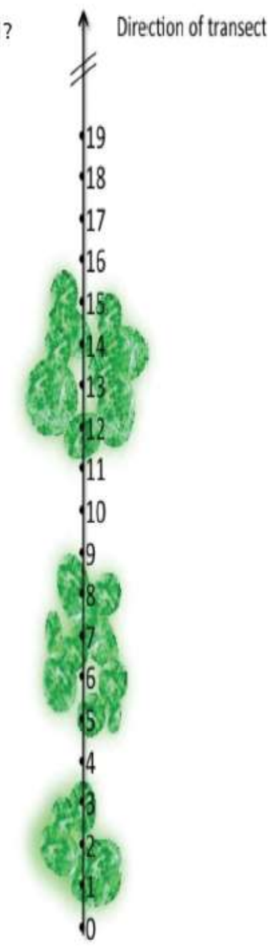

Figure 3-11: Example illustrating when to apply "in-canopy" sky when undertaking point-intercept transects

Dense lignum clumps

Where there are dense, impenetrable lignum clumps within quadrats undertake the point-intercept to the edge of the clump and simply record what points along the transect the dense lignum clumps are between (see Figure 3-12). Estimate the uppermost height of the lignum clumps along each transect and for each point that you cannot survey.

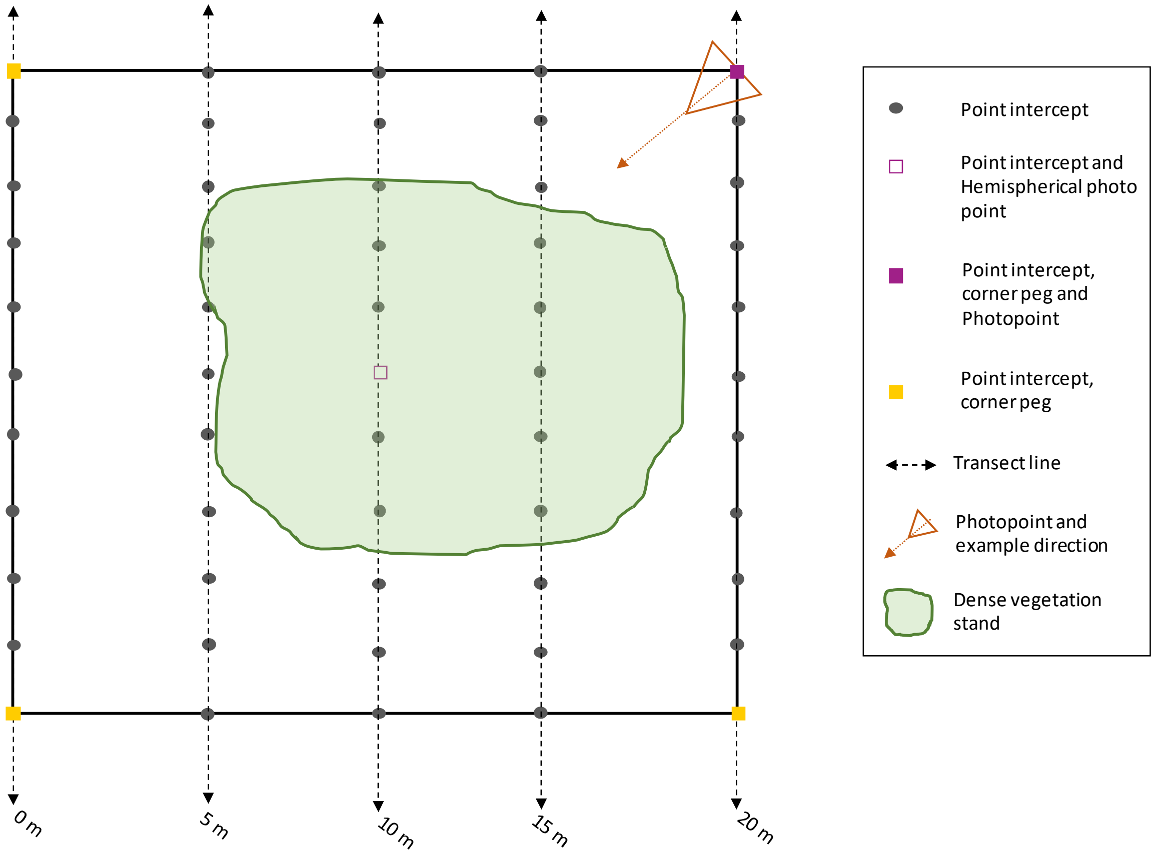

Figure 3-12: Schematic diagram illustrating sampling strategy for dense lignum clumps

2. Species richness (incidental species)

Using the **species richness** data sheet (Appendix 4 and 5), complete all relevant site information and record the presence and percent cover (added in 2018) of all living plant species (native and non-native) that are **alive** and **rooted within** the 20 m x 20 m quadrat. Complete this after finishing the vegetation structure (point-intercept method) to focus collection of data on additional plant species not recorded by the point-intercept method.

For tree/canopy species (e.g. *Eucalyptus camaldulensis*, *E. largiflorens*, *Acacia stenophylla*, etc.) indicate the life stage of the species you are recording, eg;

- MATURE – DBH > 10 cm and/or > 3 m tall
- JUVENILE – woody, DBH < 10 cm and/or < 3 m tall
- SEEDLING – non woody, usually less than 20 cm tall

There may be instances where the same species is recorded two or three times if more than one life stage is represented within the quadrat (Figure 3-13; Appendix 4 and 5).

Species
Eucalyptus camaldulensis (mature)
Eucalyptus camaldulensis (seedling)
Acacia stenophylla (juvenile)

Figure 3-13. Example of data sheet record where one species is represented by two life stages in the same 20 m x 20 m quadrat.

For unidentified species, collect a best representative sample and attach a tag to the specimen that has been labelled with a unique field number/code by the collector. Enter this information on the data sheets. After labelling, transfer specimens directly into bags to ensure individual specimens are separated pending transferring to a plant press (preferably within the field day).

3. Lignum condition

For sites where Lignum (*Duma florulenta*) is the dominant strata (e.g. Inland Shrubland vegetation categories) an additional assessment is required. Using the **Lignum** data sheet (Appendix 6; 7), assess the condition of every **Lignum clump** using the Lignum Condition Index (LCI) (Table 3-6). The condition of lignum is assessed using two rating scales that describe the percentage of above ground plant biomass that is viable (i.e. not dry/dead) and the colour of the **viable** crown (exclude the non-viable crown from the colour assessment). A 'clump' is defined as an individual plant as much as is practical; used where it is impossible to distinguish one individual plant from another.

Table 3-6. The Lignum Condition Index (LCI) used to assess the condition of lignum clumps. Adapted from Scholz et al. (2007).

% viable	score
> 95	6
75 ≤ 95	5
50 ≤ 75	4
25 ≤ 50	3
5 ≤ 25	2
0 ≤ 5	1
0	0

colour	score
all green	5
mainly green	4
half green, half yellow/brown	3
mainly yellow/brown	2
all yellow/brown	1
no viable stems	0

For each Lignum clump determine and record the gender by examining the flowers (see Figure 3-14 and Figure 3-15). Where flower buds have formed but gender is not able to be determined record this as 'BUDDING'. If there is no flowering record gender as 'UNKNOWN'. Estimate and record the abundance of flowers for each clump using the following categories;

- NONE
- SCARCE – < 10 flowers on the plant
- COMMON 10 – 50 flowers on the plant
- ABUNDANT – > 50 flowers on the plant

Figure 3-14. Low-set, star shaped **FEMALE FLOWER** of *Duma florulenta*. Female flowers are smaller than the male flowers, tri-branched style with eight barren filaments and are held close to the branch (Jensen et al. 2008). Female flowers ~4.1 mm diameter
(Chong & Walker 2005).

Figure 3-15. The **MALE FLOWER** of *Duma florulenta* is larger than the female flower and has distinctive extruding anthers. The male flowers are more obvious than the female flowers and have eight fertile stamens and a residual stigma (Jensen et al. 2008). Male flowers ~5.6 mm in diameter (Chong & Walker 2005).

Estimate and record the abundance of leaves for each Lignum clump using the following categories;

- NONE
- SCARCE – < 10 leaves on the plant
- COMMON – 10 – 50 leaves on the plant
- ABUNDANT – > 50 leaves on the plant

Determine and record the average height and width of Lignum clumps within the quadrat.

4. Soil seed bank samples

Field collection

From inside the 20m x 20m quadrat collect 10 random soil samples ~5cm depth x 10cm diameter (to a total of ~3L). Brush aside loose debris before collecting. Aggregate sample in the one bag and label with location and date.

Air-drying and storage

If soil is damp or wet air-dry to prevent the sample from going mouldy prior to storage. Suggested method for air-drying is to empty composite sample into a 4L ice-cream container and dry with the lid off (ideally inside / in a shed to prevent contamination from wind-blown seeds). If you are out in the field for a week-long trip try to allow the samples to breath to some extent (e.g. overnight in the back of the vehicle) to prevent them from going mouldy.

Sort samples to remove large debris and store air-dried samples in well labelled, sealed containers / bags at a relatively constant temperature (e.g. in an air-conditioned lab if possible).

Germination trials

Use soil from each composite sample to fill six takeaway containers (Figure 3-16). For each container take a standard equivalent volume (375mL; 1.5 cups) and place in take-away containers (~16 cm x 11 cm x 4 cm) (aluminium or plastic). For half the containers (those to be used in the damp treatment) place drainage holes in the bottom of containers. Where possible, place take-away containers within larger outer containers to help maintain damp conditions. This will give a maximum number of 360 samples at each location (e.g. Lower Murray, Mid Murray, Macquarie Marshes, Narran Lakes) reflecting 12 strata (3 vegetation categories x 4 flow return frequencies) x 5 sites nested within strata x 3 composite replicates x 2 treatments. Sample numbers will be less if not all strata are represented at locations (e.g. there is no Inland Shrubland at Mid Murray). Label each container with a unique identifier (e.g. LM_IS_C2_4_D1; Lower Murray, Inland Shrubland, Flow Category 2, Site 4, Damp treatment 1); laminated labels attached to containers are preferable.

Figure 3-16: Examples of possible containers for soil samples

Treatments – damp and submerged

Samples will be subject to one of two watering treatments; a damp treatment in which soil is kept moist for the duration of the experiment and a submerged treatment in which containers are placed within individual plastic boxes and flooded to a depth of ~5cm above the height of the soil (Figure 3-17).

Figure 3-17: Example of submerged containers used in a previous experiment (22 cm square x 12 cm deep)

For the damp treatment keep samples damp by watering daily (an automatic system of overhead sprinklers or polypipe is preferable). For the submerged treatment check water levels weekly and top-up if required.

Randomise the placement of samples in the shadehouse / glasshouse and re-randomise every three weeks.

Controls

In addition, use controls to detect the presence of seeds that might have dispersed by wind into experimental samples. Set-up and monitor six containers of sand: three within the damp watering treatment and three within the submerged watering treatment.

Timing

Run the germination experiments for 6 months. It is recommended to begin the experiments in late August and run until February.

Data recording

Observe weekly to ensure plants can be harvested upon flowering and prior to any further contribution of seeds to the sediment. When removing plants prior to seed set record species ID and abundance (count the number of individual plants of each species) (see data sheet in Appendix 8 and example of data entry set-up Figure 3-18). If species cannot be identified prior to seed set, remove and grow on in a separate pot. At the completion of the experiment undertake a final harvest and count of all species. Periodically take photos of species and sample containers.

Date	Label	Location	Veg	Flow	Site	Treat.rep	Spp A	Spp B	Spp Z
4.09.17	MM_IW_C1_3_D3	MM	IW	C1	3	D3	5	3	7

MM = Mid Murray, IW = Inland Woodland, C1 = Category 1 (near annual), D = Damp

Figure 3-18: Example of data entry set-up for soil seedbank experiments

Where possible log the daily minimum and maximum air temperatures that experimental samples are subject to inside the shadehouse / greenhouse. If this is not possible, local climatic data from BOM will be used as a surrogate.

5. Photo point images

Photo points help to document changes in vegetation community and condition over time. Consistency in photographs between surveys is essential to provide a valuable observational record of trends over time.

Using the **site summary** data sheet (front page) (Appendix 9), complete the photo point section of the survey:

1. Record photo number, direction and photographer details.
2. All photos should be taken with a high resolution (>5 megapixels) digital camera with image quality set to 'HQ', focus set to 'auto', zoom set to 'off/zero', and flash set to 'off'. If necessary, the camera should be shaded to prevent glare on the lens.
    a. Bluetooth enabled GPS and blue tooth enabled cameras, which imbed GPS coordinates into the photographs and synchronise with GIS software *are recommended*.
    b. Where possible photos are taken immediately following visual assessments.
3. From the northern most corner of the quadrat turn to **face south** and take a photo of the vegetation in the quadrat. Image should be representative of vegetation condition within the quadrat. Care should be taken to prevent direct sunlight creating glare on the lens.

a. For subsequent surveys, a compilation of reference images (**photo point reference book;** Appendix 14) with corresponding identification number, bearing and site location details is required. Photo point sites are located using a hand held GPS unit. The photographer orientates using a compass and frame alignment is achieved by referring to the original image (e.g. the camera should be positioned at the same point, pointing along the same bearing and at the same height and zoom level on each occasion).

4. Appropriate metadata must be recorded with all photographs. Essential metadata for photographs are: date and time, direction bearing in degrees (from compass), GPS coordinates, and name of site and name of photographer. Additional metadata should be recorded as required to document special or unusual conditions.

6. Hemispherical photos

The PAI is estimated from digital hemispherical photographs taken using a digital camera and fisheye lens or adaptor. For this project, photographs can be taken at any time of the day, but please take photographs as soon as possible following site-set up.

*Note: if photos have excessive sun flare, repeat process before leaving site to see if a better photo is available.

Using the **site summary** data sheet (front page) (Appendix 9), complete the hemispherical photo section of the survey;

1. Record site and photographer details.
2. Hemispherical photographs are taken using a digital camera and fisheye lens.
3. Locate the established assessment site using the location information provided.
4. Locate the marked hemispherical photo position in the centre of the quadrat (marked with a peg).
5. Adjust the camera settings (some trial and error may be required here. It is recommended that more than one photo per site is taken with a variety of settings to ensure the best quality images are recorded. A list of suggested settings per location is attached in Appendix 11)
6. Set up and level the tripod and camera at 1.3 m height.
7. Photographs must be taken with the lens pointing at 90° to the horizontal plane.
8. Capture the image/s and record the required information including the filename/number on the hemispherical photo section of the **photo** data sheet.

7. Site summary

Hydrological information

Using the **site summary** data sheet (front page) (Appendix 9), complete the hydrology and soil moisture assessment;

Record soil moisture in one of four categories

- Submerged (surface water to >1cm)
- Waterlogged (pooling of water when walking through)
- Damp (soil moist but not waterlogged)
- Dry

Provide descriptive responses to the following:

1. What is the water quality like (turbid? black? clear? algae?)
2. If water has recently receded, what height did the water get too (did the water inundate the whole quadrat? Estimate maximum water depth from height on trees/shrubs if present)

Recruitment of tree species

Using the ***site summary*** *data sheet (front* page) (Appendix 9), assess recruitment by counting the number of seedlings for each tree species present within the quadrat using the following height categories;

- <20 cm
- 20 – 50 cm
- 50 – 130 cm
- 1.3 – 3 m

Count individual seedlings where practical, however if there are more than fifty seedlings in any class, estimate the number of seedlings in that category as accurately as possible.

Site characteristics

Using the **site summary** data sheet (front page) (Appendix 9), complete the site characteristics assessment;

1. What is the topography like within the quadrat? (e.g. what is the aspect? Is there a depression?)
2. What are the dominant overstorey species?
    a. What is the general health of the overstorey? (e.g. dead, poor, moderate, good etc)
    b. Is there evidence of flowering / fruiting?
3. Is there any evidence of disturbance? (e.g. grazing, timber collection, camping, insect damage, pugging by cattle?)
4. Are there any other comments relevant to the site (either inside or outside the quadrat) that might be helpful in supporting/explaining data analysis in the future?

Significant features

On the **back page** of the site summary data sheets (Appendix 10) please draw a rough mud map of the quadrat representing significant features (e.g. trees or Lignum clumps missed in point-intercept transects).

8. Mature Tree Density

In the 2018 survey season, additional information was gathered on mature tree density.

For plots with mature trees (> 10cm DBH), record the species and diameter at breast height (DBH) at 1.3m of all individual trees. If there are large numbers of mature trees in a plot (e.g. >25) the number of trees within DBH ranges can be estimated. DBH ranges are i) 10-20 cm; ii) 20-30 cm; iii 30-50cm; iv) 50-80 cm; v) >80 cm (see Appendix 12 and 13).

3.4 Data management / analysis

It is expected that the agency conducting the assessment will collect and store data according to best practice. Once entered, data needs to be sent to the MDFRC for collation and analysis across all locations. Templates for data entry were circulated.

List of equipment for data management /analysis:

- Excel

- Word
- Access to www.anbg.gov.au/apni (publically available)

Data management points for specific components include:

Species richness

Prior to submission, species names should be corrected to the Australian Plant Name Index (APNI) https://www.anbg.gov.au/apni/ for consistency across the Murray–Darling Basin.

Photo point images

Label all photographs appropriately (e.g. site name, direction of photograph (bearing), date photo taken, name of photographer, organisation and photo number).

Create a landscape **photo point reference booklet** in Microsoft Word, with one photo per site/direction (refer to the example in Appendix 14). Where numerous photos were taken, select the best quality image (e.g. in focus and lighting not to dark, etc). Some guidelines for the photo point reference booklet are provided below:

- Create a table 3 columns x 4 rows (per page).
- Paste the images into the table as shown in Appendix 14 (e.g. one image per table cell).
- Ensure the aspect ratio of each image is locked to avoid stretching, and reduce image height size to 6.3 cm (width will adjust automatically).
- For the purposes of the photo point reference booklet, compress all images to 220 ppi (store non compressed copies of all images, labelled appropriately, so that they can be used for other purposes in the future).
- In the table row below each image, include all relevant site information; location, vegetation category, flood return frequency, site number, name and organisation of photographer, date and direction photo taken (refer Appendix 14).

In subsequent surveys, select the best quality image that also lines up with photos taken in previous surveys. Images can be cropped if necessary to replicate the previous survey. Update the photo point reference booklet by including the new image next to the previous survey image (refer Appendix 14) and following the same instructions described above.

Hemispherical photos

Label all photographs appropriately and send digital copies to MDFRC Mildura by USB or Dropbox following field surveys.

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Appendices

Appendix 1: Vegetation descriptions from GIS layers for each region and newly assigned vegetation categories (Tables A to G).

Table A: Vegetation description for Chowilla region from GIS layer (Vegetation mapping data and wetland data for Chowilla floodplain) and newly assigned vegetation categories.

BROAD_VEGD	GENFORMDES	Func_group	New category
Acacia woodland	woodland	River Coobah woodland	Inland woodland
chenopod shrubland	shrubland <1m	Terrestrial dry shrublands	N/A
chenopod shrubland	shrubland >1m	Terrestrial dry shrublands	N/A
Eucalyptus forest and woodland	forest	River Red Gum woodland	Inland woodland
Eucalyptus forest and woodland	woodland	Black Box woodland	Inland woodland
Eucalyptus forest and woodland	woodland	Black Box woodland	Inland woodland
Eucalyptus forest and woodland	woodland	Black Box woodland	Inland woodland
Eucalyptus forest and woodland	woodland	River Red Gum woodland	Inland woodland
Eucalyptus mallee forest and mallee woodland	mallee woodland	Mallee shrubland	N/A
fernland/herbland	forbland	Terrestrial dry shrublands	N/A
grassland	grassland	Emergent sedgeland	Non woody wetland
grassland	grassland	Flood dependent grasslands	Non woody wetland
hummock grassland	grassland	Flood dependent grasslands	Non woody wetland
Melaleuca forest and woodland	forest	Tea Tree woodland	N/A
rushland/sedgeland	sedgeland	Emergent sedgeland	Non woody wetland
samphire shrubland	shrubland <1m	Samphire shrublands	N/A
shrubland <1m	shrubland <1m	Samphire shrublands	N/A
shrubland >1m	shrubland >1m	Lignum shrubland	Inland shrubland
shrubland >1m	shrubland >1m	Terrestrial dry shrublands	N/A
		Wetlands	Non woody wetland

Table B: Vegetation description for Lindsay-Mulcra-Wallpolla region from GIS layer (Modelled 2005 Ecological Vegetation Class (with bioregional conservation status) and newly assigned vegetation categories.

EVC	X_EVCNAME	XGROUPNAME	WRC1	New category
103	Riverine Chenopod Woodland	Riverine Grassy Woodlands or Forests	Black box woodland	Inland Woodland
104	Lignum Swamp	Wetlands	Lignum shrubland	Inland Shrubland
106	Grassy Riverine Forest	Riverine Grassy Woodlands or Forests	Red gum forest	Inland Woodland
107	Lake Bed Herbland	Wetlands	Temporary wetlands	Non woody wetland
200	Shallow Freshwater Marsh	Wetlands	Temporary wetlands	Non woody wetland
295	Riverine Grassy Woodland	Riverine Grassy Woodlands or Forests	Red gum woodland	Inland Woodland
807	Disused Floodway Shrubby Herbland	Wetlands	Alluvial plains	Non woody wetland
808	Lignum Shrubland	Riverine Grassy Woodlands or Forests	Lignum shrubland	Inland Shrubland
809	Floodplain Grassy Wetland	Wetlands	Semipermanent wetlands	Non woody wetland
810	Floodway Pond Herbland	Wetlands	Temporary wetlands	Non woody wetland
811	Grassy Riverine Forest/Floodway Pond Herbland Complex	Riverine Grassy Woodlands or Forests	Red gum forest	Inland Woodland
813	Intermittent Swampy Woodland	Riverine Grassy Woodlands or Forests	Red gum woodland	Inland Woodland
818	Shrubby Riverine Woodland	Riparian Scrubs or Swampy Scrubs and Woodlands	Red gum woodland	Inland Woodland
819	Spike-sedge Wetland	Wetlands	Temporary wetlands	Non woody wetland
820	Sub-saline Depression Shrubland	Salt-tolerant and/or succulent Shrublands	Alluvial plains	Inland Shrubland
823	Lignum Swampy Woodland	Riverine Grassy Woodlands or Forests	Black box woodland	Inland woodland

Table C: Vegetation description for South-West New South Wales region from GIS layer (Murray Darling Basin M305 Structural Vegetation Layer. VIS_ID 917) and newly assigned vegetation categories.

Description	New Category
Barren	N/A
C.cristata - v.sparse	N/A
Chenopod shrubland	N/A
Chenopods;Grasses - v.sparse	N/A
Crops & Annual Pastures	N/A
Crops ; Annual Pastures	N/A
Crops and Annual Pastures	N/A
E.camaldulensis - sparse	Inland woodland
E.camaldulensis - v.sparse	Inland woodland
E.camaldulensis -sparse	Inland woodland
E.camaldulensis;E.largiflorens - sparse	Inland woodland
E.camaldulensis;E.largiflorens - v.sparse	Inland woodland
E.camaldulensis;E.largiflorens -isolated	Inland woodland
E.camaldulensis;E.largiflorens -sparse	Inland woodland
E.camaldulensis;E.largiflorens -very sparse	Inland woodland
E.largiflorens - isolated	Inland woodland
E.largiflorens - isolated; on Lignum	Inland woodland
E.largiflorens - very sparse	Inland woodland
E.largiflorens - very sparse; on lignum	Inland woodland
E.largiflorens -v.sparse	Inland woodland
Mosaic L & 9	N/A
Muehlenbeckia	Inland shrubland
Other Plantation	N/A
Permanent grass - v.sparse	N/A
Settlement	N/A
Water*	Non woody wetland
Wetland Herbs	Non woody wetland

*Excludes Lake Victoria

Table D: Vegetation description for Millewa region from GIS layer (Native vegetation map: Cohuna, Conargo, Echuca, Mathoura, Moulamein, Tuppal and Wanganella 1:100000 map sheets) and newly assigned vegetation categories.

Vegetation	New Category
Areas with greater than 5% native woody vegetation in cropping or urban environments	N/A
Areas with less than 5% native woody vegetation including: cropping, regrowth grassland which may have been previously cleared and/or cropped, baregr*	N/A
Grassland and/or Forbland	N/A
Grassland and/or Forbland with Isolated Trees	N/A
Mid-high Open Forest to Open Woodland	Inland woodland
Mid-high Shrubland to Sparse Shrubland	N/A
Planted natives	N/A
Tall Open Forest to Open Woodland	Inland woodland
Tall Open Forest to Sedgeland with Isolated Trees	Inland woodland
Tall Open Forest to Woodland	Inland woodland
Tall Open Shrubland and/or Open Chenopod Shrubland to Sparse Shrubland and/or Sparse Chenopod Shrubland	N/A
Tall Woodland to Open Woodland	Inland woodland
Very Tall Rushland	Non woody wetland

Table E: Vegetation description for Barmah region from GIS layer (Modelled 2005 Ecological Vegetation Class (with bioregional conservation status)) and newly assigned vegetation categories.

EVC	X_EVCNAME	X_GROUPNAM	New category
56	Floodplain Riparian Woodland	Riverine Grassy Woodlands or Forests	Inland Woodland
106	Grassy Riverine Forest	Riverine Grassy Woodlands or Forests	Inland Woodland
168	Drainage-line Aggregate	Riverine Grassy Woodlands or Forests	Inland Woodland
295	Riverine Grassy Woodland	Riverine Grassy Woodlands or Forests	Inland Woodland
334	Billabong Wetland Aggregate	Wetlands	Non woody wetland
653	Aquatic Herbland	Wetlands	Non woody wetland
803	Plains Woodland	Plains Woodlands or Forests	Inland Woodland
804	Rushy Riverine Swamp	Wetlands	Non woody wetland
809	Floodplain Grassy Wetland	Wetlands	Non woody wetland
810	Floodway Pond Herbland	Wetlands	Non woody wetland
812	Grassy Riverine Forest/Riverine Swamp Forest Complex	Riverine Grassy Woodlands or Forests	Inland Woodland
814	Riverine Swamp Forest	Riverine Grassy Woodlands or Forests	Inland Woodland
815	Riverine Swampy Woodland	Riverine Grassy Woodlands or Forests	Inland Woodland
816	Sedgy Riverine Forest	Riverine Grassy Woodlands or Forests	Inland Woodland
817	Sedgy Riverine Forest/Riverine Swamp Forest Complex	Riverine Grassy Woodlands or Forests	Inland Woodland
819	Spike-sedge Wetland	Wetlands	Non woody wetland
821	Tall Marsh	Wetlands	Non woody wetland
872	Riverine Grassy Woodland/Plains Woodland/Riverine Chenopod Woodland Complex	Riverine Grassy Woodlands or Forests	Inland Woodland
945	Floodway Pond Herbland/Riverine Swamp Forest Complex	Riverine Grassy Woodlands or Forests	Inland Woodland
975	Riverine Ephemeral Wetland	Wetlands	Non woody wetland
1015	Grassy Riverine Forest/Drainage-line Aggregate Mosaic	Riverine Grassy Woodlands or Forests	Inland Woodland

EVC	X_EVCNAME	X_GROUPNAM	New category
1016	Grassy Riverine Forest/Plains Grassy Woodland/Grassy Woodland Mosaic	Riverine Grassy Woodlands or Forests	Inland Woodland
1017	Grassy Riverine Forest/Riverine Grassy Woodland Mosaic	Riverine Grassy Woodlands or Forests	Inland Woodland
1019	Mosaic of Grassy Riverine Forest/Sedgy Riverine Forest-Riverine Swamp Forest Complex	Riverine Grassy Woodlands or Forests	Inland Woodland
1020	Mosaic of Grassy Riverine Forest/Floodway Pond Herbland-Riverine Swamp Forest Complex	Riverine Grassy Woodlands or Forests	Inland Woodland
1021	Mosaic of Drainage-line Aggregate/Grassy Riverine Forest-Riverine Swamp Forest Complex	Riverine Grassy Woodlands or Forests	Inland Woodland
1022	Drainage-line Aggregate/Riverine Swamp Forest Mosaic	Riverine Grassy Woodlands or Forests	Inland Woodland
1023	Drainage-line Aggregate/Sedgy Riverine Forest Mosaic	Riverine Grassy Woodlands or Forests	Inland Woodland
1024	Mosaic of Drainage-line Aggregate/Sedgy Riverine Forest-Riverine Swamp Forest Complex	Riverine Grassy Woodlands or Forests	Inland Woodland
1025	Drainage-line Aggregate/Tall Marsh Mosaic	Riverine Grassy Woodlands or Forests	Inland Woodland
1027	Riverine Grassy Woodland/Grassy Riverine Forest-Riverine Swamp Forest Complex	Riverine Grassy Woodlands or Forests	Inland Woodland
1028	Riverine Grassy Woodland/Riverine Swamp Forest Mosaic	Riverine Grassy Woodlands or Forests	Inland Woodland
1029	Grassy Riverine Forest/Floodway Pond Herbland Mosaic	Riverine Grassy Woodlands or Forests	Inland Woodland
1030	Grassy Riverine Forest/Riverine Swamp Forest Mosaic	Riverine Grassy Woodlands or Forests	Inland Woodland
1032	Floodplain Riparian Woodland/Riverine Grassy Woodland Mosaic	Riverine Grassy Woodlands or Forests	Inland Woodland
1033	Floodplain Riparian Woodland/Floodway Pond Herbland Mosaic	Riverine Grassy Woodlands or Forests	Inland Woodland
1034	Floodplain Riparian Woodland/Riverine Swamp Forest Mosaic	Riverine Grassy Woodlands or Forests	Inland Woodland
1035	Floodplain Riparian Woodland/Sedgy Riverine Forest Mosaic	Riverine Grassy Woodlands or Forests	Inland Woodland
1036	Mosaic of Floodplain Riparian Woodland/Sedgy Riverine Forest-Riverine Swamp Forest Complex	Riverine Grassy Woodlands or Forests	Inland Woodland
1037	Floodplain Riparian Woodland/Tall Marsh Mosaic	Riverine Grassy Woodlands or Forests	Inland Woodland
1038	Low Rises Woodland/Riverine Swampy Woodland Mosaic	Lower Slopes or Hills Woodlands	Inland Woodland
1039	Mosaic of Drainage-line Aggregate/Floodway Pond Herbland-Riverine Swamp Forest Complex	Riverine Grassy Woodlands or Forests	Inland Woodland
1040	Riverine Grassy Woodland/Riverine Swampy Woodland Mosaic	Riverine Grassy Woodlands or Forests	Inland Woodland
1041	Riverine Grassy Woodland/Sedgy Riverine Forest Mosaic	Riverine Grassy Woodlands or Forests	Inland Woodland

EVC	X_EVCNAME	X_GROUPNAM	New category
1042	Mosaic of Riverine Grassy Woodland/Floodway Pond Herbland-Riverine Swamp Forest Complex	Riverine Grassy Woodlands or Forests	Inland Woodland
1043	Aquatic Herbland/Floodplain Grassy Wetland Mosaic	Wetlands	Non woody wetland
1044	Aquatic Herbland/Floodway Pond Herbland Mosaic	Wetlands	Non woody wetland
1045	Aquatic Herbland/Riverine Swamp Forest Mosaic	Wetlands	Inland Woodland
1046	Mosaic of Aquatic Herbland/Sedgy Riverine Forest-Riverine Swamp Forest Complex	Wetlands	Inland Woodland
1047	Aquatic Herbland/Tall Marsh Mosaic	Wetlands	Non woody wetland
1048	Mosaic of Aquatic Herbland/Floodway Pond Herbland-Riverine Swamp Forest Complex	Wetlands	Inland Woodland
1049	Floodplain Grassy Wetland/Floodway Pond Herbland Mosaic	Wetlands	Non woody wetland
1050	Mosaic of Floodplain Grassy Wetland/Grassy Riverine Forest-Riverine Swamp Forest Complex	Wetlands	Inland Woodland
1051	Floodplain Grassy Wetland/Riverine Swamp Forest Mosaic	Wetlands	Inland Woodland
1052	Floodplain Grassy Wetland/Riverine Swampy Woodland Mosaic	Wetlands	Inland Woodland
1053	Mosaic of Floodplain Grassy Wetland/Sedgy Riverine Forest-Riverine Swamp Forest Complex	Wetlands	Inland Woodland
1054	Floodplain Grassy Wetland/Spike-sedge Wetland Mosaic	Wetlands	Non woody wetland
1055	Floodplain Grassy Wetland/Tall Marsh Mosaic	Wetlands	Non woody wetland
1056	Mosaic of Floodplain Grassy Wetland/Floodway Pond Herbland-Riverine Swamp Forest Complex	Wetlands	Inland Woodland
1057	Mosaic of Floodway Pond Herbland/Grassy Riverine Forest-Riverine Swamp Forest Complex	Wetlands	Inland Woodland
1058	Floodway Pond Herbland/Riverine Swamp Forest Mosaic	Wetlands	Inland Woodland
1059	Mosaic of Floodway Pond Herbland/Sedgy Riverine Forest-Riverine Swamp Forest Complex	Wetlands	Inland Woodland
1060	Floodway Pond Herbland/Tall Marsh Mosaic	Wetlands	Non woody wetland
1061	Mosaic of Grassy Riverine Forest-Riverine Swamp Forest Complex/Riverine Swamp Forest	Riverine Grassy Woodlands or Forests	Inland Woodland
1062	Grassy Riverine Forest/Riverine Swampy Woodland Mosaic	Riverine Grassy Woodlands or Forests	Inland Woodland
1063	Grassy Riverine Forest/Sedgy Riverine Forest Mosaic	Riverine Grassy Woodlands or Forests	Inland Woodland
1065	Grassy Riverine Forest/Tall Marsh Mosaic	Riverine Grassy Woodlands or Forests	Inland Woodland

EVC	X_EVCNAME	X_GROUPNAM	New category
1067	Riverine Swamp Forest/Riverine Swampy Woodland Mosaic	Riverine Grassy Woodlands or Forests	Inland Woodland
1068	Riverine Swamp Forest/Sedgy Riverine Forest Mosaic	Riverine Grassy Woodlands or Forests	Inland Woodland
1069	Riverine Swamp Forest/Sedgy Riverine Forest-Riverine Swamp Forest Complex	Riverine Grassy Woodlands or Forests	Inland Woodland
1070	Riverine Swamp Forest/Spike-sedge Wetland Mosaic	Riverine Grassy Woodlands or Forests	Inland Woodland
1071	Riverine Swamp Forest/Tall Marsh Mosaic	Riverine Grassy Woodlands or Forests	Inland Woodland
1072	Mosaic of Riverine Swamp Forest/Floodway Pond Herbland-Riverine Swamp Forest Complex	Riverine Grassy Woodlands or Forests	Inland Woodland
1073	Riverine Swampy Woodland/Sedgy Riverine Forest Mosaic	Riverine Grassy Woodlands or Forests	Inland Woodland
1074	Mosaic of Riverine Swampy Woodland/Sedgy Riverine Forest-Riverine Swamp Forest Complex	Riverine Grassy Woodlands or Forests	Inland Woodland
1075	Mosaic of Sedgy Riverine Forest/Sedgy Riverine Forest-Riverine Swamp Forest Complex	Riverine Grassy Woodlands or Forests	Inland Woodland
1076	Sedgy Riverine Forest/Spike-sedge Wetland Mosaic	Riverine Grassy Woodlands or Forests	Inland Woodland
1077	Sedgy Riverine Forest/Tall Marsh Mosaic	Riverine Grassy Woodlands or Forests	Inland Woodland
1078	Mosaic of Sedgy Riverine Forest/Floodway Pond Herbland-Riverine Swamp Forest Complex	Riverine Grassy Woodlands or Forests	Inland Woodland
1079	Mosaic of Sedgy Riverine Forest-Riverine Swamp Forest Complex/Tall Marsh	Riverine Grassy Woodlands or Forests	Inland Woodland
1080	Mosaic of Sedgy Riverine Forest-Riverine Swamp Forest Complex/Floodway Pond Herbland-Riverine Swamp Forest Complex	Riverine Grassy Woodlands or Forests	Inland Woodland
1081	Spike-sedge Wetland/Tall Marsh Mosaic	Wetlands	Non woody wetland
1082	Tall Marsh/Riverine Swamp Forest Mosaic	Wetlands	Inland Woodland
1083	Mosaic of Tall Marsh/Floodway Pond Herbland-Riverine Swamp Forest Complex	Wetlands	Inland Woodland
1084	Tall Marsh/Non-Vegetation Mosaic	Wetlands	Non woody wetland

Table F: Vegetation descriptions for Macquarie Marshes from the 2013 Plant Community Type (PCT) vegetation map (Bowen and Fontaine 2014*) and newly assigned vegetation categories.

PCT Number	NSW OEH Plant Community Type (PCT) Name	OEH Vegetation Class (Keith 2004)	EWKR Veg Class
181	Common Reed-Bush groundsel aquatic tall reedland grassland wetland	Inland Floodplain Swamps;	Non-woody wetland
182	Cumbungi rushland wetland of shallow semi-permanent water bodies and inland watercourses	Inland Floodplain Swamps;	Non-woody wetland
204	Water Couch marsh grassland wetland	Inland Floodplain Swamps;	Non-woody wetland
238	Permanent and semi-permanent freshwater lagoons	Inland Floodplain Swamps;	Non-woody wetland
53	Shallow freshwater wetland sedgeland	Inland Floodplain Swamps;	Non-woody wetland
36	River Red Gum tall to very tall open forest (wetland)	Inland Riverine Forests;	Inland Woodlands
36 - woodland	River Red Gum tall woodland (wetland)	Inland Riverine Forests;	Inland Woodlands
Baradine red gum	Baradine red gum	Inland Riverine Forests	Inland Woodlands
241	River Coobah - lignum swamp wetland	Inland Floodplain Shrublands;	Inland Shrublands
247	Lignum shrubland wetland	Inland Floodplain Shrublands;	Inland Shrublands
37	Black Box woodland wetland	North-west Floodplain Woodlands;	Inland Woodlands
40	Coolibah open woodland wetland	North-west Floodplain Woodlands;	Inland Woodlands
454	River Red Gum grassy chenopod open tall woodland (wetland)	Inland Floodplain Woodlands;	Inland Woodlands
144	Leopardwood low woodland	North-west Plain Shrublands;	N/A
144 - Lime bush	Lime bush (Citrus glauca) thickets	North-west Plain Shrublands;	N/A
145	Western Rosewood - Wilga - Belah low woodland	Western Peneplain Woodlands;	N/A
158	Old man Saltbush-mixed chenopod shrubland	Riverine Chenopod Shrublands;	N/A
206	Dirty Gum-White Cypress Pine tall woodland	North-west Alluvial Sand Woodlands;	N/A
212	Chenopod low open shrubland	Riverine Chenopod Shrublands;	N/A
250	Derived tussock grassland	Western Slopes Grasslands;	N/A
27	Weeping Myall open woodland	Riverine Plain Woodlands;	N/A
332	Tumbledown Red Gum - Black Cypress Pine - Red Stringybark woodland	Inland Rocky Hill Woodlands;	N/A
55	Belah Woodland	North-west Floodplain Woodlands;	N/A
55 - Budda	Budda thicket	North-west Floodplain Woodlands;	N/A
70	White Cypress Pine woodland	Floodplain Transition Woodlands;	N/A

98	Poplar Box - White Cypress Pine - Wilga woodland	Western Peneplain Woodlands;	N/A
Derived chenopod shrubland	Derived chenopod shrubland	Riverine Chenopod Shrublands;	N/A
Cultivated	Cultivated land	Cleared	N/A
Infrastructure	Infrastructure	Cleared	N/A
Cleared	Cleared	Cleared	N/A
watercourse	Watercourse	NA	N/A

*Bowen, S. and Fontaine, K., 2014. 2013 Vegetation Map of the Macquarie Marshes and Floodplain. NSW Office of Environment and Heritage, Sydney.

Table G: Vegetation descriptions for the Narran Lakes from the 2014 Plant Community Type (PCT) vegetation map (Eco Logical Australia 2015*) and newly assigned vegetation categories.

PCT Number	NSW OEH Plant Community Type (PCT) Name	OEH Vegetation Class (Keith 2004)	EWKR Veg Class
1000	Canegrass swamp tall grassland wetland of drainage depressions, lakes and pans of the inland plains	Inland Floodplain Swamps	Non-woody wetland
181	Common Reed - Bushy Groundsel aquatic tall reedland grassland wetland of inland river systems	Inland Floodplain Swamps	Non-woody wetland
238a	Ephemeral herbaceous vegetation of the channels of major and minor watercourses of western NSW	Inland Floodplain Swamps	Non-woody wetland
43a	Grassland - chenopod low open shrubland on floodplains in the semi-arid (hot) and arid zones	Inland Floodplain Swamps	Non-woody wetland
238	Non-woody water dependent vegetation / Ephemeral Freshwater wetlands	Inland Floodplain Swamps	Non-woody wetland
238	Permanent and semi-permanent freshwater lakes wetland of the inland slopes and plains	Inland Floodplain Swamps	Non-woody wetland
53	Shallow freshwater wetland sedgeland in depressions on floodplains on inland alluvial plains and floodplains	Inland Floodplain Swamps	Non-woody wetland
1005	Sparse saltbush forbland wetland of the irregularly inundated lakes of the arid and semi-arid (persistently hot) climate zones	Inland Floodplain Swamps	Non-woody wetland
62	Samphire saline shrubland/forbland wetland of lake beds and lake margins in the arid and semi-arid (hot) zones	Inland Saline Lakes	Non-woody wetland
247a	Lignum open shrubland wetland on regularly flooded alluvial plains	Inland Floodplain Shrublands	Inland shrublands
247	Lignum shrubland wetland on regularly flooded alluvial depressions in the Brigalow Belt South Bioregion and Darling Riverine Plains Bioregion	Inland Floodplain Shrublands	Inland shrublands
160	Nitre Goosefoot shrubland wetland on clays of the inland floodplains	Inland Floodplain Shrublands	Inland shrublands
241	River Cooba swamp wetland on the floodplains of the Darling Riverine Plains Bioregion and Brigalow Belt South Bioregion	Inland Floodplain Shrublands	Inland shrublands
36	River Red Gum tall to very tall open forest / woodland wetland on rivers on floodplains mainly in the Darling Riverine Plains Bioregion	Inland Riverine Forests	Inland woodland
38	Black Box low woodland wetland lining ephemeral watercourses or fringing lakes and clay pans of semi-arid (hot) and arid zones	North-west Floodplain Woodlands	Inland woodland
37	Black Box woodland wetland on NSW central and northern floodplains including the Darling Riverine Plains Bioregion and Brigalow Belt South Bioregion	North-west Floodplain Woodlands	Inland woodland
1005	Coolibah	North-west Floodplain Woodlands	Inland woodland
39	Coolibah - River Cooba - Lignum woodland wetland of frequently flooded floodplains mainly in the Darling Riverine Plains Bioregion	North-west Floodplain Woodlands	Inland woodland
40	Coolibah open woodland wetland with chenopod/grassy ground cover on grey and brown clay floodplains	North-west Floodplain Woodlands	Inland woodland
29	Brigalow open woodland on clay soils in the Nyngan-Bourke-Enngonia regions of the NSW north-western plains	Brigalow Clay Plains Woodlands	N/A
224	Cotton Bush - copperburr open shrubland of the arid climate zone	Gibber Chenopod Shrublands	N/A

197	Black Box - Gidgee - chenopod low open woodland wetland on alluvial clay soils in the Culgoa River region of the Darling Riverine Plains Bioregion and Mulga Lands Bioregion	Gibber Transition Shrublands	N/A
118	Gidgee chenopod woodland on red-brown clays in the semi-arid (hot) climate zone mainly in the Mulga Lands Bioregion	Gibber Transition Shrublands	N/A
55	Belah woodland on alluvial plains and low rises in the central NSW wheatbelt to Pilliga and Liverpool Plains regions	North-west Floodplain Woodlands	N/A
144	Leopardwood low woodland mainly on clayey soils in the semi-arid zone	North-west Floodplain Woodlands	N/A
207	Poplar Box grassy low woodland of drainage lines and depressions of the semi-arid (hot) and arid zone climate zones	North-west Floodplain Woodlands	N/A
212	Chenopod low open shrubland - ephemeral partly derived forbland saline wetland on occasionally flooded pale clay scalds in the NSW North Western Plains	Riverine Chenopod Shrublands	N/A
377	Copperburr low open shrubland on loam - clay flats and playas, western Brigalow Belt South Bioregion and northern Darling Riverine Plains Bioregion	Riverine Chenopod Shrublands	N/A
168	Derived Copperburr shrubland of the NSW northern inland alluvial floodplains	Riverine Chenopod Shrublands	N/A
163	Dillon Bush (Nitre Bush) shrubland of the semi-arid and arid zones	Riverine Chenopod Shrublands	N/A
158	Old Man Saltbush - mixed chenopod shrubland of the semi-arid hot (persistently dry) and arid climate zones (north-western NSW)	Riverine Chenopod Shrublands	N/A
211	Slender Saltbush - samphire - copperburr low open shrubland wetland on irregularly inundated floodplains mainly in the Darling Riverine Plains Bioregion and Brigalow Belt South Bioregion	Riverine Chenopod Shrublands	N/A
27	Weeping Myall open woodland of the Darling Riverine Plains Bioregion and Brigalow Belt South Bioregion	Riverine Plains Woodlands	N/A
1005	Grassland - chenopod low open shrubland on floodplains in the semi-arid (hot) and arid zones	Semi-arid Floodplain Grasslands	N/A
43	Mitchell Grass grassland - chenopod low open shrubland on floodplains in the semi-arid (hot) and arid zones	Semi-arid Floodplain Grasslands	N/A
146	Whitewood low open woodland of the Brigalow Belt South Bioregion and north-eastern Darling Riverine Plains Bioregion	Subtropical Semi-arid Woodlands	N/A
98	Poplar Box - White Cypress Pine - Wilga - Ironwood shrubby woodland on red sandy-loam soils in the Darling Riverine Plains Bioregion and Brigalow Belt South Bioregion	Western Peneplain Woodlands	N/A

*Eco Logical Australia 2015. Vegetation of the Barwon-Darling and Condamine-Balonne floodplain systems of New South Wales: Mapping and survey of plant community types. Prepared for Murray-Darling Basin Authority

Appendix 2: Point-Intercept data sheet (sample)

EWKR vegetation Point Intercept data sheet

Location: Lower Murray / Mid Murray / Maquarie Marshes / Narran Lakes
Vegetation: Inland woodland / Inland shrubland / Non-woody wetland
FRF: <1.5ys / 1.5 - 3 ys / 3 - 5 ys / 5 - 10 ys
Site (e.g. 1 - 5):
Date: / /
Assessor/s:
Organisation:

Transect #:

Substrate Categories	
BS	bare soil
LC	lichen crust
CWD	coarse woody debris
R	rock
LL	leaf litter (record depth, cm)
W	water (record depth, cm)
S	scats
TH	thatch (record depth, cm)
MMS	man-made structure

Canopy Categories	
ICS	in-canopy sky
sky	sky

Point	Substrate type	Species	Alive/Dead	Height (cm)	Canopy category and/or species	Alive/Dead	Height (m)

Appendix 3: Point-Intercept data sheet (sample-completed)

EWKR vegetation Point Intercept data sheet

Location: Lower Murray / Mid Murray / Maquarie Marshes / Narran Lakes

Vegetation: Inland woodland / Inland shrubland / Non-woody wetland

FRF: <1.5ys / 1.5 - 3 ys / 3 - 5 ys / 5 - 10 ys

Site (e.g. 1 - 5):	3
Date:	5 / 3 / 2017
Assessor/s:	Cherie Campell
Organisation:	MDFRC
Transect #:	

Substrate Categories	
BS	bare soil
LC	lichen crust
CWD	coarse woody debris
R	rock
LL	leaf litter (record depth, cm)
W	water (record depth, cm)
S	scats
TH	thatch (record depth, cm)
MMS	man-made structure

Canopy Categories	
ICS	in-canopy sky
sky	sky

Point	Substrate type	Species	Alive/Dead	Height (cm)	Canopy category and/or species	Alive/Dead	Height (m)
1	*BS*	*Atriplex nummularia*	*alive*	*55*	*Acacia stenophylla*	*dead*	*2.1*
2	*LL (5cm)*				*Eucalyptus largiflorens*	*alive*	*3.5*
3	*BS*				*IC - in canopy sky*		
4	*BS*	*Duma florulenta*	*alive*	*170*	*Eucalyptus largiflorens*	*alive*	*3.5*
4					*Eucalyptus camaldulensis*	*alive*	*8*
5	*BS*	*Duma florulenta*	*alive*	*150*	*sky*		

Appendix 4: Species list data sheet (sample)

EWKR vegetation species list data sheet **Date:** ____ / ____ / ____

Location: Lower Murray / Mid Murray / Maquarie Marshes / Narran Lakes **Assessor/s:** ____

Vegetation: Inland woodland / Inland shrubland / Non-woody wetland **Oranigsation:** ____

FRF: <1.5ys / 1.5 - 3 ys / 3 - 5 ys / 5 - 10 ys

Site (e.g. 1 - 5): ____

dominant species - % cover to nearest 5%
<10 individs (small forbs) = 1% cover

Species	Percent Cover (%)	Comments:

Appendix 5: Species list data sheet (sample - completed)

EWKR vegetation species list data sheet		**Date:**	06/ 03/ 2018
Location:	Lower Murray / Mid Murray / Maquarie Marshes / Narran Lakes	**Assessor/s:**	R. Durant/ L. Romanin
Vegetation:	Inland woodland / Inland shrubland / Non-woody wetland	**Oranigsation:**	MDFRC
FRF:	<1.5ys / 1.5 - 3 ys / 3 - 5 ys / 5 - 10 ys		
Site (e.g. 1 - 5):	IW_C3_2	**dominant species - % cover to nearest 5%** **<10 individs (small forbs) = 1% cover**	

Species	**Percent Cover (%)**	**Comments:**
Eucalyptus camaldulensis (mature)	30	
Eucalyptus camaldulensis (seedling)	15	
Acacia stenophylla (juvenile)	5	*few individual of Sporobolus*
Duma florulenta	10	
Lachnagrostis filiformis	5	
Sporopolus mitchelii	1	*C. cunninghamii has largely senesced*
Centipeda cunninghamii	5	

Appendix 6: Lignum Data sheet (sample)

EWKR Lignum Data sheet

Location: Lower Murray / Maquarie Marshes / Narran Lakes
Vegetation: Inland woodland / Inland shrubland / Non-woody wetland
FRF: <1.5ys / 1.5 - 3 ys / 3 - 5 ys / 5 - 10 ys
Site (e.g. 1 - 5): ____________________

Date: ______ / ______ / ______
Assessor/s: ____________________
Organisation: ____________________

Flowers	
M	Male
F	Female
B	Bud
U	Unknown

Flowers/leaves	
(NA)	None
Scarce	<10 flowers/leaves on plant
Common	10-50 flowers/leaves on plant
Abundant	>50 flowers/leaves on plant

Lignum Condition Index (LCI)

Score	% viable	Colour of viable crown
6	> 95	(NA)
5	75 < x ≤ 95	All green
4	50 < x ≤ 75	Mainly green
3	25 < x ≤ 50	Half green/ half yellow/brown
2	5 < x ≤ 25	Mainly yellow/brown
1	0 < x ≤ 5	All yellow/brown
0	0%	No viable stems

CLUMP #	%VIABLE	COLOUR	LEAVES	FLOWERS	GENDER	CLUMP HEIGHT	CLUMP WIDTH	NOTES
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								
13								
14								
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27								
28								
29								
30								
31								
32								

Appendix 7: Lignum Data sheet (sample - completed)

EWKR Lignum Data sheet

Location: Lower Murray / Maquarie Marshes / Narran Lakes
Vegetation: Inland woodland / Inland shrubland / Non-woody wetland
FRF: <1.5ys / 1.5 - 3 ys / 3 - 5 ys / 5 - 10 ys
Site (e.g. 1 - 5): *5*

Flowers	
M	Male
F	Female
B	Bud
U	Unknown

Date: 4 / *4* / *##*
Assessor/s: *Jason Nicol*
Organisation: *SARDI*

Flowers/leaves	
(NA)	None
Scarce	<10 flowers/leaves on plant
Common	10-50 flowers/leaves on plant
Abundant	>50 flowers/leaves on plant

Lignum Condition Index (LCI)		
Score	% viable	Colour of viable crown
6	> 95	(NA)
5	75 < x ≤ 95	All green
4	50 < x ≤ 75	Mainly green
3	25 < x ≤ 50	Half green/ half yellow/brown
2	5 < x ≤ 25	Mainly yellow/brown
1	0 < x ≤ 5	All yellow/brown
0	0%	No viable stems

CLUMP #	%VIABLE	COLOUR	LEAVES	FLOWERS	GENDER	CLUMP HEIGHT	CLUMP WIDTH	NOTES
1	*4*	*3*	*C*	*S*	*M*	*1.5m*	*1m*	
2	*5*	*4*	*A*	*A*	*F*	*2m*	*3m*	
3	*6*	*5*	*S*	*NA*	*U*	*0.2m*	*0.1m*	*new reshoot*
4								
5								
6								
7								
8								
9								
10								
11								
12								
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14								
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31								
32								

Appendix 8: Germination data sheet (sample)

EWKR germination data sheet Species

Date	Label	*Species Name*	Count
04/09/2018	*LM_IW_C2_2_D3*	*Mimulus repens*	6

LM_IW_C2_2_D3

Lower Murray_InlandWoodland_Category2 Flood return frequency_Site2_Damp replicate 3

Comments:

Appendix 9: Site Summary Data Sheet (sample – front page)

EWKR vegetation site summary data sheet

Location: Lower Murray / Mid Murray / Maquarie Marshes / Narran Lakes
Vegetation: Inland woodland / Inland shrubland / Non-woody wetland
FRF: <1.5ys / 1.5 - 3 ys / 3 - 5 ys / 5 - 10 ys
Site (e.g. 1 - 5): ____________
Date: ____/____/____
Assessor/s: ____________
Organisation: ____________
Time of assessment: ____________

Site Photo numbers, direction, photographer	
Photo points	
Hemi	

HYDROLOGY / SOIL MOISTURE
Is site inundated? **Y / N**
If yes, to what depth? ______
What is the water quality like?
clear / turbid / black / algae
What height did the water level get too?
____
Soil moisture
inundated / waterlogged / damp / dry

RECRUITMENT (number of woody seedlings)				
	Height class			
Tree species	<20cm	20-50cm	50-130cm	1.3-3m

SITE CHARACTERISTICS
What is the aspect/ topography of the site?
What are the dominant overstorey species? What is the general health (for each overstorey spp)? (D = Dead, P= poor, M= moderate, G=good, E=excellent)
Are overstorey (canopy) species flowering? **Y / N** Fruiting? **Y / N**
Average extent of flowering / fruiting (for each overstorey spp): NA , Scarce, Common, Abundant

Species	Flowering	Fruiting

Is their any evidence of disturbance? In particular, grazing?
(other; logging? Vehicle tracks? Cattle pugging? Pig wallowing? Fire?)
Level of disturbance (for each listed disturbance) (L=low, M=moderate, H=high)

Disturbance	Level	Disturbance	Level

Waypoint locations (easting and northing) **Datum:**

4 x quadrat corners:

Photo point: **Hemi photo:**

Appendix 10: Site Summary Data Sheet (sample – back page)

EWKR vegetation site summary data sheet - Mud Map

*for all quadrats please draw a rough map representing significant features (e.g. trees or lignum missed in point-intercept transects)

Quadrat and point-intercept lines

northern most peg

Notes /Comments (relevant to within or outside the quadrat)

(e.g. bird nests, hollows, scats - provide a degree of abundance if relevant - e.g. scarce, common, abundant)

Soil samples

Have 10 random soil samples been collected, aggregated and labelled? **Y / N**

(Need ~ 3L of soil / quadrat)

Appendix 11: Suggested camera settings for hemispherical photos

Camera settings for hemispherical photos

For all sites – if the settings are not working in the field, modify slightly to the conditions. Take care of where the sun is on the lens as excessive sunlight in the canopy or on the trunks will lead to substantial underestimates of Plant Area Index (PAI).

Mid-Murray

- Av settings:
- AV set between 0 and -2
- ISO set either at 800 or 1600
- Have F-stop set between 9.0 and 20
- TV settings:
- AV set between 0 and -2
- ISO set either at 800 or 1600
- Have F-stop between 250 and 500, the further past 500 the darker the photo gets

Lower Murray

- Av settings:
- AV set between 0 and -2
- ISO set either at 800 or 1600
- TV settings:
- AV set between 0 and -2
- ISO set either at 800 or 1600

Appendix 12: Mature Tree Density data sheet (sample – front page - completed)

EWKR Mature Tree (> 3 m height) Density data sheet

Location: Lower Murray / Mid Murray / Maquarie Marshes / Narran Lakes
Vegetation: Inland woodland / Inland shrubland / Non-woody wetland
FRF: <1.5ys / 1.5 - 3 ys / 3 - 5 ys / 5 - 10 ys
Site (e.g. 1 - 5): ***LM_IW_C3_3***
Date: *4 / 3 / 2018*
Assessor/s: *L. Romain/B. Durant*
Organisation: *MDRC*
Time of assessment: *11.50am*

Count	Instructions
0 - 25/plot	measure DBH individ tree
>25/plot	record estimate of trees within DBH range (see over page)

Count	Species	DBH @ 1.30 m
1	***Black Box***	*23.5*
2	***BB***	*17*
3	***RRG***	*43*

Appendix 13: Mature Tree Density data sheet (sample – back page - completed)

EWKR Mature Tree (> 3 m height) Density data sheet

Location: Lower Murray / Mid Murray / Maquarie Marshes / Narran Lakes
Vegetation: Inland woodland / Inland shrubland / Non-woody wetland
FRF: <1.5ys / 1.5 - 3 ys / 3 - 5 ys / 5 - 10 ys
Site (e.g. 1 - 5): *LM_IW_C2_3*
Date: 3 / 3 / 2018
Assessor/s: *L. Romain/B. Durant*
Organisation: *MDRC*
Time of assessment: *10.20am*

Count	Instructions
0 - 25 /plot	measure DBH individ tree (see over page)
>25/plot	record estimate of trees within DBH range

Species	DBH range @ 1.30 m				
	10-20 cm	20-30 cm	30-50 cm	50-80 cm	>80 cm
RRG	*10*	*30*		*5*	
BB		*8*	*20*		

Appendix 14: Photo point reference book (sample only)

Lower Murray, non-woody vegetation, 5–10 yrs, site 1 (C. Johns, MDFRC, February 2009) Direction N310

Lower Murray, non-woody vegetation, 5–10 yrs, site 1 (S. Walters, MDFRC, March 2010) Direction N310

Lower Murray, non-woody vegetation, 5–10 yrs, site 1 (G. Hayward, MDFRC, April 2011) Direction N310

Lower Murray, non-woody vegetation, 5–10 yrs, site 2 (S. Walters, MDFRC, March 2010) Direction W290

Lower Murray, non-woody vegetation, 5–10 yrs, site 2 (G. Hayward, MDFRC, April 2011) Direction W290

Lower Murray, non-woody vegetation, 5–10 yrs, site 2 (F. Freestone, MDFRC, February 2013) Direction W290

From the four corners of the Basin: Assessing vegetation responses to flow regimes

Cherie Campbell, Sam Capon, Rachael Thomas, Susan Gehrig, Jason Nicol, Casandra James, Kay Morris, Daryl Nielsen

EWKR

Environmental Water Knowledge & Research

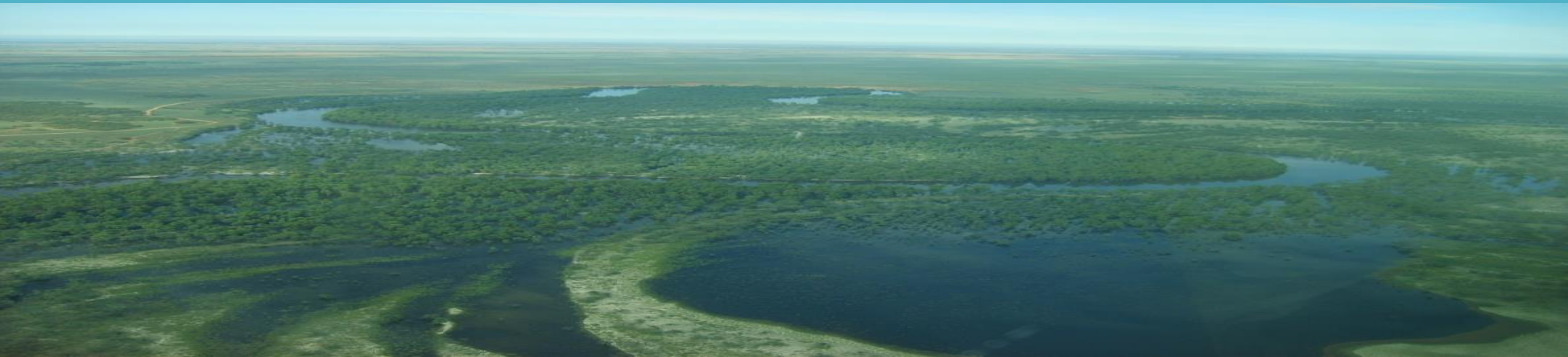
LA TROBE UNIVERSITY

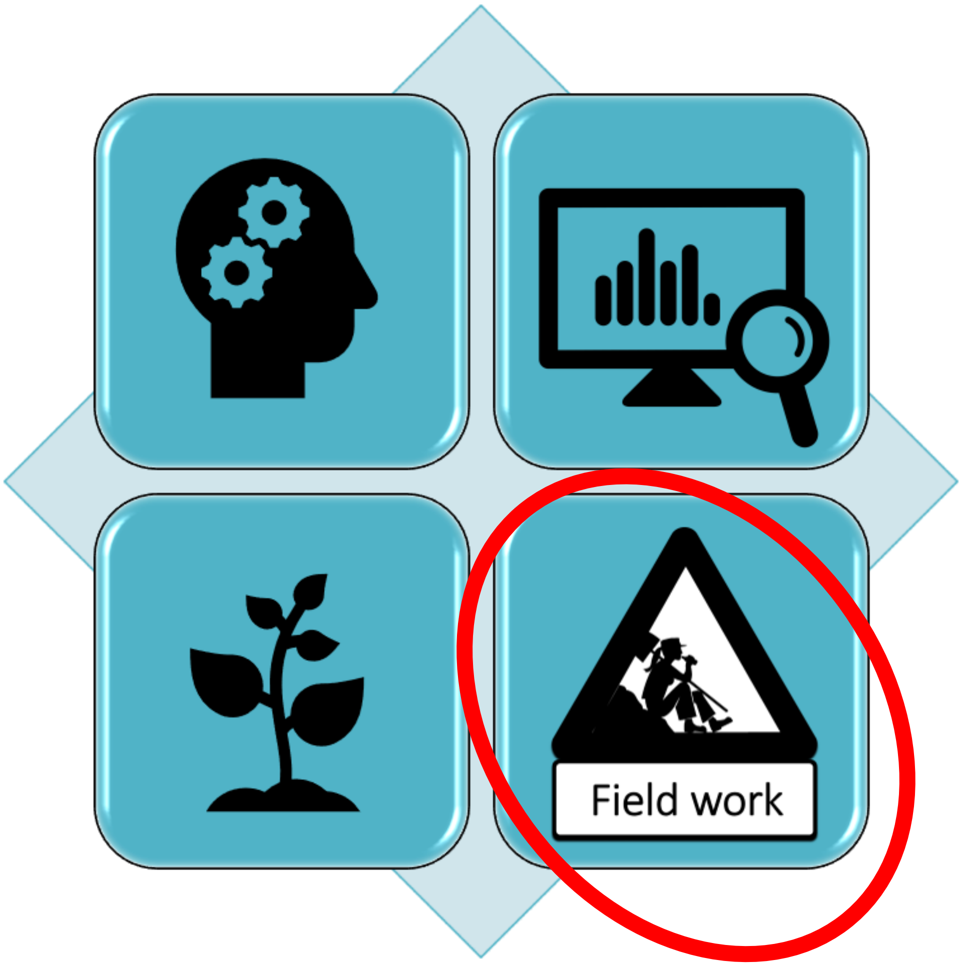
MDB EWKR is a 5 year, $10 million research project funded by the Commonwealth Environmental Water Office

The project is a collaboration between the MDFRC as lead together with 12 other research organisations

Aim to improve science to support environmental water planning and management

Address gaps in environmental watering information on waterbirds, vegetation, fish and food webs





EWKR Vegetation Theme

Research components

EWKR Vegetation Theme

Leadership Group

- MDFRC
- NSW Office of Environment and Heritage / Uni NSW
- Griffith University
- SARDI

Fieldwork and germination trials

Aims

- How do extant understorey communities and seedbank diversity differ between:
  - Vegetation structural class
  - Flooding regime
  - Location

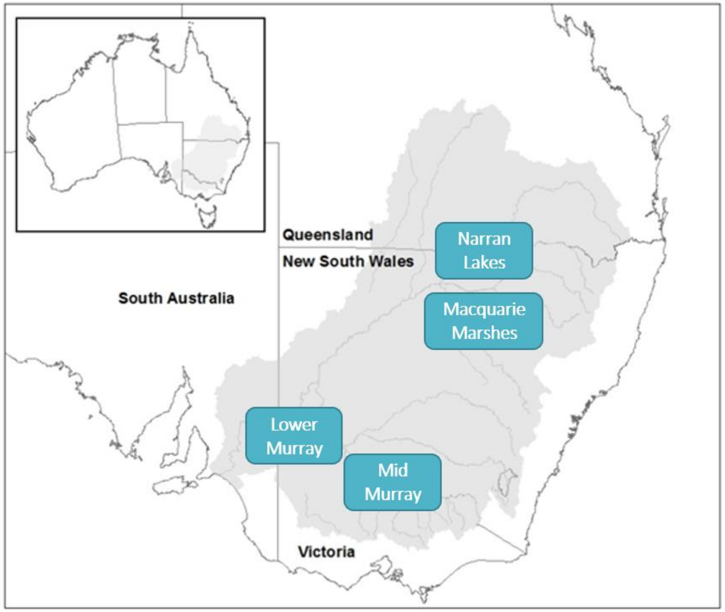


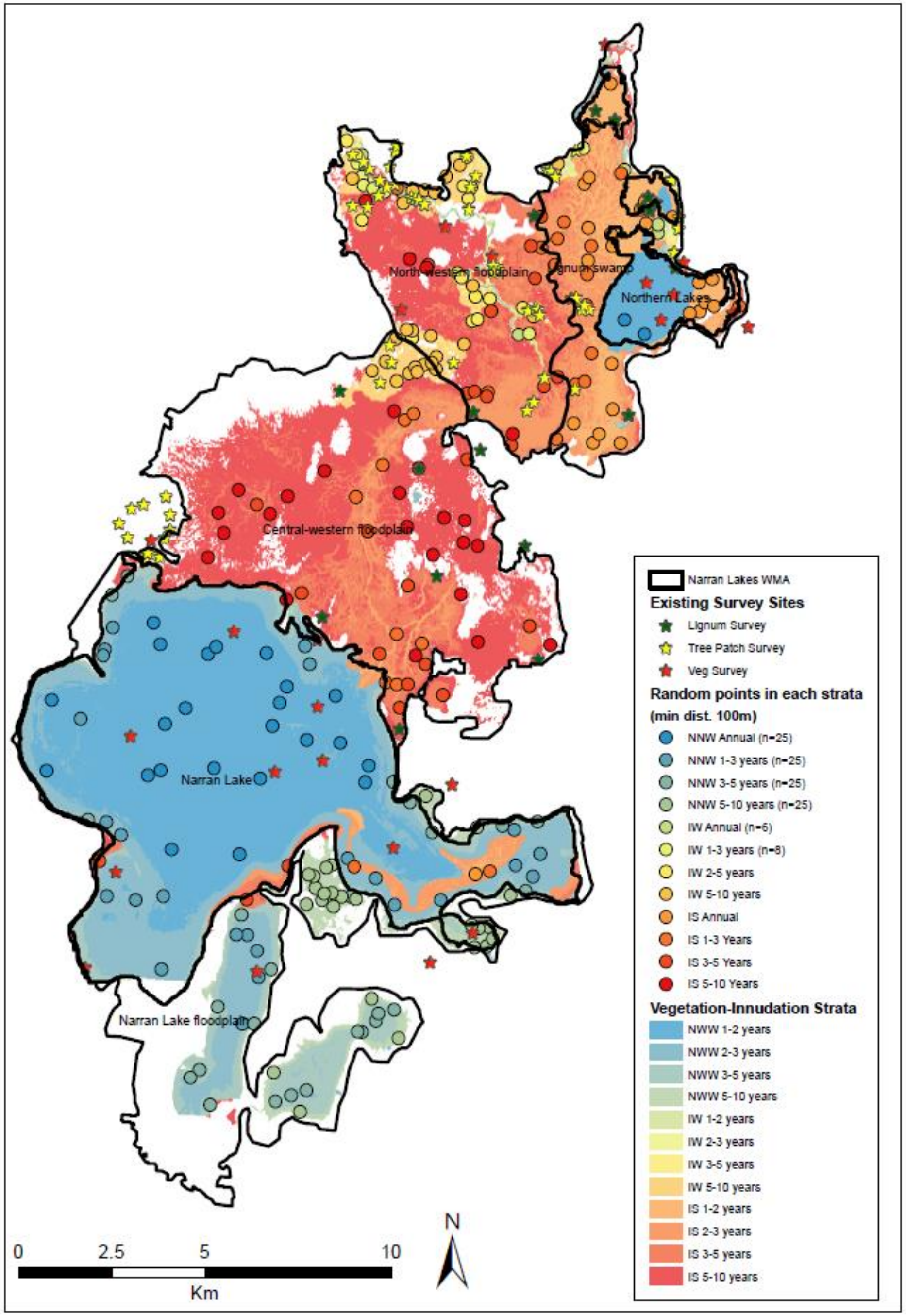
Methods

Design and site selection

Factorial design

- 4 locations
  - Narran Lakes (NL)
  - Macquarie Marshes (MQ)
  - Mid Murray (MM)
  - Lower Murray (LM)
- 4 flood intervals
  - Near annual (Cat 1)
  - 1.5-3 years (Cat 2)
  - 3-5 years (Cat 3)
  - 5-10 years (Cat 4)
- 3 vegetation structural types
  - Non-woody wetlands (NWW)
  - Inland shrublands (IS)
  - Inland woodlands (IW)





Methods

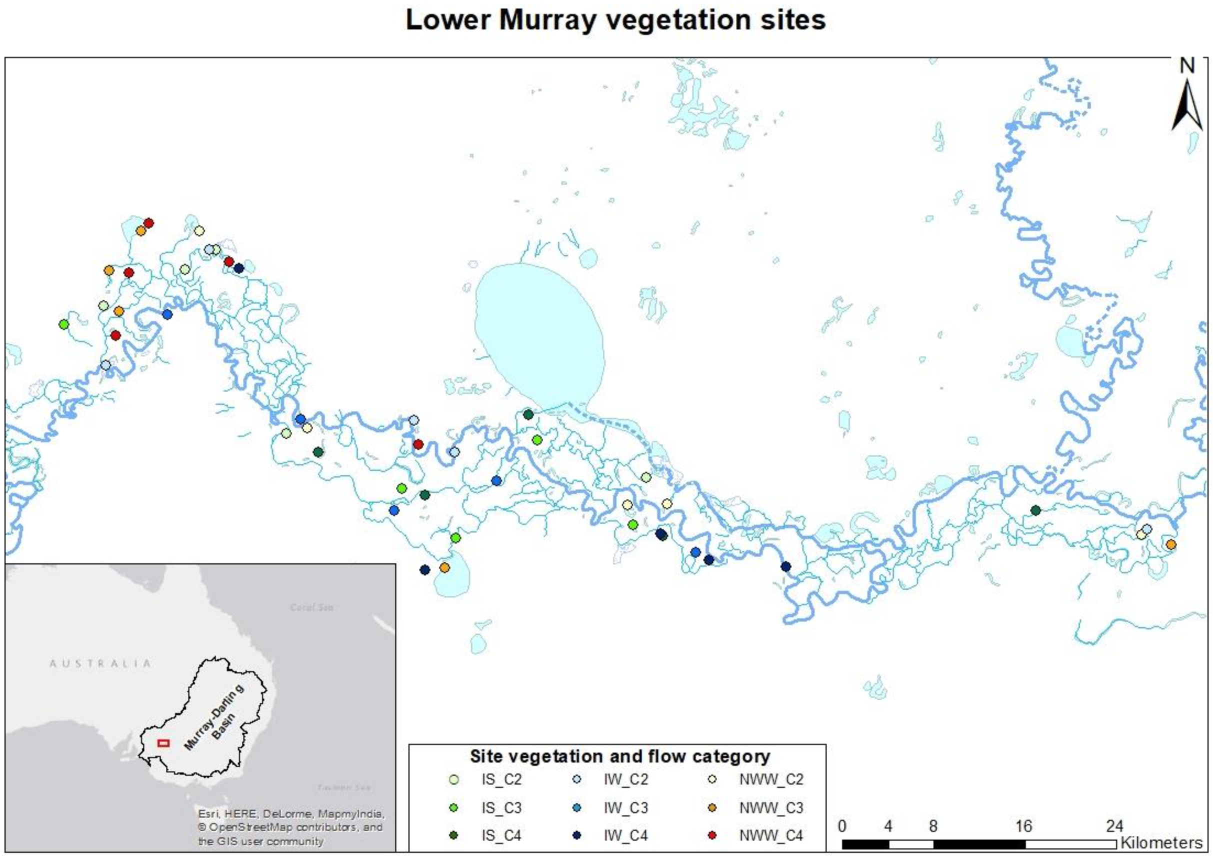
Design and site selection

Northern Sites

Narran Lakes

Rachael Thomas

NSW OEH



Methods

Design and site selection

Southern Sites

- 5 sites selected per relevant strata
- Input from local experts / managers

Methods

Design and site selection

- Not every strata relevant at every location
- 180 sites in total

Vegetation structural Category	Flood Return Frequency			
	Lower Murray	Mid Murray	Macquarie Marshes	Narran Lakes
Inland Woodland	N/A	<1.5 years (near annual)	<1 (annual)	<1.5 years (near annual)
	1.5 – 3 years	1.5 – 3 years	1-3 years	1.5-3 years
	3 – 5 years	3 – 5 years	3 – 5 years	3 – 5 years
	5 – 10 years	N/A	5 – 10 years	5 – 10 years
Inland Shrubland	N/A	N/A	N/A	<1.5 years (near annual)
	1.5 – 3 years	N/A	1-3 years	1.5-3 years
	3 – 5 years	N/A	3 – 5 years	3 – 5 years
	5 - 10 years	N/A	5 – 10 years	5 – 10 years
Non-Woody Wetland	N/A	<1.5 years (near annual)	<1 (annual)	<1.5 years (near annual)
	1.5 – 3 years	1.5 – 3 years	1-3 years	1.5-3 years
	3 – 5 years	N/A	3 – 5 years	3 – 5 years
	5 – 10 years	N/A	5 – 10 years	5 – 10 years

Methods
Monitoring

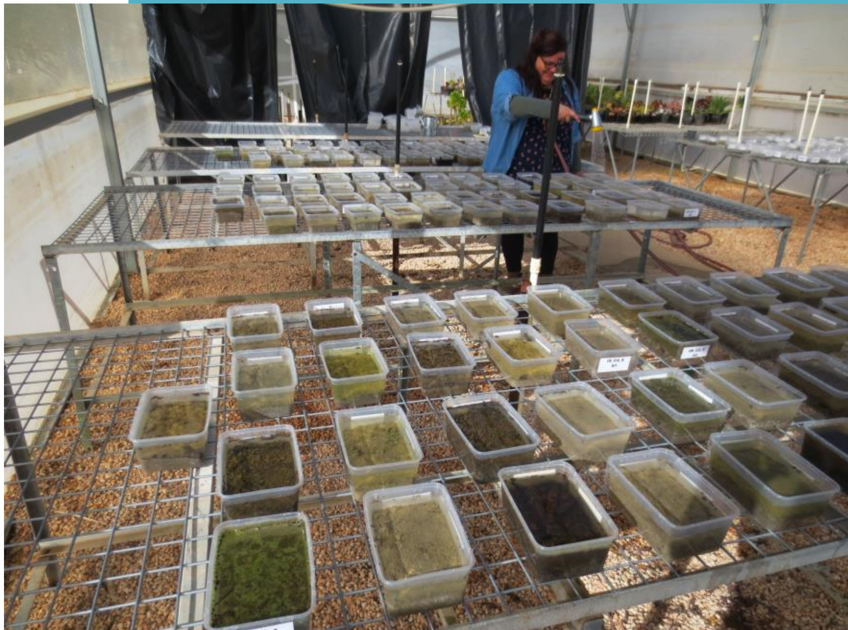
- Surveyed
  - Autumn 2017
  - Autumn 2018
- Each site: 20 x 20 m plot
- Point intercept transects
  - Characterise structure:
    - *substrate; low-, mid- and upper- strata*
- Species richness + composition
- Woody recruitment
- Canopy cover (PAI)
- Tree density
- Lignum condition
- Site condition
  - Other pressures (grazing, disturbance)


Jason Nicol (SARDI)

Methods

Germination trials

- Soil collected
  - Autumn 2017
- Treatments
  - Damp
  - Submerged
- Set-up in August 2017
  - Ran for 6 months
- Undertaken in 4 locations
  - Brisbane, Sydney, Albury, Mildura




Non-woody Wetland – Mid Murray
MDFRC 2017


Inland Shrubland – Lower Murray
J. Nicol 2017

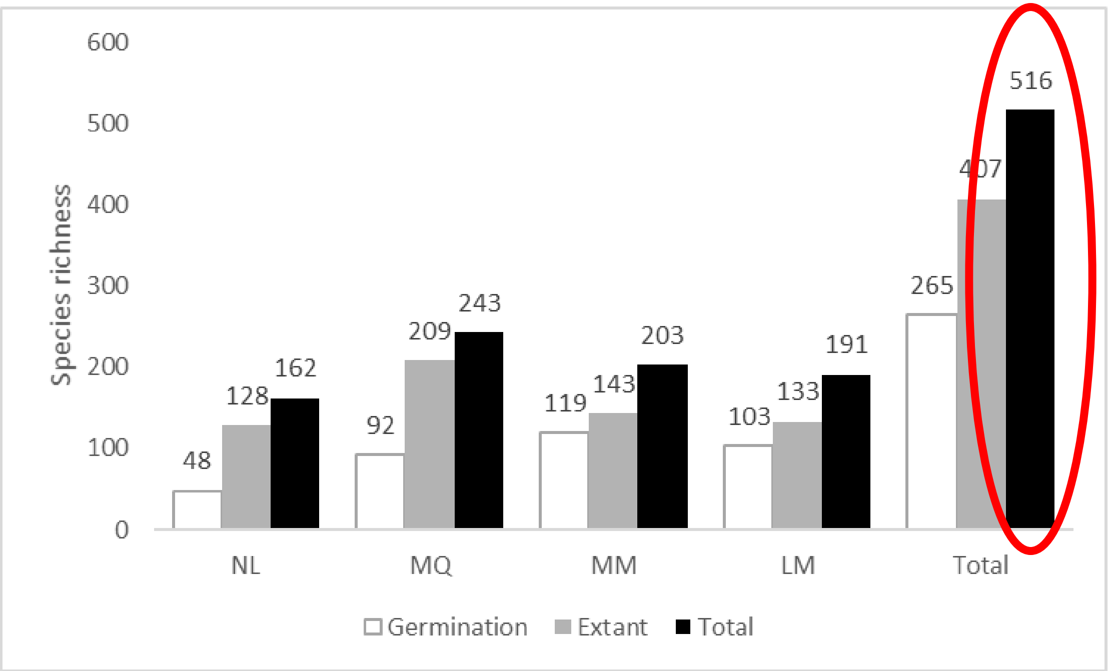
Results


R. Thomas 2017
Inland Woodland – Mac Marshes

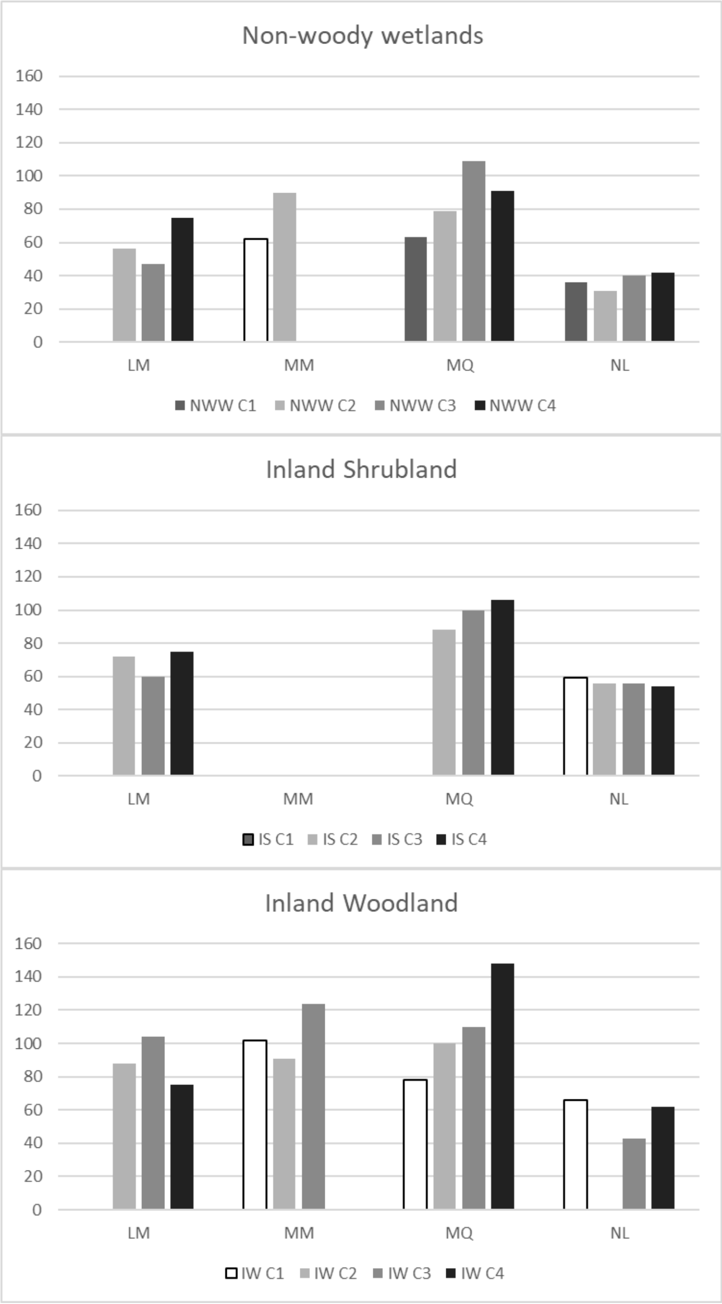

Griffith Uni 2017
Non-woody wetland – Narran Lakes

Results

Species richness



- ~30% of species recorded in both field surveys and germination trials
- ~65% of species recorded from a single location
  - 67% field, 74% germination



Results

Species richness

- ~30% of species recorded from a single location-veg-flow strata
  - 34% field, 25% germination

Common across germination trials

- *Cyperus difformis*
- *Ammannia multiflora*
- *Centipeda cunninghamii*
- *Alternanthera denticulata*
- *Elatine gratioloides*

Results

Opportunities for expression

Germination trials only

- *Bergia ammannioides* (LM + MQ)
- *Callitriche sonderi* (LM + MM)
- *Eleocharis pallens* (LM + NL)
- *Isolepis australiensis* (LM)
- *Lipocarpha microcephala* (LM + MM)
- *Myosurus australis* (LM)

Results

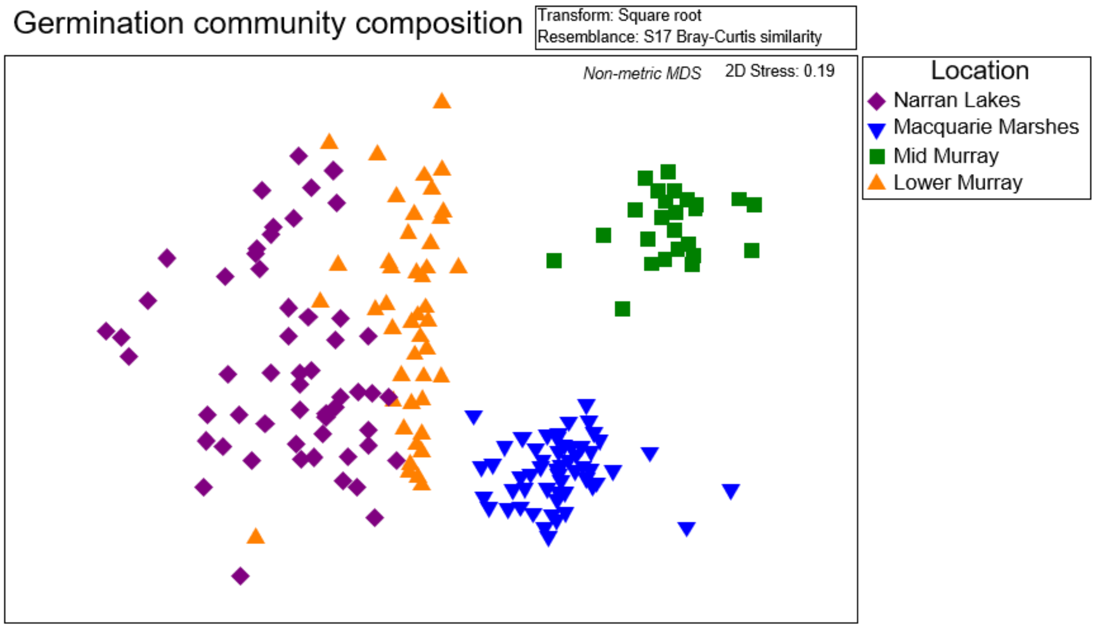
Lying dormant

- *Schoenoplectiella dissachantha*
- Lower Murray (Chowilla, SA)
- Non-woody wetland
- Flow Cat 4: 5 – 10 years

- Last recorded occurrence in Southern Basin in 1994
    - Lyrup, SA (flooded ground)

Results

Hidden gems

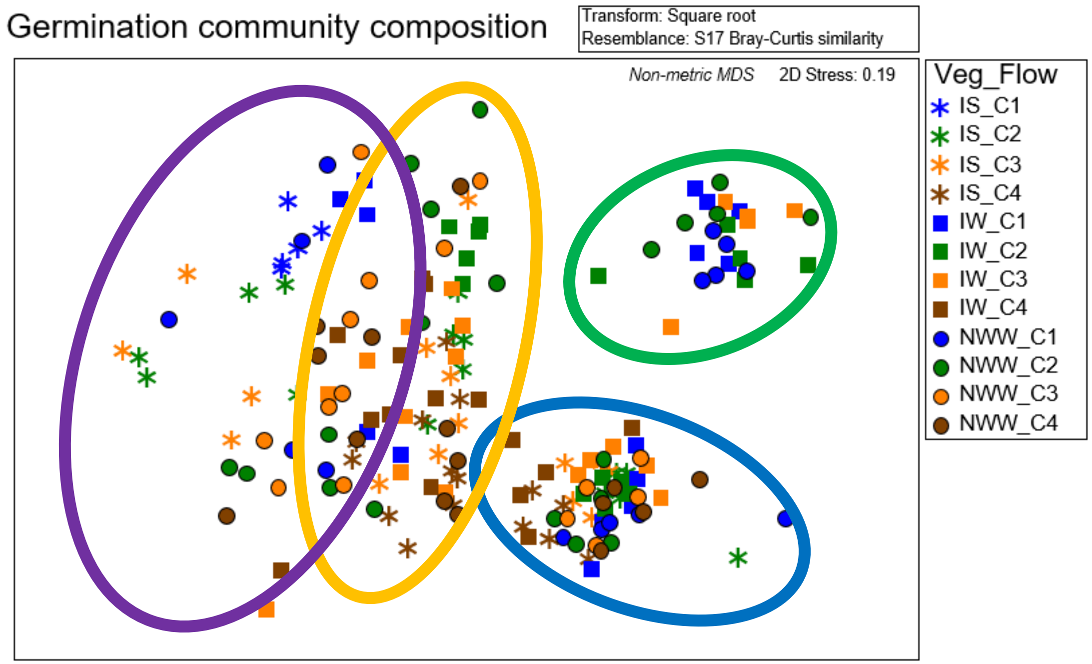


Results

Germination: Community composition

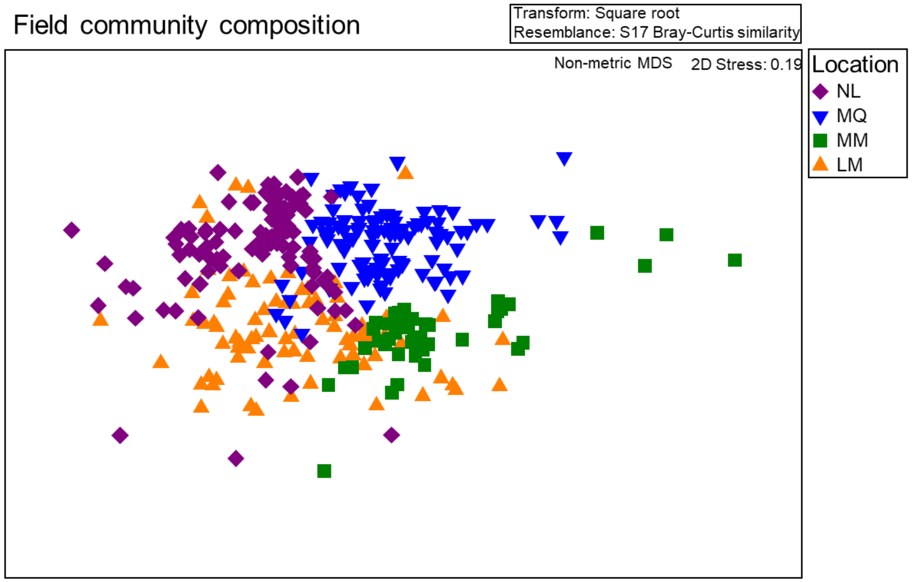
Results

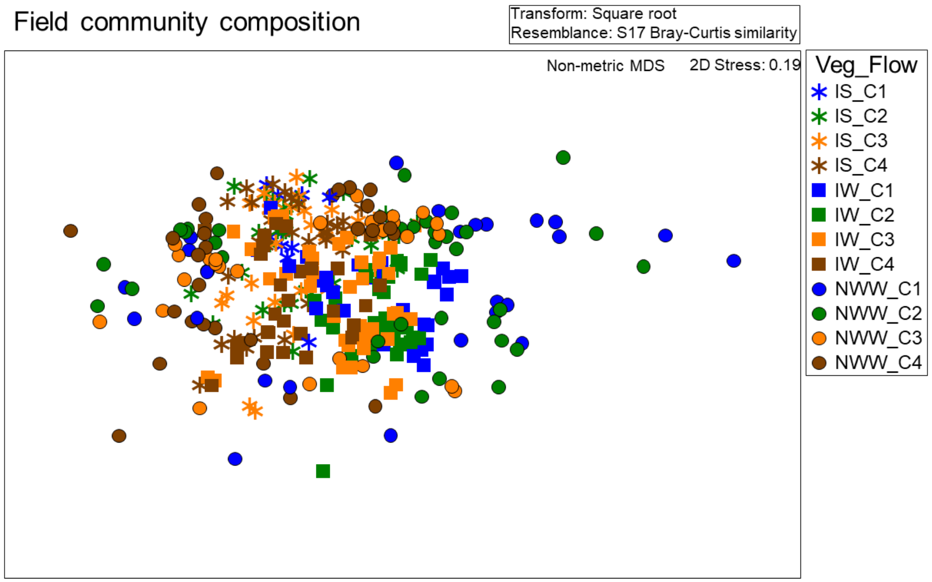
Germination: Community composition



Significant interaction

LocationxVegetationxFlow, p=0.0001





Results

Field: Community composition

Significant interaction

LocationxVegetationxFlow, p=0.0001

11 outliers removed

Main points

- Very strong influence of location
  - ➢ Both field and germination
- Species have broad distribution ranges
  - ➢ Dispersal limited?
  - ➢ Site-specific constraints?
  - ➢ Responses are time-bound
    - Short life cycles
    - Similar short to longer-term flow regimes
    - Challenges comparing current regime / hydrological state

What next

Further work

- Only species richness and composition at this stage
  - ➢ Inundation
  - ➢ Structural responses
  - ➢ Functional group / trait responses
  - ➢ Native vs exotic
  - ➢ Modifiers
    - Canopy cover, tree density, site disturbances
  - ➢ Influence of flow and vegetation structure within locations
- What this space for more results ☺

Watering entering Clear Lake

Griffiths Uni 2017

Thankyou

For more information

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Website: http://ewkr.com.au/

Facebook: https://www.facebook.com/LaTrobeCFE/

Project Collaborators

Appendix V3.3: Seed bank germination paper

N.B. This is a full manuscript in preparation for submission to a scientific journal for publication. Inclusion as an output in this technical report doesn't preclude the ability to publish.

Vulnerability of resilient systems to the Anthropocene

Campbell, C.J. [1]✉, Thomas, R.F.[2,3], Capon, S.J.[4], James, C.S. [5], Nicol, J.M. [6], Gehrig, S. L.[1], Morris, K. [7], Nielsen, D. [8,9], Palmer, G.J.[4], Durant, R.A.[8]

✉ Cherie Campbell, Research Centre for Freshwater Ecosystems, La Trobe University, Mildura, Victoria, Australia 3500

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Introduction

Herbaceous plant communities in arid wetland systems are considered to be resilient due to their large, long-lived and persistent (*sensu* Leck & Brock, 2000; Thompson & Grime, 1979) soil seed banks; giving them the ability to persist in hydrologically variable systems. These species often have widespread distributions attributed to multiple dispersal vectors (e.g. wind (e.g. Soons 2006, Soomers et al. 2013), waterbirds (e.g. Clausen et al. 2002, Reynolds and Cumming 2016, Coughlan et al. 2017), flooding (e.g. Nilsson et al. 1991, Kehr et al. 2014, Cubley and Brown 2016) that operate over large spatial scales. The soil seed banks of arid wetlands are a legacy of the recent and historical hydrological and land management regimes (Dawson et al. 2017a, Dawson et al. 2017b, Dawson et al. 2017c). Anthropogenic changes to hydrology (e.g. dams and extraction for domestic and agricultural uses), climate change and vegetation structure influence seed bank dynamics that result in seed gains or losses at the wetland scale. These seed bank dynamics include seed production, germination, change in viability, predation and dispersal (Figure 1, Table 1). Therefore, understanding patterns of seed bank composition and structure at the landscape and basin scale is important to inform vulnerability and prioritisation of management interventions such as the delivery of environmental water.

Studies within wetlands have frequently found spatial patterns of seed bank species richness and density related to flood history (e.g. Holzel and Otte 2001, Capon and Brock 2006). For example, there is often a humped distribution of species richness along flooding frequency gradients with low species richness in areas frequently or permanently flooded and in areas rarely flooded with highest species richness in intermediate areas, in line with the intermediate disturbance hypothesis (Connell 1978). In addition, there can be zonation of the seed bank with respect to elevation that reflects the distribution of species in the extant vegetation (*sensu* Spence, 1982).

The presence of overstorey or perennial shrubs can also influence seed bank transactions. Trees and shrubs compete with herbaceous vegetation for water and nutrients and reduce light availability, which affects reproductive success. However, the dappled shade provided by floodplain eucalypts reduces soil and air temperature whilst allowing light penetration. Similarly, the shrub *Duma florulenta* (Meisn.) T.M.Schust. offers protection from grazing and can provide nursery habitat for herbaceous species (James, Capon, & Quinn, 2015). The provision of leaf litter by overstorey species has both positive and negative impacts; litter reduces evaporation of soil moisture and reduces soil temperature but may prevent seeds from germinating by providing a physical barrier or allelopathic compounds (particularly floodplain eucalypts) (May & Ash, 1990; Moradshahi, Ghadiri, & Ebrahimikia, 2003; Sasikumar, Vijayalakshmi, & Parthiban, 2002). The presence of perennial vegetation may also influence water movement during floods. Dense reed beds and shrubs reduce flow velocity resulting in deposition of sediment (and seeds) and localised turbulence from water moving around the trunks of trees can cause erosion (authors pers. obs.).

Despite the high degree of resilience (due to the seed bank) of arid wetland herbaceous vegetation, there are potential risks such as changes to the hydrological regime, climate change and vegetation clearing. Very few studies have investigated seed banks over large spatial scales or considered combined influences of extant vegetation structure and hydrology (but see Capon and Brock 2006; Dawson et al. 2017; Higgisson et al 2018). In this study, we investigated patterns of soil seed bank species diversity and distribution at regional scales and within four large wetland complexes in the Murray-Darling Basin. We explore the influence of flood frequency and extant vegetation structure (e.g. canopy cover) and, the potential vulnerability of these wetlands to climate change and anthropogenic disturbances.

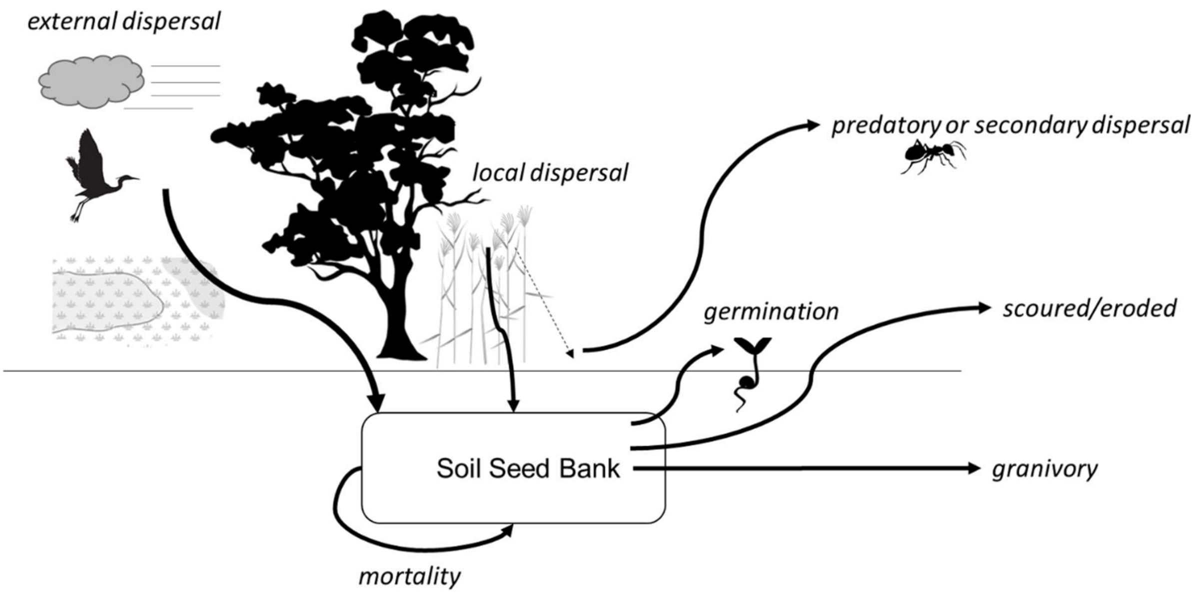

Figure 1. Conceptual representation of seed bank dynamics

Table 1. Expected influence of increased flooding frequency and increased canopy cover on individual seed bank transactions and processes

Transaction	Process	Expected Outcome	
		Increasing flooding	Increasing canopy
Deposit (gain)	External dispersal		
	Wind	Movement of buoyant seeds	Increase
	Flood	Increased potential for hydrochory	Increase/Decrease
	Animal (e.g. waterbirds)	Increased potential for seed dispersal and deposition via increased faunal visitation	Increased potential for seed deposition (perching/roosting habitat)
	Local dispersal		
	Seed deposition	Increased potential for localised hydrochory	Increase/Decrease
Withdrawal (loss)	Predation	Decrease/increase	No impact
	Secondary dispersal	Potential for increased export (via water and animals)	
	Germination	Increased germination post flood recession	Increase/Decrease
	Scouring/erosion	Increased export	Increase/Decrease
	Granivory	Decreased granivory during periods of inundation	Increase
	Mortality (loss of viability)	Increase/Decrease	Decrease (reduced soil temperatures)

Materials and Methods

Study area, climate and flow regime

The Murray-Darling Basin (the Basin) is a large river basin (>1million km^2) located in south-eastern Australia and encompasses the catchments of the Murray and Darling Rivers and their tributaries (Figure 2). Rainfall across the Basin is spatially and temporally variable averaging 457mm annually with more in the south-east and eastern margin, and less in the west (<300 mm) (CSIRO, 2008). There is a seasonal rainfall gradient with summer dominance in the north and winter dominance in the south. Most regions experience warm to hot semi-arid conditions. Evaporation is four times higher than rainfall and only a small proportion (6%) of rainfall generates surface runoff (CSIRO, 2008). The Basin supports the most important agricultural region in Australia sustained by river regulation, a catalyst for high water demand causing competing water use but with detrimental ecological impacts (Swirepik et al. 2016; Kingsford et al. 2015). The Basin also supports high ecological values across diverse ecosystems with about 300,000 wetlands. Of these, 16 wetlands, or wetland complexes are listed as internationally important under the Ramsar Convention on Wetlands (Pittock and Finlayson 2011). Four wetland complexes are the focus of our study: Narran Lakes (1) and the Macquarie Marshes (2) in the northern Basin and, the Mid-Murray (3) and Lower-Murray (4) in the southern Basin (Figure 2 and Table 2). Each of these four locations encompasses Ramsar listed wetlands. Each wetland system relies on flooding regimes from highly variable river flows that are regulated by large upstream dams and in channel weirs (Kingsford 2000), altering the natural flow regime (Arthington 2012).

The Narran Lakes floodplain wetland complex is located in the semi-arid region at the terminus of the Narran River, a distributary of the Condamine-Balonne River system, with a highly variable south-west flow that is summer dominated. Narran Lakes are characterised by a series of ephemeral lakes dominated by herbfields in dry periods, extensive areas of the perennial shrub lignum (*Duma florulenta*) on a complex network of braided channels and, riparian open forest and floodplain woodlands (James et al. 2007).

The Macquarie Marshes are a large (~200,000 ha) floodplain wetland system located on the lower reaches of the north flowing Macquarie River which has highly variable flows for a flooding regime that is winter-spring dominated (Thomas et al. 2015). They are characterised by a complex mosaic of diverse vegetation types including vast reed beds of *Phragmites australis* interspersed by open water lagoons, meadows of water couch (*Paspalum* *distichum*), segdeland swamps and river red gum (*Eucalyptus camaldulensis*) forests and woodlands with lignum (*Duma florulenta*) shrublands and river cooba (*Acacia stenophylla*) (Thomas and Ocock 2016). Coolibah (*E. coolabah*) and black box (*E. largiflorens*) woodlands occur at higher elevations on the floodplain (Paijmans 1981). Both the Narran Lakes and Macquarie Marshes wetland systems provide important waterbird habitat, especially breeding habitat for colonial nesting waterbirds (Brandis et al. 2011; Kingsford and Auld 2005).

The Mid-Murray location includes Barmah Forest in Victoria and Millewa Forest in NSW. Barmah–Millewa Forest is situated along the Edwards and Murray Rivers between the towns of Tocumwal, Echuca and Deniliquin covering ~66,000 hectares of floodplain. Barmah–Millewa Forest supports the largest river red gum forest in Australia and is the largest and most intact freshwater floodplain system along the River Murray (MDBA 2012). It has a wide variety of ecosystem types and is characterised by swamps, marshes, reedbeds, deeper lakes and billabongs, open grassland plains, river red gum forest, black box woodland and deep creek channels which distribute water throughout the forest and back to the river.

The Lower-Murray location encompasses Lindsay-Mulcra-Wallpolla (LMW) Islands in Victoria, Chowilla Floodplain in South Australia, and floodplain properties in south-west NSW, covering ~50,000 hectares. These areas support aquatic, riparian, and floodplain habitats including Ramsar-listed wetlands and a diversity of ecologically valuable species. They are dominated by river red gum woodlands, lignum shrublands, black box woodlands, chenopod shrublands, herblands and grasslands (MDBC 2006, Sharley and Huggan 1995).

The Lower-Murray has been severely impacted by river regulation and abstraction. A series of 11 low level (~3 m high) weirs were constructed between Mildura and Blanchetown that are typically managed to maintain stable water levels for irrigation and navigation. Regulation by weirs coupled with upstream abstraction, has resulted in almost complete loss of small to medium sized floods from the Lower Murray with long periods of stable water levels (Maheshwari et al. 1995). Nevertheless, an overbank flood peaking at 106,000 ML day^{-1} at Wentworth and 95,000 ML day^{-1} at the South Australian Border in late spring 2016, prior to collection of sediment samples, inundated approximately 80% of the floodplain.

Size, climate characteristics and inundation conditions at the time of sampling at the four locations are summarised in Table 2.

Table 2. Description of wetland systems including location, total size, Ramsar area, average rainfall, minimum and maximum temperatures, total evaporation and sampling conditions during soil collection.

Wetland System	Location		Climate^			Recent hydrological conditions and percent of sites flooded when sampled in autumn 2017
	Lat/Long	Size (ha) (Ramsar site)	Rainfall (Average) (mm)	Temperature Min; max (degrees Celsius)	Total Evaporation (mm)	
Narran Lakes	29°46'24"S 147°23'11"E	32,700 (8,847)	A: 400-600 W: 50-100 S: 100-200	A: 12-15; 24-27 W: 3-6;18-21 S: 18-21; 33-36	A: 1800-2000 W: 200-300 S: 700-800	No recent flooding 0% inundated
Macquarie Marshes	30°45'17"S 147°32'07"E	200,000 (19,850)	A: 400-600 W: 50-100 S: 100-200	A: 9-12; 24-27 W: 3-6;15-18 S: 18-21; 33-36	A: 2000-2400 W: 200-300 S: 800-900	Recent flooding of entire floodplain 20% inundated
Mid-Murray (Barmah-Millewa Forest)	35°49'03"S; 144° 58'00" E	66,000 (66,000)	A: 400-600 W: 100-200 S: 50-100	A: 9-12; 21-24 W: 3-6;12-15 S: 12-15; 30-33	A: 1600-1800 W: 100-200 S: 700-800	Recent partial flooding of floodplain 25% inundated
Lower-Murray (Chowilla Floodplain, Lindsay-Mulcra-Wallpolla Islands, NSW floodplain)	33°53'S to 34°11'S; 140°59'03.9"E	50,000 (17,700)	A: 239-288 W: 50-100 S: 50-100	A: 9-12; 24-27 W: 3-4;15-16 S: 15-16; 32-34	A: 2190 W: 100-200 S: 700-800	Recent partial flooding of floodplain 9% inundated

^Climate data taken from the Bureau of Meteorology (BOM) climate averages website based on a standard 30 year climatology (1961-1990), A = annual, W = winter, S = summer

http://www.bom.gov.au/jsp/ncc/climate_averages/rainfall/index.jsp?period=win&area=oz#maps

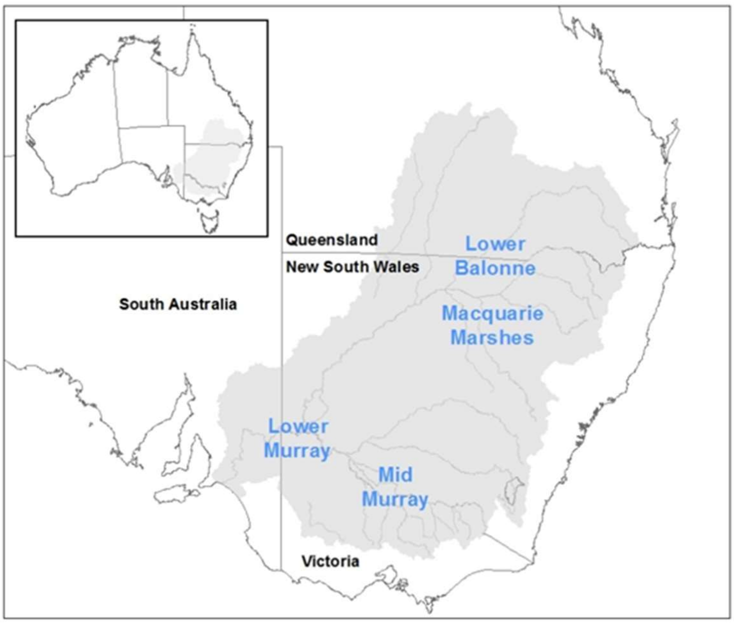

Figure 2. Locations of four floodplain wetland systems: Narran Lakes (Lower Balonne), Macquarie Marshes, Mid-Murray and Lower Murray in the Murray-Darling Basin, Australia, for field soil sampling of seed banks.

Sampling design

Within each of the four wetland systems soil sampling was carried out during Autumn (March-May) 2017 using a stratified random sampling design in structural vegetation types with different flood frequency return intervals. We defined three broad structural vegetation classes differentiated along a gradient of increasing canopy cover: non-woody wetlands (NWW), inland shrublands (IS), inland woodlands (IW). The distribution of these vegetation classes were derived from the best available state-based vegetation mapping (Narran Lakes 2014: EcoLogical Australia (2015); Macquarie Marshes 2013: NSW OEH VIS_ID 4892; Mid-Murray: Barmah-Modelled 2005 Ecological Vegetation Class (DEPI 2008), Millewa-Deniliquin NVMP VIS_ID 874 (NSW OEH 2010b); Lower-Murray: LMW-Modelled 2005 Ecological Vegetation Class (DEPI 2008), Chowilla-Vegetation and wetland mapping data (Department for Environment and Water 2016), SW NSW-Murray Darling Basin M305_Structural Vegetation Layer VIS_ID 917 (NSW OEH 2010a)). Mapped plant communities were allocated to each structural vegetation class (Supplementary Material 1, Tables S.1 to S.7).

We selected four flood frequency return interval categories: near annual (Cat1), 1 in 1.5 – 3 years (Cat2), 1 in 3 – 5 years (Cat3), 1 in 5 – 10 years (Cat4), representative of the indicative ranges of known water requirements for different floodplain wetland plant species (Roberts and Marston 2011).

For the two northern wetland systems, we mapped inundation frequency to determine the average return interval of floods across the floodplains of the Narran Lakes and Macquarie Marshes. Independent inundation events were initially determined from river flow peaks (Macquarie: DS Marebone Weir July 1988 to June 2013; Narran: Wilby Wilby January 1988 to December 2012) using the peak over threshold (POT) method (Thomas 2019). Inundation maps classified from the archive of Landsat satellite image observations (1988-2013) (Thomas et al. 2015) were then allocated to each event according to the map date. Inundation maps were then aggregated with pixels recoded to a value of one to create inundation event maps: 30 in total for the Macquarie Marshes and 16 for the Narran Lakes (Thomas 2019; Thomas et al. 2016). Using ERDAS Imagine software (ERDAS 2015) we counted the number of times a location (pixel) was inundated by an event. To determine the number of years between floods, the return interval, we divided the total number of observation years (25 for the Macquarie Marshes and 26 for the Narran Lakes) by the number of times flooded. These values were then allocated to each flood frequency category.

For the two southern sites, Mid-Murray and Lower-Murray, flood-return-frequency was calculated using flow data and CSIROs River Murray Flood Inundation Mapping (RiMFIM) (Overton et al. 2006; Sims et al. 2014). Flow data was accessed from various gauging stations along the Murray River corresponding with the regions of interest. Peak annual flow was extracted from daily flow data collected between 1988 and 2010 for flow across the Victorian–South Australian border (Chowilla; data provided by Jason Nicol, SARDI), Lock 9 (LMW and south-west NSW; data provided by Andrew Keogh, MDBA) and at Tocumwal (Barmah–Millewa; data downloaded from MDBA website, 30/6/2016). To calculate flood recurrence interval, peak annual flow was ranked according to magnitude (such that the highest peak flow volume = 1, second highest peak annual flow = 2 and so on) (Fuller 1914, cited by McConnell and Abel 2015). Recurrence interval (RI) was calculated using number of years (n) and relative ranking (m) such that; $RI=(n+1)/m$.

Peak annual flow and recurrence interval (on a LOG scale) were plotted and a logarithmic line of best fit added. The formula resulting from the line of best fit was then used to calculate flow at a required return interval. In ArcGIS, flow information contained in River Murray Flood Inundation Mapping (RiMFIM) (Overton et al. 2006; Sims et al. 2014) layers applicable to the regions; Chowilla (Zone 16 and 17), LMW and SW NSW (Zones 13 – 16) and Barmah (Zone 3) and Millewa (EW02) were used to determine areas corresponding to flood return frequency. Additionally, more recent flood mapping (up to flows of 65 000 ML.day-1), undertaken by the MDBA for Barmah-Millewa, was incorporated (data provided by Andrew Keogh, MDBA).

To map the vegetation-flood frequency strata classes (three vegetation classes x four flood frequency categories, Table 3) we used a spatial overlay analysis (intersection) between the structural vegetation class map and flood frequency category maps within each wetland system. Not all wetland systems had the full complement of strata classes (Table 3). Twenty-five potential sites within each stratum were randomly generated with a 100m minimum distance using ArcGIS (ESRI, 1995-2010). For each wetland system a maximum of five replicate sites per strata were selected following consultation with, and expert input from local managers, and consideration of the following criteria: the likelihood of inundation with managed flows (including weir pool manipulations); previous inundation with managed flows (including weir pool manipulations); availability of supporting data (existing monitoring or complementary data); known waterbird breeding sites; access / landholder consent; pixel area (very small areas are likely to be unrepresentative and potentially inaccurate); and, any known inaccuracies with the spatial mapping (i.e. misalignment between vegetation categories and/or flood return frequency).

Table 3. Strata classes of structural vegetation classes with different flood frequency categories for the Lower Murray, Mid Murray, Macquarie Marshes and Narran Lakes wetland systems.

	Flood Frequency Category				
Structural Vegetation Class	Lower Murray	Mid Murray	Macquarie Marshes	Narran Lakes	Strata Nomenclature
Inland Woodland	N/A	<1.5 years (near annual)	<1 (annual)	<1.5 years (near annual)	IW-CAT1
	1.5 – 3 years	1.5 – 3 years	1-3 years	1.5-3 years	IW-CAT2
	3 – 5 years	3 – 5 years	3 – 5 years	3 – 5 years	IW-CAT3
	5 – 10 years	N/A	5 – 10 years	5 – 10 years	IW-CAT4
Inland Shrubland	N/A	N/A	N/A	<1.5 years (near annual)	IS-CAT1
	1.5 – 3 years	N/A	1-3 years	1.5-3 years	IS-CAT2

	3 – 5 years	N/A	3 – 5 years	3 – 5 years	IS-CAT3
	5 - 10 years	N/A	5 – 10 years	5 – 10 years	IS-CAT4
Non-Woody Wetland	N/A	<1.5 years (near annual)	<1 (annual)	<1.5 years (near annual)	NWW-CAT1
	1.5 – 3 years	1.5 – 3 years	1-3 years	1.5-3 years	NWW-CAT2
	3 – 5 years	N/A	3 – 5 years	3 – 5 years	NWW-CAT3
	5 – 10 years	N/A	5 – 10 years	5 – 10 years	NWW-CAT4

Collection of soil samples

Composite soil samples were collected at each field site, in Autumn 2017, from a 20m x 20m quadrat. Within each quadrat 10 random samples were taken, ~5cm deep x 10cm diameter, and combined to form a single composite sample per site. Samples were allowed to air dry and were then stored in air-tight containers until the start of the germination trials.

Germination trials

Germination trials were undertaken simultaneously in four separate locations: Brisbane (Narran Lakes), Sydney (Macquarie Marshes), Albury (Mid-Murray) and Mildura (Lower Murray). Standard plastic takeaway containers (~16 cm x 11 cm x 4 cm) were used to germinate soil. Each container was filled with a standard equivalent volume (375mL; 1.5 cups) to a depth of ~2cm. Two treatments were applied; damp and submerged. For the damp treatment drainage holes were drilled in the bottom of each container and containers were kept moist via overhead sprinklers or dripper systems. For the submerged treatments containers were placed inside larger pots and inundated to ~5cm above the soil. Containers were randomly placed in the shadehouse / glasshouse and were re-randomise 3 times throughout the experiment (e.g. every two months). Control pots (containing sand) were included in each treatment. The germination trials ran for six months, from August 2017 to February 2018.

Species were identified prior to seed set, with all individuals counted and removed from containers. Where species were unable to be identified, these were removed, re-potted and grown-on until identification was possible. Species richness and abundance are reported cumulatively as the total number of species and counts of individuals plants recorded in a container during the six months of the experiment.

Results

Floristic descriptions

A total of 259 species germinated across all four locations (Table 4, Appendix 1), with species richness varying substantially between the four locations, from 48 species at Narran Lakes to 118 species in the Mid-Murray. The vast majority of species are native (71%). Almost half the species recorded are native forbs (117 species), followed by sedges, grasses and sub-shrubs, with few records of trees and shrubs (Table 4). Sixty-three species (24.3%) were only recorded from one site (i.e. from a single sampling stratum), with no species recorded across all 36 strata sampled (Appendix 1). The most widely distributed species (based on presence) was *Cyperus difformis*, which was recorded at every location in almost every strata (31/36). Other widely distributed species include: *Alternanthera denticulata, Ammannia multiflora, Centipeda cunninghamii, Elatine gratioloides, Polygonum plebeium,* and *Schenkia australis*.

Average species richness and abundance, varied according to location, flow category and vegetation class (Figure 3), but was notably lower at Narran Lakes.

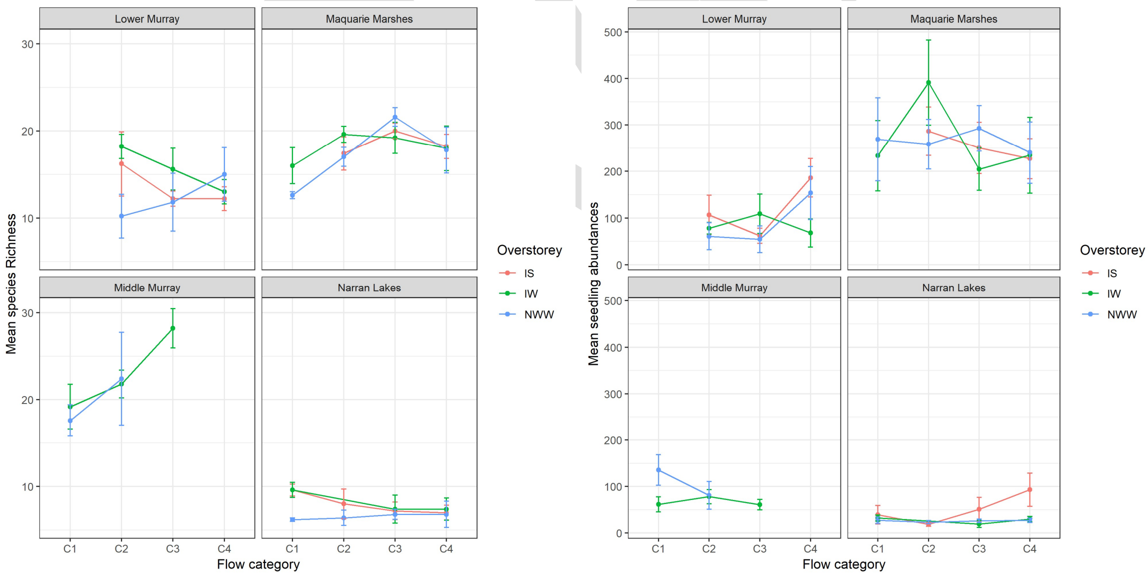

Figure 3. Mean (a) species richness and (b) abundance at the four locations, in each of three vegetation classes (NWW = Non-woody wetlands, IS = Inland Shrubland and IW = Inland Woodland) and four flow categories (C1 = near annual, C2 = 1 flow in 1.5 to 3 years, C3 = 1 flow in 3 to 5 years, C4 = 1 flow in 5 to 10 years).

Table 4. Species richness in total, for each of the four locations, and according to origin, life-history, and life-form

Location	Total species	Native	Exotic	Annual	Perennial	Forb	Grass	Sedge	Subshrub	Shrub	Tree
All locations	259	184	56	126	103	175	27	32	23	0	2
Narran Lakes	48	42 (87.5%)	4 (8.3%)	28	18	36	3	7	2	0	0
Macquarie Marshes	90	64 (71.1%)	23 (25.6%)	50	33	60	11	7	12	0	0
Mid Murray	118	75 (63.6%)	26 (22.0%)	41	53	85	11	15	6	0	1
Lower Murray	103	89 (86.4%)	14 (13.6%)	60	41	65	9	15	12	0	2

N.B. Unassigned origin = 19 species (ID to genus or family level); unassigned life-history = 30 species (ID to genus or family level)

The germinating seed bank communities at all locations are largely dominated by native species (Figure 4), with exotic abundance and richness (Figure 5) particularly low at both Narran Lakes and Lower Murray.

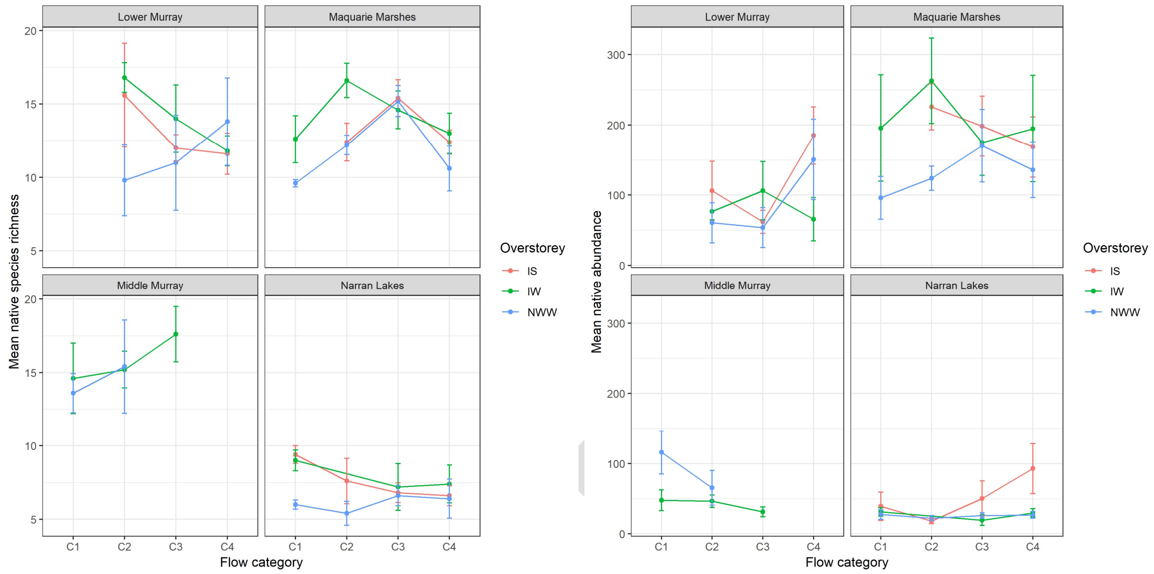

Figure 4. Mean (a) native species richness and (b) native abundance at the four locations, in each of three vegetation classes (NWW = Non-woody wetlands, IS = Inland Shrubland and IW = Inland Woodland) and four flow categories (C1 = near annual, C2 = 1 flow in 1.5 to 3 years, C3 = 1 flow in 3 to 5 years, C4 = 1 flow in 5 to 10 years).

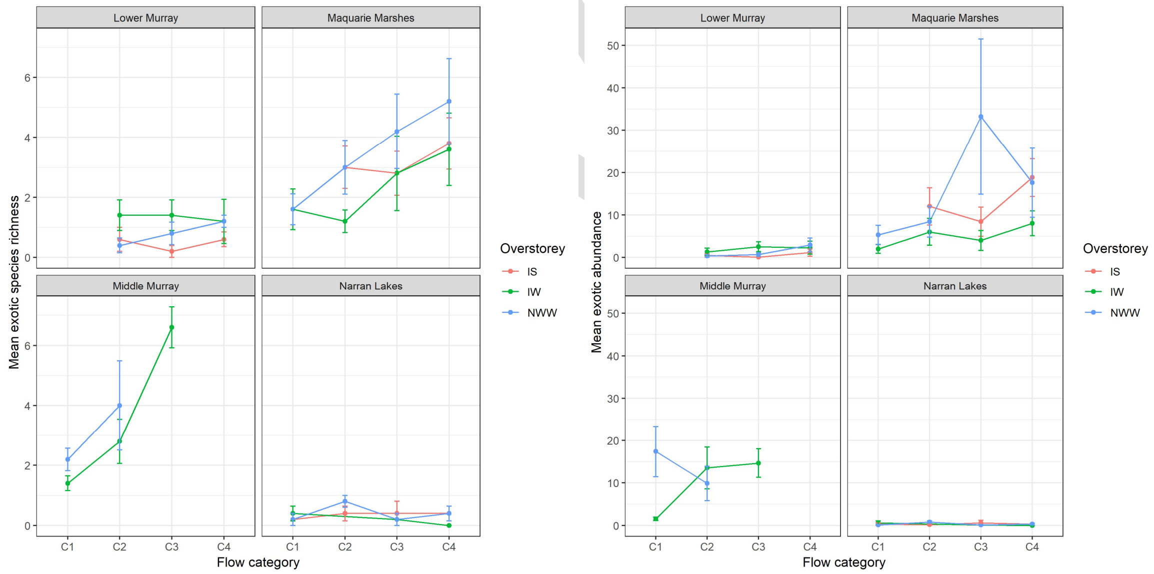

Figure 5. Mean (a) exotic species richness and (b) exotic abundance at the four locations, in each of three vegetation classes (NWW = Non-woody wetlands, IS = Inland Shrubland and IW = Inland Woodland) and four flow categories (C1 = near annual, C2 = 1 flow in 1.5 to 3 years, C3 = 1 flow in 3 to 5 years, C4 = 1 flow in 5 to 10 years).

Richness and abundance of different life-forms varies between location, vegetation class and flow category (Appendix 2, Figures A2.1:A2.5). Mid-Murray and Macquarie Marshes had a comparatively high proportion of grasses (Figure 6) The Macquarie Marshes also had a comparatively high abundance of sedges, though this was quite variable (Figure 7).

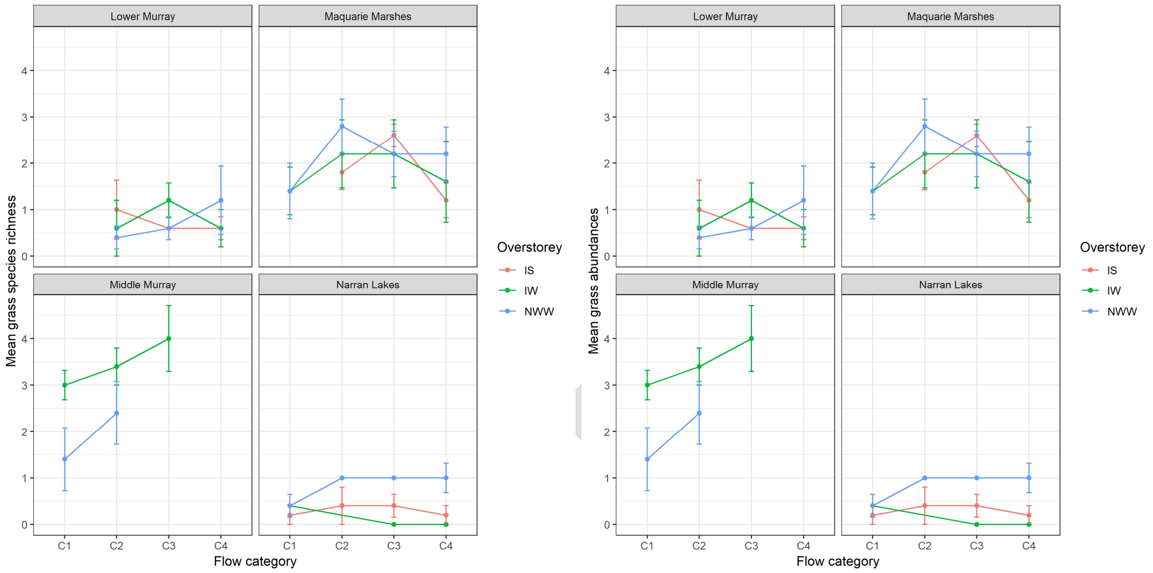

Figure 6. Mean grass (a) richness and (b) abundance at the four locations, in each of three vegetation classes (NWW = Non-woody wetlands, IS = Inland Shrubland and IW = Inland Woodland) and four flow categories (C1 = near annual, C2 = 1 flow in 1.5 to 3 years, C3 = 1 flow in 3 to 5 years, C4 = 1 flow in 5 to 10 years).

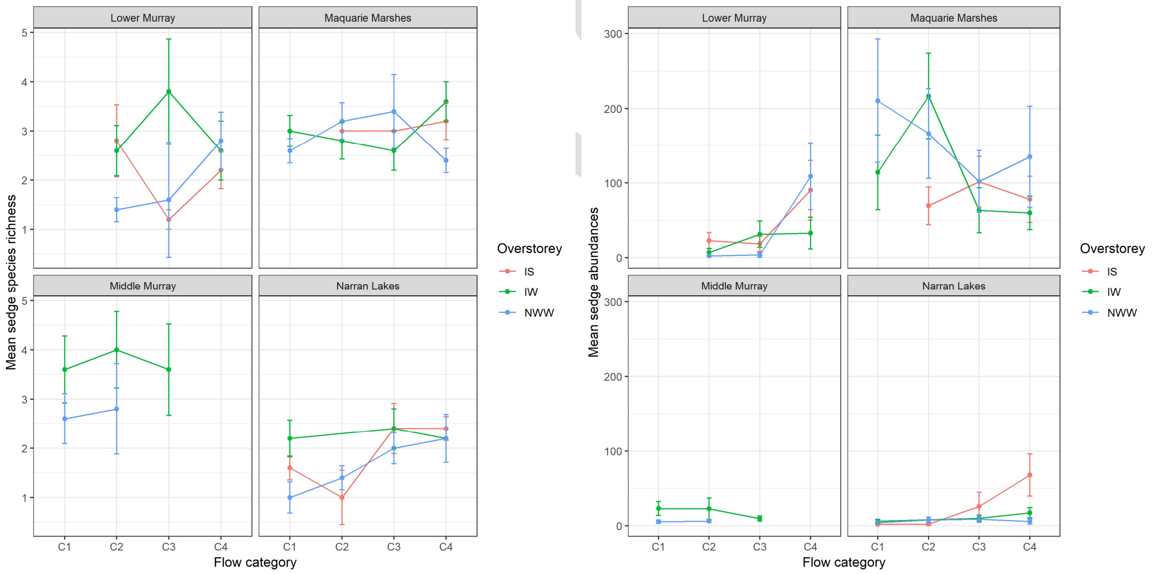

Figure 7. Mean sedge (a) richness and (b) abundance at the four locations, in each of three vegetation classes (NWW = Non-woody wetlands, IS = Inland Shrubland and IW = Inland Woodland) and four flow categories (C1 = near annual, C2 = 1 flow in 1.5 to 3 years, C3 = 1 flow in 3 to 5 years, C4 = 1 flow in 5 to 10 years).

Community composition

There was a strong influence of location on the germinable seed bank community, for both species presence/absence (Figure 8) and abundance (Figure 9) with distinct assemblages apparent for each of the four locations. There is greater dispersion in both the Narran Lakes and Lower Murray communities, particularly along the y-axis. While there is a clear influence of location (Figure 8 and 9 (a)), there are only weak relationships with vegetation class (Figure 8 and 9 (b)), flow category (Figure 8 and 9 (c)) and veg-flow strata (Figure 8 and 9 (d)) at this combined scale.

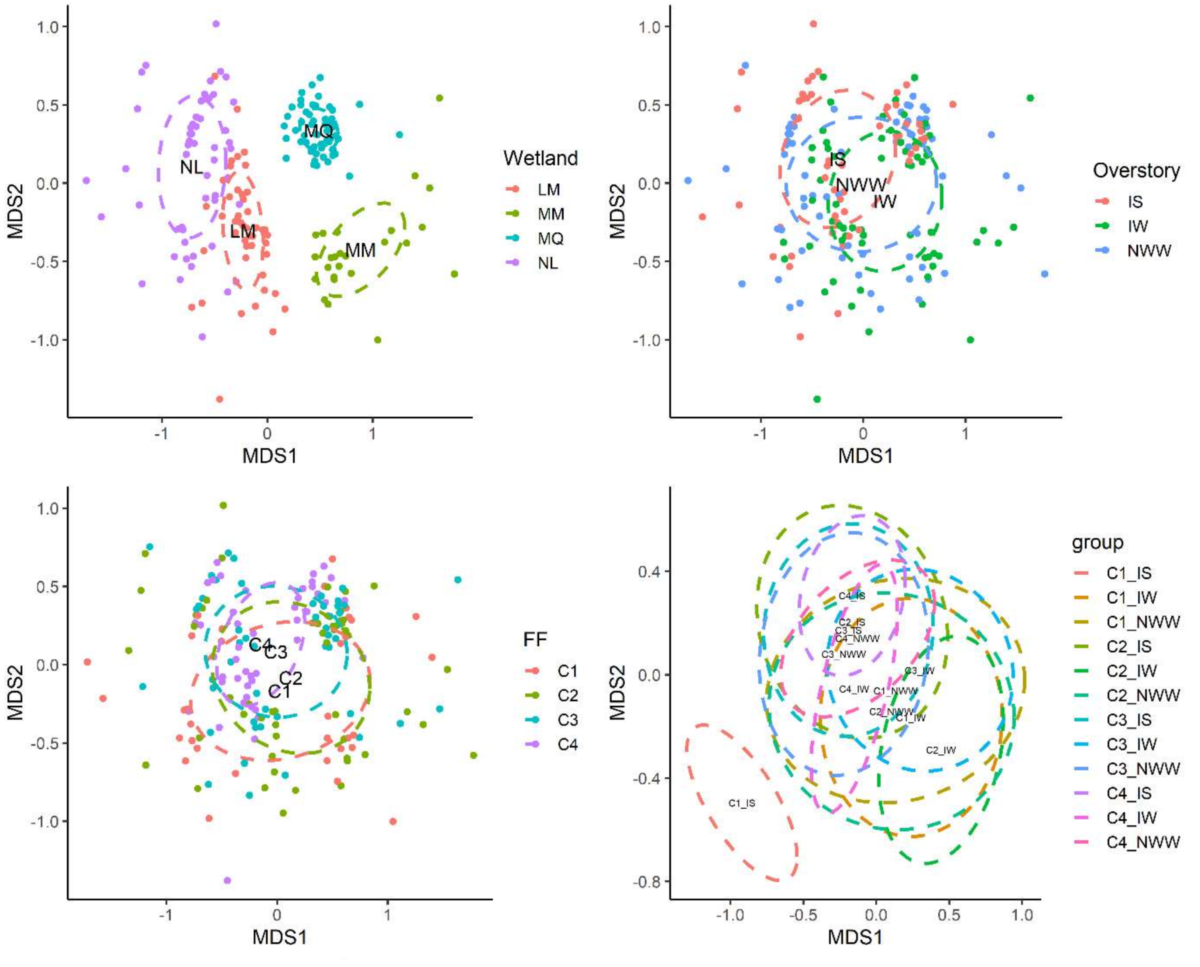

Figure 8. nMDS plots of the four locations based on species presence/absence: a) by location (LM = Lower Murray, MM = Mid Murray, MQ = Macquarie Marshes, NL = Narran Lakes); b) by vegetation class (IS = Inland Shrubland, IW = Inland Woodland, NWW = Non-woody wetland; c) by flow category (C1 = near annual, C2 = 1 flow in 1.5 to 3 years, C3 = 1 flow in 3 to 5 years, C4 = 1 flow in 5 to 10 years); and d) by veg-flow strata.

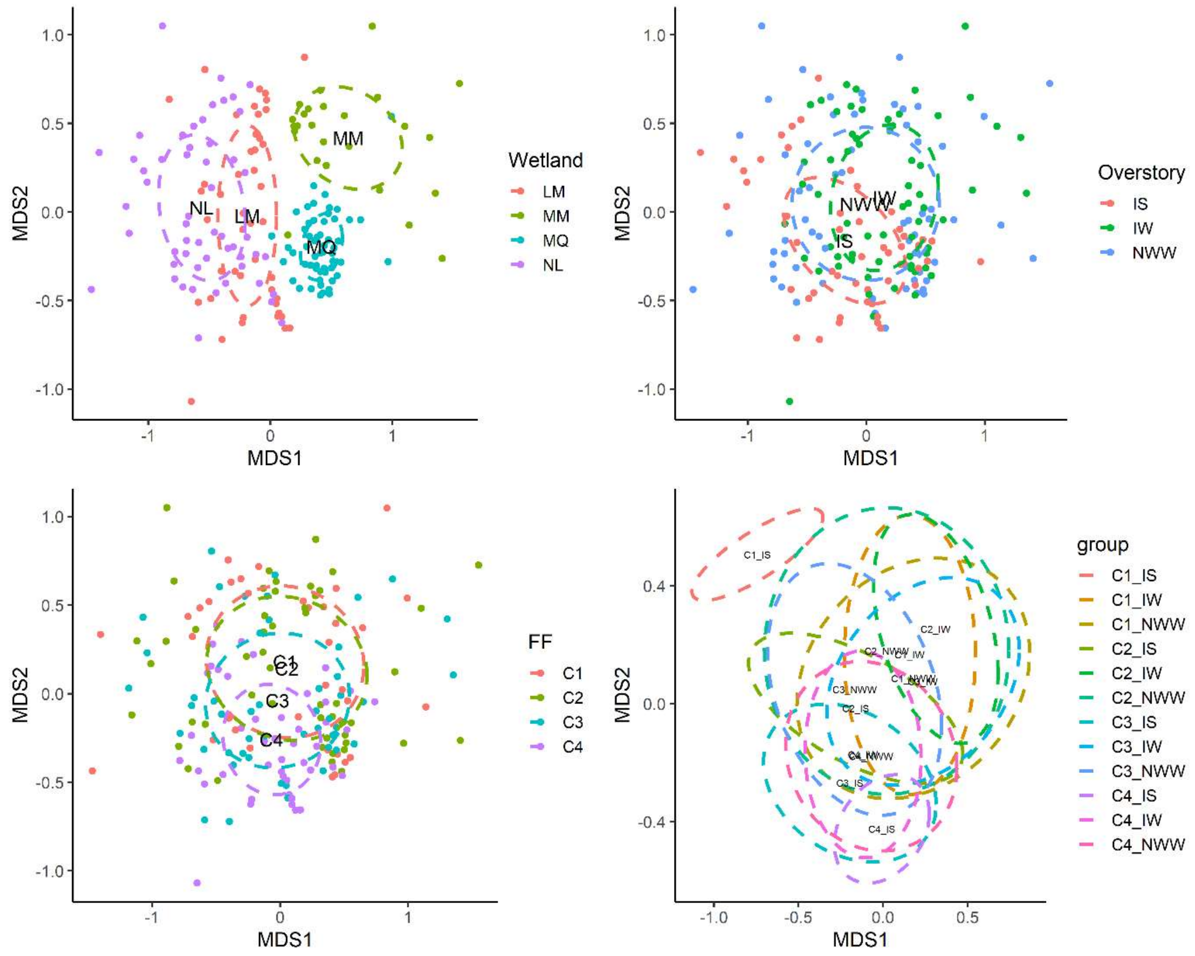

Figure 9. nMDS plots of the four locations based on species abundance: a) by location (LM = Lower Murray, MM = Mid Murray, MQ = Macquarie Marshes, NL = Narran Lakes); b) by vegetation class (IS = Inland Shrubland, IW = Inland Woodland, NWW = Non-woody wetland; c) by flow category (C1 = near annual, C2 = 1 flow in 1.5 to 3 years, C3 = 1 flow in 3 to 5 years, C4 = 1 flow in 5 to 10 years); and d) by veg-flow strata.

Within each location the influence of vegetation class and/or flow category on seed bank response is apparent, with different relationships evident at the different locations. For example, there is a strong relationship between flood frequency and seed bank responses at Macquarie Marshes (Figure 10(b)) and to a lesser extent Mid Murray (Figure 10(c)), and relatively weak relationships at Narran Lakes (Figure 10(a)) and Lower Murray (Figure 10(d)).

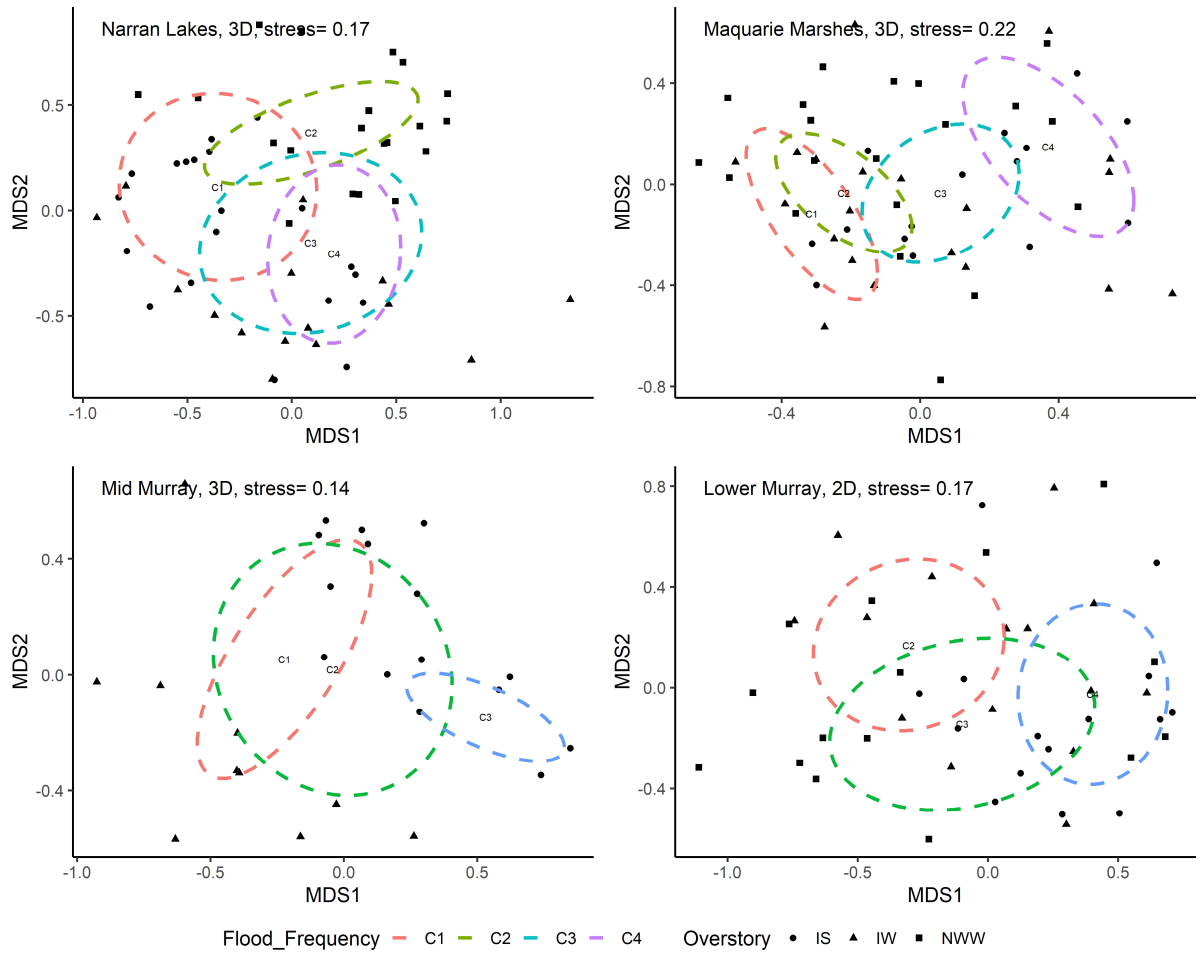

Figure 10. nMDS plots displaying relationships between flow category (C1 = near annual, C2 = 1 flow in 1.5 to 3 years, C3 = 1 flow in 3 to 5 years, C4 = 1 flow in 5 to 10 years) and seed bank assemblage within each of four locations: a) Narran Lakes; b) Macquarie Marshes; c) Mid Murray; and d) Lower Murray.

In relation to vegetation structure and seed bank responses, there were strong relationships at Narran Lakes (Figure 11(a)) and Mid Murray (Figure 11(c)) and relatively weak relationships at Macquarie Marshes (Figure 11(b)) and Lower Murray (Figure 11(d)). The relationships between seed bank response and flow category and/or vegetation class are summarised in Table 5, along with a descriptive indication of recent hydrological conditions. Where there has been no recent flooding, there is a stronger relationship with vegetation class, and where there has been recent, complete inundation there is a stronger relationship with flow category. However, these relationships do not hold true when there has been only partial flooding, with only weak relationships observed with both flow category and vegetation class at Lower Murray, and moderate to strong relationships with both flow category and vegetation class at Mid Murray (noting there are fewer veg-flow strata represented).

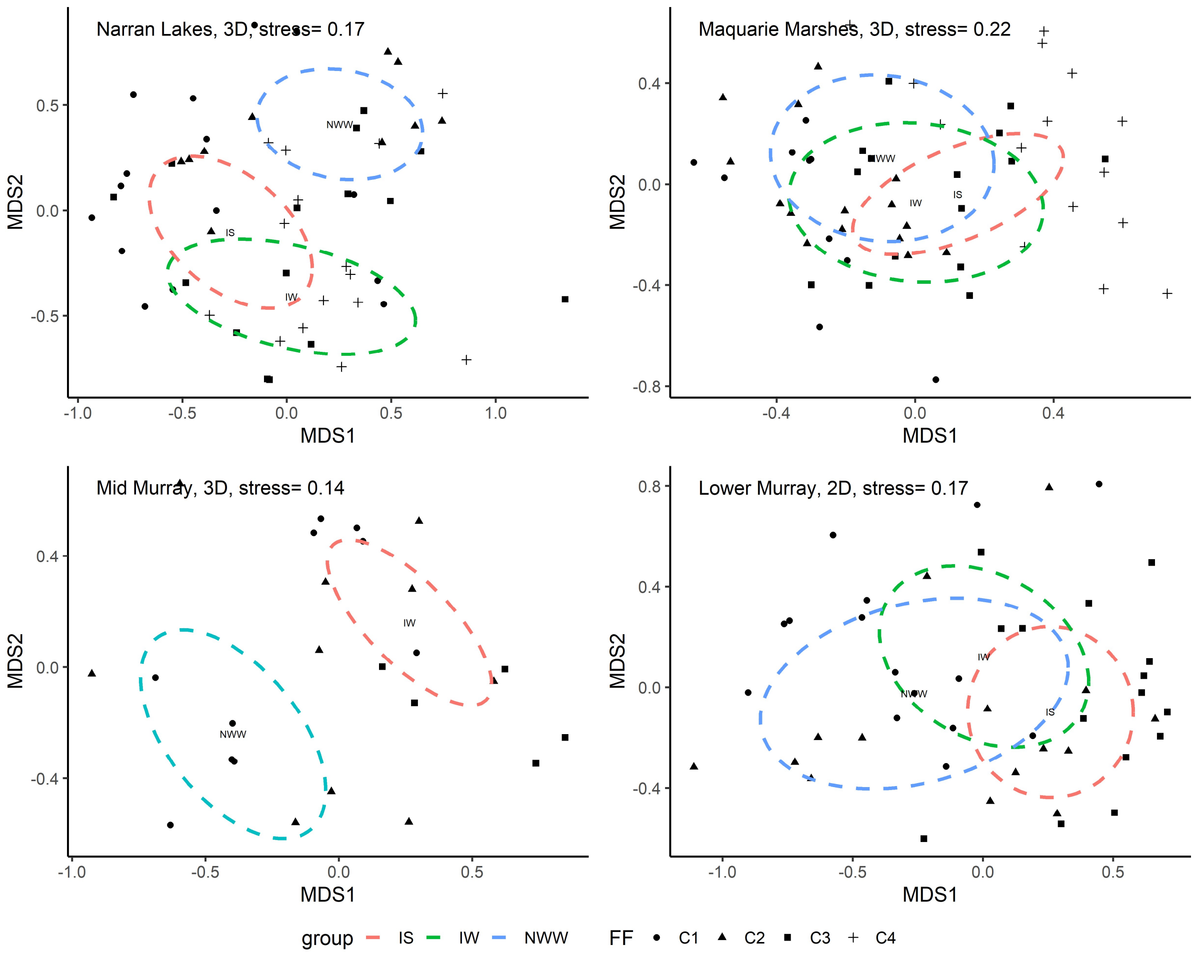

Figure 11. nMDS plots displaying relationships between vegetation class (IS = Inland Shrubland, IW = Inland Woodland, NWW = Non-woody wetland); and seed bank assemblage within each of four locations: a) Narran Lakes; b) Macquarie Marshes; c) Mid Murray; and d) Lower Murray.

Table 5. Summary of visually assessed relationships between seed bank assemblage, flow category and vegetation class in relation to recent hydrological conditions

Location	Recent hydrological conditions	Relationship with flow category	Visually assessed relationship with vegetation class
Narran Lakes	No recent flooding	Weak	Strong
Macquarie Marshes	Complete flooding	Strong	Weak
Mid Murray	Partial flooding	Moderate	Strong
Lower Murray	Partial flooding	Weak	Weak

Discussion

Understanding patterns of seed bank composition and structure at the landscape and basin scale is important to inform vulnerability and prioritisation of management interventions such as the delivery of environmental water.

The distinct assemblages observed across the four wetland complexes highlights the importance of location, and potentially local drivers, on the composition and expression of soil seed banks. This has implications for water management decisions in terms of prioritising areas of inundation to maximise the potential diversity at a Basin-scale, with the inference being that greater spatial representativeness will likely lead to greater Basin-scale diversity. However, spatial representativeness needs to be coupled with the influence of temporal flow regimes. At Macquarie Marshes, for example, native species abundance was greatest in the C2 or C3 flow categories, depending on vegetation class, and dropped off in the drier category (C4). This may relate to the persistence of certain species within seedbanks and their ability to survive dry periods. Conversely, there was typically a steady increase in exotic species abundance with increasing dryness (from C1-C4) in all three vegetation classes. Temporal flow regimes influence soil seed banks through the number of opportunities for germination, seed set and dispersal and soil carbon processes. Depending on the availability of water, the need for spatial representativeness at a Basin-scale may need to be balanced with the requirement for temporal flow regimes that are appropriate to maintain resilience within the native wetland flora at smaller scales.

Richness and abundance characteristics of the soil seed bank including nativeness, exoticness, life-history, and life-form were highly variable among locations, which is in line with the strong influence of location. The drier western sites, Narran Lakes and Lower Murray, did display lower exotic species richness and abundance which is likely explained by less favourable conditions such as lower rainfall and higher temperatures, though exotic richness and abundance may also be influenced by the intensity of past land-use. Grasses were a feature of the Mid-Murray and Macquarie Marshes, while sedges were more dominant at the Lower Murray. Interestingly Narran Lakes recorded relatively low species richness and abundance. It is unclear what is influencing this outcome. The near complete absence of woody seedlings, trees and shrubs, was common to all four locations and reflects the lack of a persistent seed bank in the majority of species common to these life-forms (Chong and Walker 2005, Jensen et al 2008).

Within each location, there were different influences on the community seed bank response, potentially linked to recent conditions. For example, at the Macquarie Marshes, where there was complete inundation of all surveyed sites in the recent history, there is a strong influence of flood frequency on understory vegetation communities, but only a weak influence of vegetation structure. Conversely, at Narran Lakes, where there was no recent flooding prior to surveys in 2017, there is a strong influence of vegetation structure on understory vegetation communities, and only a weak influence of flood frequency. While in the Lower Murray, where there was partial inundation, there were only weak relationships with both flood frequency and vegetation structure, suggesting other influences dominate. These relationships, however, need to be tested in a broader range of locations and at different scales (individual sites, vs across the wetland complex).

While seed banks display a high degree of resilience, anthropogenic disturbances such as changes to the hydrological regime, climate change and vegetation clearing, are potential risks. A review of seed bank studies (Roberts et al. 2017) highlights the variability in both seed bank diversity and density in Australian wetlands. Average richness in the order of 10 – 20 species per sampled strata, per location, may place our results in the mid to lower end of the spectrum, with the exception of Narran Lakes which typically has less than 10 species. These comparisons, however, need to be interpreted with caution (Roberts et al. 2017). At a wetland complex scale our results ranged from 48 to 118 species, with a total of 259 species for the entire study. Unique seed bank assemblages occur at the Basin-scale, representing high landscape-scale diversity. While seed banks are adapted to withstand natural wetting and drying, there are limits to their resilience. Seed banks are likely to be vulnerable to changes in temperature, changes in rainfall patterns, changes to seed-rain and retention capabilities through alterations to vegetation structure and frequency of inundation.

Management implications

Outcomes from this research inform environmental watering event planning and implementation, including considerations such as what are the key components of the flow regime or how should non-flow drivers be considered to achieve target responses. These outcomes can be used to better predict responses to environmental watering events and use those predictions to help plan or prioritise watering actions.

The overwhelming influence of location highlights the diversity of seed bank communities in space and time at a landscape scale. This has implications for water management decisions in terms of prioritising areas for inundation to maximise the potential diversity at a Basin-scale. There will inevitably still be trade-off questions that arise in long-term planning such as should we maximise the extent of inundation to potentially maximise diversity spatially or should we build up resilience and temporal diversity at a more limited suite of locations? Basin-scale management should aim to be equitable and representative of a large number of vegetation types in a range of areas over time (cumulative spatial representativeness across multiple years), while retaining the flexibility to build resilience and temporal diversity at identified locations (targeted, multi-year waterings). The key is to balance outcomes over time.

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Appendix 1. Species list by location, vegetation class and flow category, including classification of species into origin, life history and life form

				Lower Murray									Macquarie Marshes											Mid Murray					Narran Lakes											# of strata
				IS			IW			NWW			IS			IW				NWW				IW			NWW		IS				IW			NWW				
Species	Exotic.Native	Life.history	Life.form	C2	C3	C4	C2	C3	C4	C2	C3	C4	C2	C3	C4	C1	C2	C3	C4	C1	C2	C3	C4	C1	C2	C3	C1	C2	C1	C2	C3	C4	C1	C3	C4	C1	C2	C3	C4	
Acacia stenophylla	Native	perennial	Tree				X																																	1
Aeschynomene indica	Native	annual	Sub-shrub											X	X	X		X					X																	5
Alternanthera denticulata	Native	annual	Sub-shrub	X			X	X		X			X	X		X	X	X	X		X	X	X	X	X	X	X	X					X	X	X					21
Alternanthera nodiflora	Native	annual	Sub-shrub								X																													1
Amaranthus macrocarpus	Native	annual	Forb																X		X		X																	3
Amaranthus mitchellii	Native	annual	Forb												X			X	X			X																		4
Ammannia multiflora	Native	annual	Forb	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X		X	X			X	X	X	X					28
Amphibromus nervosus	Native	perennial	Grass	X								X	X	X		X	X	X			X	X																		9
Aphanes australiana	Native	annual	Forb																					X	X	X														3
Arctotheca calendula	Exotic	annual	Forb																							X														1
Arthropodium sp	Native	perennial	Forb																					X																1
Asphodelus fistulosus	Exotic	annual	Forb			X																																		1
Aster subulatus	Exotic	annual	Sub-shrub													X					X																			2
Asteraceae			Forb																					X																1
Atriplex leptocarpa	Native	perennial	Sub-shrub				X	X																																2
Atriplex semibaccata	Native	perennial	Sub-shrub					X																																1
Atriplex sp.	Native	perennial	Sub-shrub																X			X																		2
Avena fatua	Exotic	annual	Forb																									X												1
Azolla filiculoides	Native	perennial	Forb										X	X	X	X	X			X	X	X				X	X	X												11
Bergia ammannioides	Native	annual	Forb	X	X	X	X	X	X		X	X		X			X	X				X	X																	13
Brachyscome ciliaris var. lanuginosa	Native	perennial	Forb						X																															1
Brachyscome lineariloba	Native	annual	Forb		X	X		X	X			X																												5
Brachyscome multifida	Native	annual	Forb																														X		X		X			3
Brachyscome sp.	Native		Forb												X						X																			2
Brassica sp.			Forb																																		X			1
Brassicaceae			Forb																							X														1
Bulbine bulbosa	Native	perennial	Forb																											X	X			X						3
Bulbine semibarbata	Native	annual	Forb		X							X																												2
Bulbostylis barbata	Native	annual	Sedge/rush																										X				X						X	3
Calandrinia balonensis	Native	annual	Forb																														X							1
Calandrinia eremaea	Native	annual	Forb			X	X	X	X			X																												5
Callitriche sonderi	Native	annual	Forb	X			X			X	X	X												X	X		X	X												9
Callitriche stagnalis	Exotic	perennial	Forb																					X	X		X	X												4
Calotis latiuscula	Native	perennial	Forb																											X	X			X	X	X				5
Calotis scabiosifolia var. integrifolia	Native	perennial	Forb	X	X	X		X	X		X	X																												7
Calotis scapigera	Native	perennial	Forb																										X	X	X		X							4
Capsella bursa-pastoris	Exotic	annual	Forb											X	X				X				X																	4
Cardamine paucijuga	Native	annual	Forb																					X	X	X														3
Carex appressa	Native	perennial	Sedge/rush																					X																1
Carex inversa	Native	perennial	Sedge/rush																							X		X												2
Carex sp			Sedge/rush																						X		X	X												3
Carex tereticaulis	Native	perennial	Sedge/rush																					X																1
Centaurium tenuiflorum	Exotic	annual	Forb																												X	X								2
Centipeda cunninghamii	Native	perennial	Forb	X	X	X	X	X	X	X	X	X												X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	24
Centipeda minima	Native	annual	Forb	X	X		X	X		X	X		X	X	X	X	X	X	X	X	X		X	X			X													18
Charophyte	Native		Forb										X	X	X	X	X	X	X	X	X	X	X	X			X	X												14
Chenopodiaceae			Forb												X	X						X	X		X															5
Convolvulus erubescens	Native	perennial	Forb										X										X																	2
Conyza bonariensis	Exotic	annual	Sub-shrub											X		X		X	X		X		X		X	X														8
Conyza sp	Exotic	perennial	Sub-shrub																					X		X	X													3
Cotula australis	Native	annual	Forb											X				X	X			X		X	X	X		X												8
Cotula bipinnata	Exotic	annual	Forb																						X	X		X												3
Cotula coronpifolia	Exotic	annual	Forb																									X												1

Cotula sp			Forb																									X												1
Craspedia chrysantha	Native	annual	Forb	X	X				X		X	X																												5
Craspedia pleiocephala	Native	annual	Forb																										X											1
Crassula decumbens	Native	annual	Forb																					X	X	X		X												4
Crassula helmsii	Native	annual	Forb																						X	X	X	X												4
Crassula peduncularis	Native	annual	Forb																						X	X	X	X												4
Crassula sieberiana	Native	annual	Forb	X	X	X	X	X	X		X	X							X						X	X														11
Cucumis sp			Forb																						X															1
Cullen cinereum	Native	annual	Sub-shrub											X								X										X				X	X		X	6
Cuscuta campestris	Exotic	annual	Forb				X			X																														2
Cycnogeton sp	Native	annual	Forb																					X																1
Cynodon dactylon	Native	perennial	Grass																							X	X	X												3
Cyperus bifax	Native	perennial	Sedge/rush																															X	X				X	3
Cyperus difformis	Native	annual	Sedge/rush	X	X	XX	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X						X	X	X	X	X	X	X	X	X	X	31
Cyperus eragrostis	Exotic	perennial	Sedge/rush																							X														1
Cyperus gilesii	Native	annual	Sedge/rush																											X	X	X	X	X	X	X	X	X	X	10
Cyperus gymnocaulos	Native	perennial	Sedge/rush					X			X	X																												3
Cyperus pygmaeus	Native	annual	Sedge/rush					X																					X				X			X				4
Cyperus sp			Sedge/rush																					X	X	X	X	X												5
Dactyloctenium aegyptium	Exotic	annual	Grass										X										X																	2
Damasonium minus	Native	annual	Forb										X			X	X				X	X		X	X			X												8
Daucus glochidiatus	Native	annual	Forb																										X	X	X	X	X	X	X					7
Digitaria divaricatissima	Native	perennial	Grass																											X									X	2
Digitaria sp			Grass																						X	X	X	X												4
Diplachne fusca	Native	annual	Grass									X																												1
Disphyma crassifolium subsp. clavellatum	Native	annual	Forb			X			X																															2
Dysphania glomulifera	Native	annual	Forb	X			X		X		X																													4
Dysphania pumilio	Native	annual	Sub-shrub				X		X	X	X		X	X		X	X		X	X	X	X	X	X	X	X	X	X												18
Echinochloa colona	Native	annual	Grass											X	X		X	X	X	X	X		X																	8
Echium plantagineum	Exotic	annual	Forb																						X	X														2
Eclipta platyglossa	Native	annual	Forb										X	X	X		X	X	X			X	X			X								X	X					11
Einadia nutans	Native	perennial	Sub-shrub				X						X	X	X	X	X	X	X							X														9
Elatine gratioloides	Native	annual	Forb			X				X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X												19
Eleocharis acuta	Native	perennial	Sedge/rush	X		X		X	X															X	X	X		X	X	X	X	X	X	X	X			X	X	17
Eleocharis pallens	Native	perennial	Sedge/rush	X	X	X	X	X	X	X	X	X																		X	X			X						12
Eleocharis plana	Native	perennial	Sedge/rush										X	X	X	X	X	X	X	X	X	X	X	X	X	X	X													15
Eleocharis pusilla	Native	perennial	Sedge/rush	X					X			X																												3
Eleocharis sp	Native		Sedge/rush																					X	X	X	X	X												5
Eleocharis sphacelata	Native	perennial	Sedge/rush																					X																1
Enchylaena tomentosa	Native	perennial	Sub-shrub											X								X																		2
Eragrostis dielsii	Native	annual	Grass				X				X																													2
Eragrostis lacunaria	Native	perennial	Grass				X																																	1
Eragrostis parviflora	Native	annual	Grass											X				X	X																					3
Erigeron sp			Forb																							X														1
Erodium crinitum	Native	annual	Forb	X								X			X																									3
Eucalyptus camaldulensis	Native	perennial	Tree				X																			X		X												3
Euchiton involucratus	Native	perennial	Forb																						X															1
Euchiton sphaericus	Native	annual	Forb				X	X									X		X			X																		5
Euphorbia dallachyana	Native	perennial	Forb	X	X	X	X	X			X	X											X			X														9
Fimbristylis velata	Native	annual	Sedge/rush								X																													1
Geranium sp			Forb																							X	X	X												3
Glinus lotoides	Native	annual	Forb				X	X		X	X								X	X			X																	7
Glinus oppositifolius	Native	annual	Forb				X	X																																2
Glossostigma elatinoides	Native	perennial	Forb				X		X	X				X	X	X		X		X			X				X	X												11
Glycyrrhiza acanthocarpa	Native	perennial	Sub-shrub				X				X	X																												3
Goodeniaceae	Native		Forb																X																					1
Gratiola sp.	Native	perennial	Forb				X			X																														2
Heliotropium europaeum	Native	annual	Forb																											X							X	X		3
Heliotropium supinum	Exotic	annual	Forb	X			X		X		X	X																												5
Hordeum leporinum	Exotic	annual	Grass												X				X		X		X																	4
Hypochaeris albiflora	Exotic	perennial	Forb											X								X																		2
Hypochaeris glabra	Exotic	annual	Forb					X	X																	X		X												4

Isoetopsis graminifolia	Native	annual	Forb		X	X	X	X		X		X																											6
Isolepis australiensis	Native	annual	Sedge/rush	X	X		X			X	X	X																											6
Isolepis hookeriana	Native	annual	Sedge/rush									X																											1
Isolepis marginata	Exotic	annual	Sedge/rush				X																																1
Juncus aridicola	Native	perennial	Sedge/rush										X	X	X	X	X	X	X		X	X																	9
Juncus bufonius	Native	annual	Sedge/rush								X	X	X		X				X			X	X																7
Juncus holoschoenus	Native	perennial	Sedge/rush																X		X																		2
Juncus ingens	Native	perennial	Sedge/rush																									X											1
Juncus sp.			Sedge/rush										X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X											16
Juncus usitatus	Native	perennial	Sedge/rush	X			X	X																															3
Lachnagrostis filiformis	Native	annual	Grass	X			X	X				X		X	X	X	X	X	X		X	X	X	X	X	X	X	X											18
Lactuca serriola	Exotic	annual	Forb										X	X					X	X			X			X		X											7
Lemna disperma	Native	perennial	Forb										X	X	X	X	X		X	X	X	X	X																10
Lepidium fasciculatum	Exotic	annual	Forb															X	X				X																3
Lepidium sp.			Forb																														X						1
Limosella australis	Native	perennial	Forb		X		X		X	X	X	X													X	X	X	X	X										11
Lipocarpha microcephala	Native	annual	Sedge/rush					X			X	X														X		X											5
Ludwigia octovalvis	Native	annual	Forb											X		X	X	X			X	X	X																7
Ludwigia palustris	Exotic	perennial	Forb																						X	X		X											3
Ludwigia peploides var. montevidensis	Native	perennial	Forb							X			X			X	X	X		X	X	X			X	X	X	X											12
Lysimachia arvensis	Exotic	perennial	Forb					X											X			X																	3
Lythrum hyssopifolia	Native	annual	Forb																									X					X						2
Lythrum wilsonii	Native	annual	Forb										X	X	X		X		X			X																	6
Malva parviflora	Exotic	annual	Forb										X		X				X			X	X																5
Marsilea costulifera	Native	perennial	Forb			X						X																											2
Marsilea drummondii	Native	perennial	Forb			X				X		X																				X							4
Medicago laciniata	Exotic	annual	Forb											X	X				X			X	X																5
Medicago minima	Exotic	annual	Forb												X		X	X	X			X	X																6
Medicago polymorpha	Exotic	annual	Forb								X	X	X	X	X		X	X	X	X	X	X	X			X	X			X								X	16
Medicago sp	Exotic	annual	Forb																							X		X											2
Melilotus indicus	Exotic	annual	Forb									X																											1
Mesembryanthemum nodiflorum	Exotic	annual	Forb		X	X		X	X			X																											5
Mimulus gracilis	Native	perennial	Forb												X		X	X	X			X							X		X	X	X	X	X						11
Mimulus repens	Native	perennial	Forb	X	X	X	X	X	X	X		X																											8
Myosurus australis	Native	annual	Forb	X			X		X	X	X																												5
Myriophyllum caput-medusae	Native	perennial	Forb																					X		X	X	X											4
Myriophyllum crispatum	Native	perennial	Forb																								X												1
Myriophyllum papillosum	Native	perennial	Forb										X	X	X	X	X	X	X	X	X	X	X	X	X		X	X											15
Myriophyllum variifolium	Native	perennial	Forb										X	X		X	X			X		X					X	X											8
Myriophyllum verrucosum	Native	perennial	Forb							X																	X	X	X	X					X	X	X	X	X	10
Nitella sp	Native	annual	Forb																					X			X												2
Nymphoides crenata	Native	perennial	Forb													X	X	X		X							X	X											6
Ottelia ovalifolia	Native	annual	Forb																					X				X											2
Oxalis perennans	Native	perennial	Forb										X	X	X	X	X	X	X	X	X	X	X			X													12
Oxalis sp.			Forb					X																X															2
Panicum decompositum var decompositum	Native	perennial	Grass																							X		X											2
Panicum queenslandicum	Native	perennial	Grass																												X								1
Panicum sp	Native	perennial	Grass																					X	X														2
Paspalidium jubiflorum	Native	perennial	Grass					X																X	X	X	X	X											6
Paspalidium sp.	Native		Grass											X																									1
Paspalum distichum	Native	perennial	Grass														X				X																		2
Persicaria decipiens	Native	perennial	Forb							X						X	X			X	X																		5
Persicaria lapathifolia	Native	annual	Sub-shrub							X																													1
Persicaria prostrata	Native	perennial	Forb				X																																1
Persicaria sp	Native		Forb																									X											1
Phragmites australis	Native	perennial	Grass																									X											1
Phyla canescens	Exotic	perennial	Forb										X	X	X	X	X	X			X	X	X																9
Phyllanthus lacunarius	Native	annual	Forb		X	X																																	2
Plagiobothrys plurisepaleus	Native	annual	Forb	X	X	X		X		X	X	X																											7
Plantago cunninghamii	Native	annual	Forb	X	X	X						X		X				X	X			X	X											X						10
Plantago drummondii	Native	annual	Forb	X	X	X		X																															4
Plantago sp	Native		Forb																					X		X													2

Poa sp	Native	perennial	Grass																					X															1
Poaceae			Grass										X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X											16
Polygonaceae			Forb																					X															1
Polygonum aviculare	Exotic	annual	Forb																						X	X	X												3
Polygonum plebeium	Native	annual	Forb	X	X		X			X	X	X		X			X	X	X	X	X								X	X	X		X		X	X	X	X	X	21
Polypogon lutosus	Exotic	perennial	Grass															X				X																	2
Polypogon monspeliensis	Exotic	annual	Grass										X	X		X	X	X	X	X	X	X	X																10
Portulaca filifolia	Native	annual	Forb																														X						1
Potamogeton ochreatus	Native	perennial	Forb														X				X																		2
Potamogeton sulcatus	Native	perennial	Forb																					X	X			X											3
Pratia concolor	Native	perennial	Forb											X	X	X		X						X	X	X		X											8
Pseudognaphalium luteoalbum	Native	annual	Forb	X			X				X				X		X	X	X			X						X	X	X			X	X	X		X			15
Pseudoraphis spinescens	Native	perennial	Grass																					X			X	X											3
Ranunculus inundatus	Native	perennial	Forb																					X	X	X													3
Ranunculus muricatus	Exotic	perennial	Forb																									X											1
Ranunculus pentandrus var. platycarpus	Native	annual	Forb	X	X			X																															3
Ranunculus sceleratus	Exotic	annual	Forb										X	X	X	X		X		X	X	X																	8
Ranunculus undosus	Native	perennial	Forb										X		X	X		X				X																	5
Rhagodia spinescens	Native	perennial	Sub-shrub				X																																1
Romulea sp	Exotic	perennial	Forb																						X														1
Rorippa eustylis	Native	annual	Forb					X																							X		X						3
Rorippa laciniata	Native	perennial	Forb										X				X	X			X	X							X	X			X	X	X					10
Rorippa palustris	Exotic	annual	Forb																						X	X													2
Rorippa sp	Native		Forb																					X	X	X		X											4
Rumex brownii	Native	perennial	Forb	X			X			X														X		X		X	X	X	X		X			X			X	12
Rumex conglomeratus	Exotic	perennial	Forb																					X	X	X		X											4
Rumex crystallinus	Native	annual	Forb																										X	X	X	X	X			X		X	X	8
Rumex sp			Forb																					X	X														2
Rumex tenax	Native	perennial	Forb							X			X	X	X	X	X	X	X	X	X	X	X		X			X											14
Rytidosperma setaceum	Native	perennial	Grass			X			X			X														X													4
Schenkia australis	Native	annual	Forb								X			X	X		X	X	X			X	X					X	X	X	X	X	X	X	X	X		X	X	19
Schismus barbatus	Exotic	annual	Grass					X																															1
Schoenoplectiella dissachantha	Native	annual	Sedge/rush									X																											1
Scleranthus minusculus	Native	annual	Forb			X																																	1
Sclerolaena brachyptera	Native	annual	Sub-shrub		X	X			X			X																											4
Sclerolaena sp.	Native	perennial	Sub-shrub												X		X	X				X																	4
Sclerolaena tricuspis	Native	perennial	Sub-shrub									X																											1
Senecio cunninghamii var. cunninghamii	Native	perennial	Sub-shrub	X	X	X	X		X																														5
Senecio glossanthus	Native	annual	Forb											X	X		X		X			X	X																6
Senecio quadridentatus	Native	annual	Forb															X																					1
Senecio runcinifolius	Native	annual	Forb				X																																1
Sesbania cannabina	Native	annual	Forb																																X	X				2
Sisymbrium erysimoides	Exotic	annual	Forb	X			X	X		X								X																					5
Sisymbrium sp	Exotic		Forb																					X															1
Solanum sp			Forb																					X															1
Solenogyne sp	Native	perennial	Forb																							X													1
Soliva sessilis	Exotic	annual	Forb													X			X		X																		3
Sonchus oleraceus	Exotic	annual	Forb												X			X		X		X															X	X		6
Spergularia rubra	Native	perennial	Forb	X	X	X	X		X	X	X	X																											8
Sphaeromorphaea australis	Native	perennial	Forb																						X		X												2
Sphaeromorphaea littoralis	Native	perennial	Forb	X			X	X	X		X	X																											6
Sporobolus mitchellii	Native	perennial	Grass	X	X	X		X	X	X	X	X																	X	X	X	X	X			X	X	X	X	17
Stellaria angustifolia	Native	perennial	Forb										X	X	X	X	X	X		X	X	X									X			X						11
Stellaria media	Exotic	annual	Forb																					X	X	X		X											4
Stellaria pallida	Exotic	annual	Forb																					X	X	X													3
Stellaria sp	Native	perennial	Forb																					X	X			X											3
Stemodia florulenta	Native	perennial	Forb	X	X		X	X	X		X	X																					X		X				X	10
Symphiotrichum subulatus	Exotic	annual	Forb															X	X		X	X																	4
Tecticornia sp.	Native		Forb	X																																			1
Tetragonia moorei	Native	annual	Forb						X																														1
Tetragonia tetragonioides	Native	annual	Forb															X	X																				2
Trachymene glaucifolia	Native	annual	Forb																										X	X			X						3

Trifolium campestre var. campestre	Exotic	annual	Forb																						X		X													2
Trifolium glomeratum	Exotic	annual	Forb																						X		X													2
Trifolium repens	Exotic	perennial	Forb																							X														1
Trifolium resupinatum	Exotic	annual	Forb																						X															1
Triglochin calcitrapa	Native	annual	Forb									X																												1
Triglochin hexagonum	Native	annual	Forb									X																												1
Trigonella suavissima	Native	annual	Forb																									X	X	X	X				X	X	X			7
Typha domingensis	Native	perennial	Sedge/rush																	X		X																		2
Typha sp.	Native	perennial	Sedge/rush	X		X	X	X	X	X														X	X	X	X	X												11
Vallisneria australis	Native	perennial	Forb																					X	X		X	X												4
Verbena gaudichaudii	Native	perennial	Forb										X	X	X			X	X			X	X																	7
Verbena sp			Forb																							X		X												2
Verbena supina	Exotic	annual	Forb						X																				X	X	X		X	X		X			X	8
Veronica peregrina	Exotic	annual	Forb				X																																	1
Vittadinia gracilis	Native	perennial	Sub-shrub																							X														1
Vittadinia sp	Native	perennial	Forb																						X	X		X												3
Wahlenbergia fluminalis	Native	perennial	Forb	X	X	X	X	X	X			X														X														8
Wahlenbergia sp	Native		Forb																					X	X	X	X	X												5
Xanthium occidentale	Exotic	annual	Sub-shrub																				X																	1
Xanthium spinosum	Exotic	annual	Sub-shrub															X			X																			2
Number of species				**39**	**29**	**31**	**49**	**41**	**34**	**31**	**35**	**47**	**36**	**48**	**44**	**37**	**44**	**51**	**53**	**29**	**40**	**56**	**44**	**52**	**55**	**69**	**41**	**70**	**21**	**23**	**20**	**14**	**27**	**19**	**19**	**15**	**13**	**12**	**17**	**259**

Appendix 2. Plots of mean species richness and abundance for annual and perennial species, forbs and sub-shrubs.

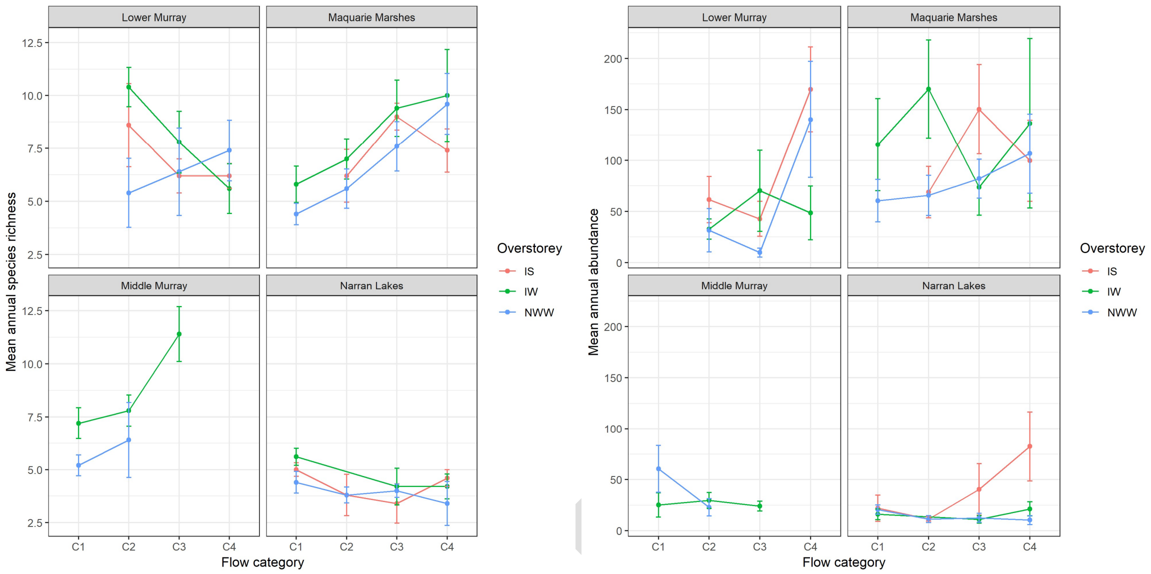

Figure A2.1. Mean annual (a) species richness and (b) abundance at the four locations, in each of three vegetation classes (NWW = Non-woody wetlands, IS = Inland Shrubland and IW = Inland Woodland) and four flow categories (C1 = near annual, C2 = 1 flow in 1.5 to 3 years, C3 = 1 flow in 3 to 5 years, C4 = 1 flow in 5 to 10 years).

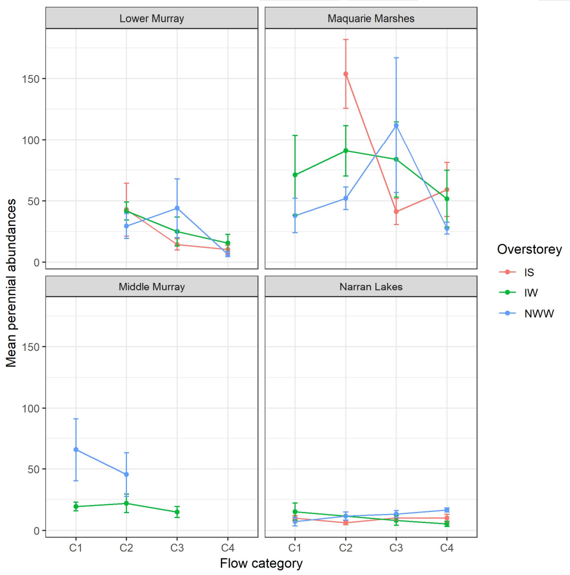

Figure A2.2. Mean perennial (a) species richness and (b) abundance at the four locations, in each of three vegetation classes (NWW = Non-woody wetlands, IS = Inland Shrubland and IW = Inland Woodland) and four flow categories (C1 = near annual, C2 = 1 flow in 1.5 to 3 years, C3 = 1 flow in 3 to 5 years, C4 = 1 flow in 5 to 10 years).

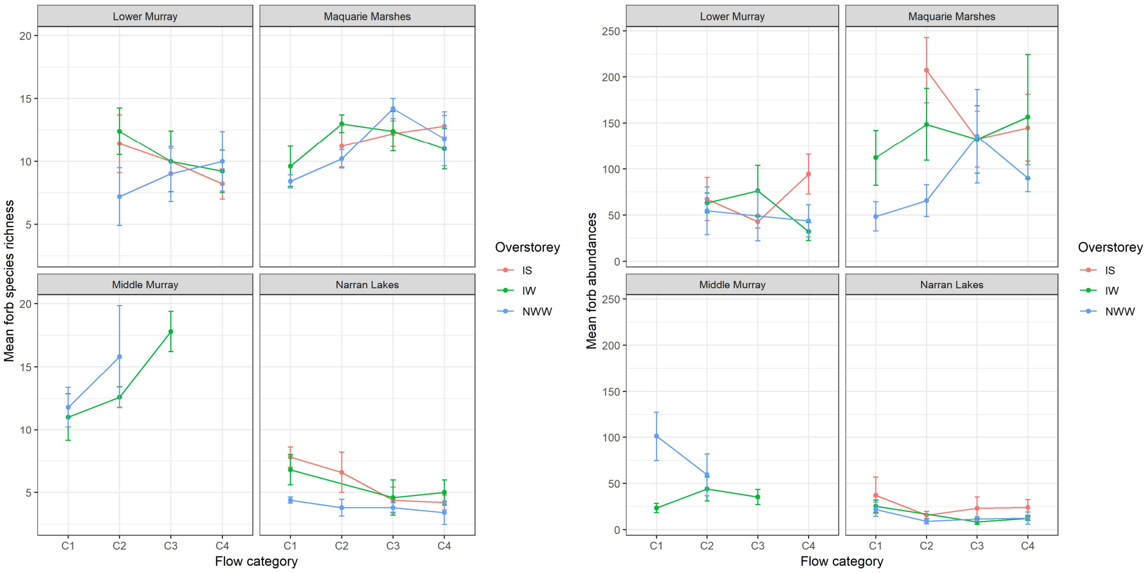

Figure A2.3. Mean forb (a) species richness and (b) abundance at the four locations, in each of three vegetation classes (NWW = Non-woody wetlands, IS = Inland Shrubland and IW = Inland Woodland) and four flow categories (C1 = near annual, C2 = 1 flow in 1.5 to 3 years, C3 = 1 flow in 3 to 5 years, C4 = 1 flow in 5 to 10 years).

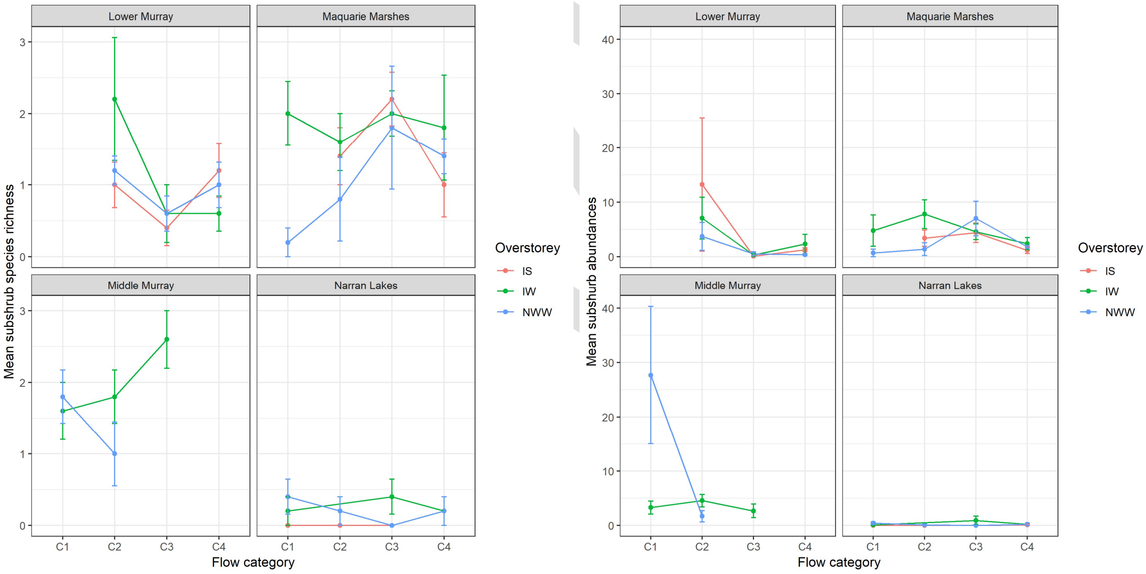

Figure A2.4. Mean subshrub (a) species richness and (b) abundance at the four locations, in each of three vegetation classes (NWW = Non-woody wetlands, IS = Inland Shrubland and IW = Inland Woodland) and four flow categories (C1 = near annual, C2 = 1 flow in 1.5 to 3 years, C3 = 1 flow in 3 to 5 years, C4 = 1 flow in 5 to 10 years).

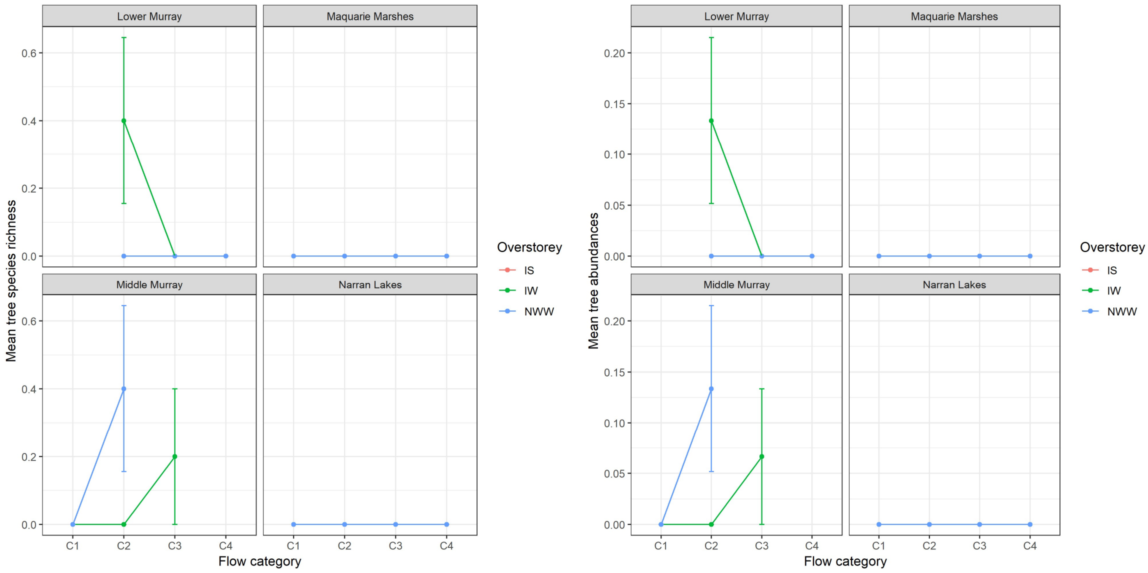

Figure A2.5. Mean tree (a) species richness and (b) abundance at the four locations, in each of three vegetation classes (NWW = Non-woody wetlands, IS = Inland Shrubland and IW = Inland Woodland) and four flow categories (C1 = near annual, C2 = 1 flow in 1.5 to 3 years, C3 = 1 flow in 3 to 5 years, C4 = 1 flow in 5 to 10 years).

Supplementary Information 1: Vegetation descriptions from GIS layers for each region and newly assigned vegetation categories (Tables S.1 to S.7).

Table S.1: Vegetation description for Chowilla region from GIS layer (Vegetation mapping data and wetland data for Chowilla floodplain) and newly assigned vegetation categories.

BROAD_VEGD	GENFORMDES	Func_group	New category
Acacia woodland	woodland	River Coobah woodland	Inland woodland
chenopod shrubland	shrubland <1m	Terrestrial dry shrublands	N/A
chenopod shrubland	shrubland >1m	Terrestrial dry shrublands	N/A
Eucalyptus forest and woodland	forest	River Red Gum woodland	Inland woodland
Eucalyptus forest and woodland	woodland	Black Box woodland	Inland woodland
Eucalyptus forest and woodland	woodland	Black Box woodland	Inland woodland
Eucalyptus forest and woodland	woodland	Black Box woodland	Inland woodland
Eucalyptus forest and woodland	woodland	River Red Gum woodland	Inland woodland
Eucalyptus mallee forest and mallee woodland	mallee woodland	Mallee shrubland	N/A
fernland/herbland	forbland	Terrestrial dry shrublands	N/A
grassland	grassland	Emergent sedgeland	Non woody wetland
grassland	grassland	Flood dependent grasslands	Non woody wetland
hummock grassland	grassland	Flood dependent grasslands	Non woody wetland
Melaleuca forest and woodland	forest	Tea Tree woodland	N/A
rushland/sedgeland	sedgeland	Emergent sedgeland	Non woody wetland
samphire shrubland	shrubland <1m	Samphire shrublands	N/A
shrubland <1m	shrubland <1m	Samphire shrublands	N/A
shrubland >1m	shrubland >1m	Lignum shrubland	Inland shrubland
shrubland >1m	shrubland >1m	Terrestrial dry shrublands	N/A
		Wetlands	Non woody wetland

Table S.2: Vegetation description for Lindsay-Mulcra-Wallpolla region from GIS layer (Modelled 2005 Ecological Vegetation Class (with bioregional conservation status) and newly assigned vegetation categories.

EVC	X_EVCNAME	XGROUPNAME	WRC1	New category
103	Riverine Chenopod Woodland	Riverine Grassy Woodlands or Forests	Black box woodland	Inland Woodland
104	Lignum Swamp	Wetlands	Lignum shrubland	Inland Shrubland
106	Grassy Riverine Forest	Riverine Grassy Woodlands or Forests	Red gum forest	Inland Woodland
107	Lake Bed Herbland	Wetlands	Temporary wetlands	Non woody wetland
200	Shallow Freshwater Marsh	Wetlands	Temporary wetlands	Non woody wetland
295	Riverine Grassy Woodland	Riverine Grassy Woodlands or Forests	Red gum woodland	Inland Woodland
807	Disused Floodway Shrubby Herbland	Wetlands	Alluvial plains	Non woody wetland
808	Lignum Shrubland	Riverine Grassy Woodlands or Forests	Lignum shrubland	Inland Shrubland
809	Floodplain Grassy Wetland	Wetlands	Semipermanent wetlands	Non woody wetland
810	Floodway Pond Herbland	Wetlands	Temporary wetlands	Non woody wetland
811	Grassy Riverine Forest/Floodway Pond Herbland Complex	Riverine Grassy Woodlands or Forests	Red gum forest	Inland Woodland
813	Intermittent Swampy Woodland	Riverine Grassy Woodlands or Forests	Red gum woodland	Inland Woodland
818	Shrubby Riverine Woodland	Riparian Scrubs or Swampy Scrubs and Woodlands	Red gum woodland	Inland Woodland
819	Spike-sedge Wetland	Wetlands	Temporary wetlands	Non woody wetland
820	Sub-saline Depression Shrubland	Salt-tolerant and/or succulent Shrublands	Alluvial plains	Inland Shrubland
823	Lignum Swampy Woodland	Riverine Grassy Woodlands or Forests	Black box woodland	Inland woodland

Table S.3: Vegetation description for South-West New South Wales region from GIS layer (Murray Darling Basin M305 Structural Vegetation Layer. VIS_ID 917) and newly assigned vegetation categories.

Description	New Category
Barren	N/A
C.cristata - v.sparse	N/A
Chenopod shrubland	N/A
Chenopods;Grasses - v.sparse	N/A
Crops & Annual Pastures	N/A
Crops ; Annual Pastures	N/A
Crops and Annual Pastures	N/A
E.camaldulensis - sparse	Inland woodland
E.camaldulensis - v.sparse	Inland woodland
E.camaldulensis -sparse	Inland woodland
E.camaldulensis;E.largiflorens - sparse	Inland woodland
E.camaldulensis;E.largiflorens - v.sparse	Inland woodland
E.camaldulensis;E.largiflorens -isolated	Inland woodland
E.camaldulensis;E.largiflorens -sparse	Inland woodland
E.camaldulensis;E.largiflorens -very sparse	Inland woodland
E.largiflorens - isolated	Inland woodland
E.largiflorens - isolated; on Lignum	Inland woodland
E.largiflorens - very sparse	Inland woodland
E.largiflorens - very sparse; on lignum	Inland woodland
E.largiflorens -v.sparse	Inland woodland
Mosaic L & 9	N/A
Muehlenbeckia	Inland shrubland
Other Plantation	N/A
Permanent grass - v.sparse	N/A
Settlement	N/A
Water*	Non woody wetland
Wetland Herbs	Non woody wetland

*Excludes Lake Victoria

Table S.4: Vegetation description for Millewa region from GIS layer (Native vegetation map: Cohuna, Conargo, Echuca, Mathoura, Moulamein, Tuppal and Wanganella 1:100000 map sheets) and newly assigned vegetation categories.

Vegetation	New Category
Areas with greater than 5% native woody vegetation in cropping or urban environments	N/A
Areas with less than 5% native woody vegetation including: cropping, regrowth grassland which may have been previously cleared and/or cropped, baregr*	N/A
Grassland and/or Forbland	N/A
Grassland and/or Forbland with Isolated Trees	N/A
Mid-high Open Forest to Open Woodland	Inland woodland
Mid-high Shrubland to Sparse Shrubland	N/A
Planted natives	N/A
Tall Open Forest to Open Woodland	Inland woodland
Tall Open Forest to Sedgeland with Isolated Trees	Inland woodland
Tall Open Forest to Woodland	Inland woodland
Tall Open Shrubland and/or Open Chenopod Shrubland to Sparse Shrubland and/or Sparse Chenopod Shrubland	N/A
Tall Woodland to Open Woodland	Inland woodland
Very Tall Rushland	Non woody wetland

Table S.5: Vegetation description for Barmah region from GIS layer (Modelled 2005 Ecological Vegetation Class (with bioregional conservation status)) and newly assigned vegetation categories.

EVC	X_EVCNAME	X_GROUPNAM	New category
56	Floodplain Riparian Woodland	Riverine Grassy Woodlands or Forests	Inland Woodland
106	Grassy Riverine Forest	Riverine Grassy Woodlands or Forests	Inland Woodland
168	Drainage-line Aggregate	Riverine Grassy Woodlands or Forests	Inland Woodland
295	Riverine Grassy Woodland	Riverine Grassy Woodlands or Forests	Inland Woodland
334	Billabong Wetland Aggregate	Wetlands	Non woody wetland
653	Aquatic Herbland	Wetlands	Non woody wetland
803	Plains Woodland	Plains Woodlands or Forests	Inland Woodland
804	Rushy Riverine Swamp	Wetlands	Non woody wetland
809	Floodplain Grassy Wetland	Wetlands	Non woody wetland
810	Floodway Pond Herbland	Wetlands	Non woody wetland
812	Grassy Riverine Forest/Riverine Swamp Forest Complex	Riverine Grassy Woodlands or Forests	Inland Woodland
814	Riverine Swamp Forest	Riverine Grassy Woodlands or Forests	Inland Woodland
815	Riverine Swampy Woodland	Riverine Grassy Woodlands or Forests	Inland Woodland
816	Sedgy Riverine Forest	Riverine Grassy Woodlands or Forests	Inland Woodland
817	Sedgy Riverine Forest/Riverine Swamp Forest Complex	Riverine Grassy Woodlands or Forests	Inland Woodland
819	Spike-sedge Wetland	Wetlands	Non woody wetland
821	Tall Marsh	Wetlands	Non woody wetland
872	Riverine Grassy Woodland/Plains Woodland/Riverine Chenopod Woodland Complex	Riverine Grassy Woodlands or Forests	Inland Woodland
945	Floodway Pond Herbland/Riverine Swamp Forest Complex	Riverine Grassy Woodlands or Forests	Inland Woodland
975	Riverine Ephemeral Wetland	Wetlands	Non woody wetland
1015	Grassy Riverine Forest/Drainage-line Aggregate Mosaic	Riverine Grassy Woodlands or Forests	Inland Woodland

EVC	X_EVCNAME	X_GROUPNAM	New category
1016	Grassy Riverine Forest/Plains Grassy Woodland/Grassy Woodland Mosaic	Riverine Grassy Woodlands or Forests	Inland Woodland
1017	Grassy Riverine Forest/Riverine Grassy Woodland Mosaic	Riverine Grassy Woodlands or Forests	Inland Woodland
1019	Mosaic of Grassy Riverine Forest/Sedgy Riverine Forest-Riverine Swamp Forest Complex	Riverine Grassy Woodlands or Forests	Inland Woodland
1020	Mosaic of Grassy Riverine Forest/Floodway Pond Herbland-Riverine Swamp Forest Complex	Riverine Grassy Woodlands or Forests	Inland Woodland
1021	Mosaic of Drainage-line Aggregate/Grassy Riverine Forest-Riverine Swamp Forest Complex	Riverine Grassy Woodlands or Forests	Inland Woodland
1022	Drainage-line Aggregate/Riverine Swamp Forest Mosaic	Riverine Grassy Woodlands or Forests	Inland Woodland
1023	Drainage-line Aggregate/Sedgy Riverine Forest Mosaic	Riverine Grassy Woodlands or Forests	Inland Woodland
1024	Mosaic of Drainage-line Aggregate/Sedgy Riverine Forest-Riverine Swamp Forest Complex	Riverine Grassy Woodlands or Forests	Inland Woodland
1025	Drainage-line Aggregate/Tall Marsh Mosaic	Riverine Grassy Woodlands or Forests	Inland Woodland
1027	Riverine Grassy Woodland/Grassy Riverine Forest-Riverine Swamp Forest Complex	Riverine Grassy Woodlands or Forests	Inland Woodland
1028	Riverine Grassy Woodland/Riverine Swamp Forest Mosaic	Riverine Grassy Woodlands or Forests	Inland Woodland
1029	Grassy Riverine Forest/Floodway Pond Herbland Mosaic	Riverine Grassy Woodlands or Forests	Inland Woodland
1030	Grassy Riverine Forest/Riverine Swamp Forest Mosaic	Riverine Grassy Woodlands or Forests	Inland Woodland
1032	Floodplain Riparian Woodland/Riverine Grassy Woodland Mosaic	Riverine Grassy Woodlands or Forests	Inland Woodland
1033	Floodplain Riparian Woodland/Floodway Pond Herbland Mosaic	Riverine Grassy Woodlands or Forests	Inland Woodland
1034	Floodplain Riparian Woodland/Riverine Swamp Forest Mosaic	Riverine Grassy Woodlands or Forests	Inland Woodland
1035	Floodplain Riparian Woodland/Sedgy Riverine Forest Mosaic	Riverine Grassy Woodlands or Forests	Inland Woodland
1036	Mosaic of Floodplain Riparian Woodland/Sedgy Riverine Forest-Riverine Swamp Forest Complex	Riverine Grassy Woodlands or Forests	Inland Woodland
1037	Floodplain Riparian Woodland/Tall Marsh Mosaic	Riverine Grassy Woodlands or Forests	Inland Woodland
1038	Low Rises Woodland/Riverine Swampy Woodland Mosaic	Lower Slopes or Hills Woodlands	Inland Woodland
1039	Mosaic of Drainage-line Aggregate/Floodway Pond Herbland-Riverine Swamp Forest Complex	Riverine Grassy Woodlands or Forests	Inland Woodland
1040	Riverine Grassy Woodland/Riverine Swampy Woodland Mosaic	Riverine Grassy Woodlands or Forests	Inland Woodland
1041	Riverine Grassy Woodland/Sedgy Riverine Forest Mosaic	Riverine Grassy Woodlands or Forests	Inland Woodland

EVC	X_EVCNAME	X_GROUPNAM	New category
1042	Mosaic of Riverine Grassy Woodland/Floodway Pond Herbland-Riverine Swamp Forest Complex	Riverine Grassy Woodlands or Forests	Inland Woodland
1043	Aquatic Herbland/Floodplain Grassy Wetland Mosaic	Wetlands	Non woody wetland
1044	Aquatic Herbland/Floodway Pond Herbland Mosaic	Wetlands	Non woody wetland
1045	Aquatic Herbland/Riverine Swamp Forest Mosaic	Wetlands	Inland Woodland
1046	Mosaic of Aquatic Herbland/Sedgy Riverine Forest-Riverine Swamp Forest Complex	Wetlands	Inland Woodland
1047	Aquatic Herbland/Tall Marsh Mosaic	Wetlands	Non woody wetland
1048	Mosaic of Aquatic Herbland/Floodway Pond Herbland-Riverine Swamp Forest Complex	Wetlands	Inland Woodland
1049	Floodplain Grassy Wetland/Floodway Pond Herbland Mosaic	Wetlands	Non woody wetland
1050	Mosaic of Floodplain Grassy Wetland/Grassy Riverine Forest-Riverine Swamp Forest Complex	Wetlands	Inland Woodland
1051	Floodplain Grassy Wetland/Riverine Swamp Forest Mosaic	Wetlands	Inland Woodland
1052	Floodplain Grassy Wetland/Riverine Swampy Woodland Mosaic	Wetlands	Inland Woodland
1053	Mosaic of Floodplain Grassy Wetland/Sedgy Riverine Forest-Riverine Swamp Forest Complex	Wetlands	Inland Woodland
1054	Floodplain Grassy Wetland/Spike-sedge Wetland Mosaic	Wetlands	Non woody wetland
1055	Floodplain Grassy Wetland/Tall Marsh Mosaic	Wetlands	Non woody wetland
1056	Mosaic of Floodplain Grassy Wetland/Floodway Pond Herbland-Riverine Swamp Forest Complex	Wetlands	Inland Woodland
1057	Mosaic of Floodway Pond Herbland/Grassy Riverine Forest-Riverine Swamp Forest Complex	Wetlands	Inland Woodland
1058	Floodway Pond Herbland/Riverine Swamp Forest Mosaic	Wetlands	Inland Woodland
1059	Mosaic of Floodway Pond Herbland/Sedgy Riverine Forest-Riverine Swamp Forest Complex	Wetlands	Inland Woodland
1060	Floodway Pond Herbland/Tall Marsh Mosaic	Wetlands	Non woody wetland
1061	Mosaic of Grassy Riverine Forest-Riverine Swamp Forest Complex/Riverine Swamp Forest	Riverine Grassy Woodlands or Forests	Inland Woodland
1062	Grassy Riverine Forest/Riverine Swampy Woodland Mosaic	Riverine Grassy Woodlands or Forests	Inland Woodland
1063	Grassy Riverine Forest/Sedgy Riverine Forest Mosaic	Riverine Grassy Woodlands or Forests	Inland Woodland
1065	Grassy Riverine Forest/Tall Marsh Mosaic	Riverine Grassy Woodlands or Forests	Inland Woodland

EVC	X_EVCNAME	X_GROUPNAM	New category
1067	Riverine Swamp Forest/Riverine Swampy Woodland Mosaic	Riverine Grassy Woodlands or Forests	Inland Woodland
1068	Riverine Swamp Forest/Sedgy Riverine Forest Mosaic	Riverine Grassy Woodlands or Forests	Inland Woodland
1069	Riverine Swamp Forest/Sedgy Riverine Forest-Riverine Swamp Forest Complex	Riverine Grassy Woodlands or Forests	Inland Woodland
1070	Riverine Swamp Forest/Spike-sedge Wetland Mosaic	Riverine Grassy Woodlands or Forests	Inland Woodland
1071	Riverine Swamp Forest/Tall Marsh Mosaic	Riverine Grassy Woodlands or Forests	Inland Woodland
1072	Mosaic of Riverine Swamp Forest/Floodway Pond Herbland-Riverine Swamp Forest Complex	Riverine Grassy Woodlands or Forests	Inland Woodland
1073	Riverine Swampy Woodland/Sedgy Riverine Forest Mosaic	Riverine Grassy Woodlands or Forests	Inland Woodland
1074	Mosaic of Riverine Swampy Woodland/Sedgy Riverine Forest-Riverine Swamp Forest Complex	Riverine Grassy Woodlands or Forests	Inland Woodland
1075	Mosaic of Sedgy Riverine Forest/Sedgy Riverine Forest-Riverine Swamp Forest Complex	Riverine Grassy Woodlands or Forests	Inland Woodland
1076	Sedgy Riverine Forest/Spike-sedge Wetland Mosaic	Riverine Grassy Woodlands or Forests	Inland Woodland
1077	Sedgy Riverine Forest/Tall Marsh Mosaic	Riverine Grassy Woodlands or Forests	Inland Woodland
1078	Mosaic of Sedgy Riverine Forest/Floodway Pond Herbland-Riverine Swamp Forest Complex	Riverine Grassy Woodlands or Forests	Inland Woodland
1079	Mosaic of Sedgy Riverine Forest-Riverine Swamp Forest Complex/Tall Marsh	Riverine Grassy Woodlands or Forests	Inland Woodland
1080	Mosaic of Sedgy Riverine Forest-Riverine Swamp Forest Complex/Floodway Pond Herbland-Riverine Swamp Forest Complex	Riverine Grassy Woodlands or Forests	Inland Woodland
1081	Spike-sedge Wetland/Tall Marsh Mosaic	Wetlands	Non woody wetland
1082	Tall Marsh/Riverine Swamp Forest Mosaic	Wetlands	Inland Woodland
1083	Mosaic of Tall Marsh/Floodway Pond Herbland-Riverine Swamp Forest Complex	Wetlands	Inland Woodland
1084	Tall Marsh/Non-Vegetation Mosaic	Wetlands	Non woody wetland

Table S.6: Vegetation descriptions for Macquarie Marshes from the 2013 Plant Community Type (PCT) vegetation map (Bowen and Fontaine 2014*) and newly assigned vegetation categories.

PCT Number	NSW OEH Plant Community Type (PCT) Name	OEH Vegetation Class (Keith 2004)	EWKR Veg Class
181	Common Reed-Bush groundsel aquatic tall reedland grassland wetland	Inland Floodplain Swamps;	Non-woody wetland
182	Cumbungi rushland wetland of shallow semi-permanent water bodies and inland watercourses	Inland Floodplain Swamps;	Non-woody wetland
204	Water Couch marsh grassland wetland	Inland Floodplain Swamps;	Non-woody wetland
238	Permanent and semi-permanent freshwater lagoons	Inland Floodplain Swamps;	Non-woody wetland
53	Shallow freshwater wetland sedgeland	Inland Floodplain Swamps;	Non-woody wetland
36	River Red Gum tall to very tall open forest (wetland)	Inland Riverine Forests;	Inland Woodlands
36 - woodland	River Red Gum tall woodland (wetland)	Inland Riverine Forests;	Inland Woodlands
Baradine red gum	Baradine red gum	Inland Riverine Forests	Inland Woodlands
241	River Coobah - lignum swamp wetland	Inland Floodplain Shrublands;	Inland Shrublands
247	Lignum shrubland wetland	Inland Floodplain Shrublands;	Inland Shrublands
37	Black Box woodland wetland	North-west Floodplain Woodlands;	Inland Woodlands
40	Coolibah open woodland wetland	North-west Floodplain Woodlands;	Inland Woodlands
454	River Red Gum grassy chenopod open tall woodland (wetland)	Inland Floodplain Woodlands;	Inland Woodlands
144	Leopardwood low woodland	North-west Plain Shrublands;	N/A
144 - Lime bush	Lime bush (Citrus glauca) thickets	North-west Plain Shrublands;	N/A
145	Western Rosewood - Wilga - Belah low woodland	Western Peneplain Woodlands;	N/A
158	Old man Saltbush-mixed chenopod shrubland	Riverine Chenopod Shrublands;	N/A
206	Dirty Gum-White Cypress Pine tall woodland	North-west Alluvial Sand Woodlands;	N/A

212	Chenopod low open shrubland	Riverine Chenopod Shrublands;	N/A
250	Derived tussock grassland	Western Slopes Grasslands;	N/A
27	Weeping Myall open woodland	Riverine Plain Woodlands;	N/A
332	Tumbledown Red Gum - Black Cypress Pine - Red Stringybark woodland	Inland Rocky Hill Woodlands;	N/A
55	Belah Woodland	North-west Floodplain Woodlands;	N/A
55 - Budda	Budda thicket	North-west Floodplain Woodlands;	N/A
70	White Cypress Pine woodland	Floodplain Transition Woodlands;	N/A
98	Poplar Box - White Cypress Pine - Wilga woodland	Western Peneplain Woodlands;	N/A
Derived chenopod shrubland	Derived chenopod shrubland	Riverine Chenopod Shrublands;	N/A
Cultivated	Cultivated land	Cleared	N/A
Infrastructure	Infrastructure	Cleared	N/A
Cleared	Cleared	Cleared	N/A
watercourse	Watercourse	NA	N/A

*Bowen, S. and Fontaine, K., 2014. 2013 Vegetation Map of the Macquarie Marshes and Floodplain. NSW Office of Environment and Heritage, Sydney.

Table S.7: Vegetation descriptions for the Narran Lakes from the 2014 Plant Community Type (PCT) vegetation map (Eco Logical Australia 2015*) and newly assigned vegetation categories.

PCT Number	NSW OEH Plant Community Type (PCT) Name	OEH Vegetation Class (Keith 2004)	EWKR Veg Class
1000	Canegrass swamp tall grassland wetland of drainage depressions, lakes and pans of the inland plains	Inland Floodplain Swamps	Non-woody wetland
181	Common Reed - Bushy Groundsel aquatic tall reedland grassland wetland of inland river systems	Inland Floodplain Swamps	Non-woody wetland
238a	Ephemeral herbaceous vegetation of the channels of major and minor watercourses of western NSW	Inland Floodplain Swamps	Non-woody wetland
43a	Grassland - chenopod low open shrubland on floodplains in the semi-arid (hot) and arid zones	Inland Floodplain Swamps	Non-woody wetland
238	Non-woody water dependent vegetation / Ephemeral Freshwater wetlands	Inland Floodplain Swamps	Non-woody wetland
238	Permanent and semi-permanent freshwater lakes wetland of the inland slopes and plains	Inland Floodplain Swamps	Non-woody wetland
53	Shallow freshwater wetland sedgeland in depressions on floodplains on inland alluvial plains and floodplains	Inland Floodplain Swamps	Non-woody wetland
1005	Sparse saltbush forbland wetland of the irregularly inundated lakes of the arid and semi-arid (persistently hot) climate zones	Inland Floodplain Swamps	Non-woody wetland
62	Samphire saline shrubland/forbland wetland of lake beds and lake margins in the arid and semi-arid (hot) zones	Inland Saline Lakes	Non-woody wetland
247a	Lignum open shrubland wetland on regularly flooded alluvial plains	Inland Floodplain Shrublands	Inland shrublands
247	Lignum shrubland wetland on regularly flooded alluvial depressions in the Brigalow Belt South Bioregion and Darling Riverine Plains Bioregion	Inland Floodplain Shrublands	Inland shrublands
160	Nitre Goosefoot shrubland wetland on clays of the inland floodplains	Inland Floodplain Shrublands	Inland shrublands
241	River Cooba swamp wetland on the floodplains of the Darling Riverine Plains Bioregion and Brigalow Belt South Bioregion	Inland Floodplain Shrublands	Inland shrublands
36	River Red Gum tall to very tall open forest / woodland wetland on rivers on floodplains mainly in the Darling Riverine Plains Bioregion	Inland Riverine Forests	Inland woodland
38	Black Box low woodland wetland lining ephemeral watercourses or fringing lakes and clay pans of semi-arid (hot) and arid zones	North-west Floodplain Woodlands	Inland woodland
37	Black Box woodland wetland on NSW central and northern floodplains including the Darling Riverine Plains Bioregion and Brigalow Belt South Bioregion	North-west Floodplain Woodlands	Inland woodland

1005	Coolibah	North-west Floodplain Woodlands	Inland woodland
39	Coolibah - River Cooba - Lignum woodland wetland of frequently flooded floodplains mainly in the Darling Riverine Plains Bioregion	North-west Floodplain Woodlands	Inland woodland
40	Coolibah open woodland wetland with chenopod/grassy ground cover on grey and brown clay floodplains	North-west Floodplain Woodlands	Inland woodland
29	Brigalow open woodland on clay soils in the Nyngan-Bourke-Enngonia regions of the NSW north-western plains	Brigalow Clay Plains Woodlands	N/A
224	Cotton Bush - copperburr open shrubland of the arid climate zone	Gibber Chenopod Shrublands	N/A
197	Black Box - Gidgee - chenopod low open woodland wetland on alluvial clay soils in the Culgoa River region of the Darling Riverine Plains Bioregion and Mulga Lands Bioregion	Gibber Transition Shrublands	N/A
118	Gidgee chenopod woodland on red-brown clays in the semi-arid (hot) climate zone mainly in the Mulga Lands Bioregion	Gibber Transition Shrublands	N/A
55	Belah woodland on alluvial plains and low rises in the central NSW wheatbelt to Pilliga and Liverpool Plains regions	North-west Floodplain Woodlands	N/A
144	Leopardwood low woodland mainly on clayey soils in the semi-arid zone	North-west Floodplain Woodlands	N/A
207	Poplar Box grassy low woodland of drainage lines and depressions of the semi-arid (hot) and arid zone climate zones	North-west Floodplain Woodlands	N/A
212	Chenopod low open shrubland - ephemeral partly derived forbland saline wetland on occasionally flooded pale clay scalds in the NSW North Western Plains	Riverine Chenopod Shrublands	N/A
377	Copperburr low open shrubland on loam - clay flats and playas, western Brigalow Belt South Bioregion and northern Darling Riverine Plains Bioregion	Riverine Chenopod Shrublands	N/A
168	Derived Copperburr shrubland of the NSW northern inland alluvial floodplains	Riverine Chenopod Shrublands	N/A
163	Dillon Bush (Nitre Bush) shrubland of the semi-arid and arid zones	Riverine Chenopod Shrublands	N/A

158	Old Man Saltbush - mixed chenopod shrubland of the semi-arid hot (persistently dry) and arid climate zones (north-western NSW)	Riverine Chenopod Shrublands	N/A
211	Slender Saltbush - samphire - copperburr low open shrubland wetland on irregularly inundated floodplains mainly in the Darling Riverine Plains Bioregion and Brigalow Belt South Bioregion	Riverine Chenopod Shrublands	N/A
27	Weeping Myall open woodland of the Darling Riverine Plains Bioregion and Brigalow Belt South Bioregion	Riverine Plains Woodlands	N/A
1005	Grassland - chenopod low open shrubland on floodplains in the semi-arid (hot) and arid zones	Semi-arid Floodplain Grasslands	N/A
43	Mitchell Grass grassland - chenopod low open shrubland on floodplains in the semi-arid (hot) and arid zones	Semi-arid Floodplain Grasslands	N/A
146	Whitewood low open woodland of the Brigalow Belt South Bioregion and north-eastern Darling Riverine Plains Bioregion	Subtropical Semi-arid Woodlands	N/A
98	Poplar Box - White Cypress Pine - Wilga - Ironwood shrubby woodland on red sandy-loam soils in the Darling Riverine Plains Bioregion and Brigalow Belt South Bioregion	Western Peneplain Woodlands	N/A

*Eco Logical Australia 2015. Vegetation of the Barwon-Darling and Condamine-Balonne floodplain systems of New South Wales: Mapping and survey of plant community types. Prepared for Murray-Darling Basin Authority

Appendix V3.4: Field Site Assessment and Germination Trial Research Activity Report

Authors alphabetically (and institution): Cherie Campbell (LTU), Sam Capon (Griffith University), Susan Gehrig (MDFRC), Cassandra James (James Cook University), Kay Morris (Arthur Rylah Institute), Jason Nicol (SARDI), Daryl Nielsen (CSIRO), Gary Palmer (Griffith University), Rachael Thomas (NSW OEH / UNSW)

Document purpose: Summary document to capture the outputs and outcomes from the Field Site Assessment and Germination Trial component of the MDB EWKR Vegetation theme. This document complements and refers to other outputs rather than duplicates information.

- ## Research Question

This field site assessment and germination trial component sought to address the broad research question '*what drives vegetation responses to watering actions?*' by assessing the influence of location, flood-return-frequency and vegetation structure on extant understory communities and seed bank diversity.

- ## Methods

For detailed methods refer to Campbell et al. 2019 Field Assessment Experimental Design report (Appendix V3.1) as well as methods described in Campbell et al. (draft), *Vulnerability of resilient systems to the Anthropocene* (Appendix V3.3). This section provides a summary of the methods and highlights any deviations from the methods document above.

The component primarily addressed understory diversity, however data was also collected on woody recruitment and survival and condition of long-lived woody vegetation and lignum.

The field site assessments and germination trials addressed the broad research question 'w*hat drives vegetation responses to watering actions?'* This study assessed the influence of location, flood-return-frequency and vegetation structure on understory plant assemblages. There was emerging evidence to suggest location is a key driver of vegetation assemblages (LTIM and TLM data). The influence of location has important implications for the transferability of predications and prioritisation processes at the Basin-scale. Wetland vegetation is typically sensitive to hydrologic changes, and studies within wetlands have frequently found spatial patterns of seed bank species richness and density related to flood history (e.g. Holzel and Otte 2001, Capon and Brock 2006). Structural vegetation classes for this study were defined along an assumed canopy-cover gradient from none to low fringing cover in non-woody wetlands, to lignum woody cover in inland shrublands, to highest canopy cover in inland woodlands. The presence of overstory or perennial shrubs can influence extant vegetation and seed banks. Trees and shrubs compete with herbaceous vegetation for water and nutrients and reduce light availability, which affects reproductive success. However, the dappled shade provided by floodplain eucalypts reduces soil and air temperature whilst allowing light penetration and the protection from grazing offered by the shrub *Duma florulenta* (Meisn.) T.M.Schust. can provide nursery habitat for herbaceous species (James et al., 2015). The provision of leaf litter by overstory species has both positive and negative impacts; litter reduces evaporation of soil moisture and reduces soil temperature but may prevent seeds from germinating by providing a physical barrier or allelopathic compounds (particularly floodplain eucalypts) (May and Ash, 1990; Moradshahi et al., 2003; Sasikumar et al., 2002). Due to the influence of the existing canopy, recruitment may not always occur in woodland environments and may occur in neighbouring open patches

A total of 180 sites were surveyed applying a factorial design with five sites in all levels of each factor: four geographical regions (Mid-Murray, Lower-Murray, Macquarie Marshes and Narran Lakes) of the Murray-Darling Basin (MDB), three flood intervals (near annual, 1.5-3 years, 3-5 years and 5-10 years) and three vegetation structural types (non-woody, inland shrublands and inland woodlands). Sites were surveyed on two occasions, in autumn 2017 and 2018. The hydrological phase of each wetland at the time of survey (e.g. inundated, flow recession, dry) varied between the wetlands.

Not all strata were present or represented at sufficient spatial scale to be relevant at all four locations (Table 1). Where strata were relevant and sampled, five replicate sites were established within each strata.

For the seed bank component, we germinated the soil under both damp and submerged treatments for approximately six months. All seed bank samples were a composite of 10 random soil samples, from the top five cm of soil, collected within quadrats. Due to differences in the total volume of soil collected (once air-dried), the Lower Murray and Mid Murray had three replicate pots per treatment, per strata, Narran Lakes had two replicate pots and Macquarie Marshes had one.

To address our overarching research priorities for both non-woody wetland vegetation as well as woody seedling recruitment, we looked at the responses of these aspects separately. Due to the structural importance of lignum to processes such as waterbird breeding, we also looked at the structural response of lignum.

Data analysis

Extant understory vegetation

Plant species recorded in both the point-intercept surveys and as incidental records were combined to represent the full compliment of species recorded in the 20m x 20m plots. Non-metric multidimensional scaling (nMDS) was used to display differences or similarities in community composition. This was undertaken in Primer V7 using a Bray-Curtis resemblance matrix derived from the square-root transformed plant species data.

SIMPER (similarity percentages) analysis was undertaken in Primer V7 (Clarke and Gorley 2015) to determine the species contributing to ~70% of the similarity within strata (e.g. within Lower Murray-Inland Shrubland-flow category C2).

Woody seedlings

Counts of woody seedlings recorded in the <20cm size category were graphed in Microsoft Excel.

Lignum structure

Measurements of lignum height and width for individual clumps were used to estimate the volume of lignum, assuming a cylindrical shape (Volume = $\pi r^2 h$). Data from the autumn 2018 survey was used. Where sites were unable to be accessed in 2018, data from 2017 was substituted. As all clumps within a 20m x 20m plot were measured, estimates of lignum volume per hectare were calculated (20m x 20m plots = 0.04 ha x 25). We acknowledge this is likely to be an overestimate of volume as clumps probably tend to resemble more of an elliptical shape in cross section. We also acknowledge that the density of clumps is unlikely to be uniform across a hectare.

Lignum is only a minor component of the vegetation communities in the Mid-Murray so this location was excluded from the analysis. Lignum was only recorded from a handful of sites in the Non-woody wetland vegetation class, so results are only presented for Inland Shrubland and Inland Woodland.

Table 1: Flow and vegetation strata monitored at each of the four locations. Blank cells represent un- or insufficiently represented strata within the location

Vegetation structural category	Flood Return Frequency			
	Lower Murray	Mid Murray	Macquarie Marshes	Narran Lakes
		Near annual	Near annual	Near annual

Non-Woody Wetland	1.5 – 3 years	1.5 – 3 years	1.5 – 3 years	1.5 – 3 years
	3 – 5 years		3 – 5 years	3 – 5 years
	5 – 10 years		5 – 10 years	5 – 10 years
Inland Shrubland				Near annual
	1.5 – 3 years		1.5 – 3 years	1.5 – 3 years
	3 – 5 years		3 – 5 years	3 – 5 years
	5 – 10 years		5 – 10 years	5 – 10 years
Inland Woodland		Near annual	Near annual	Near annual
	1.5 – 3 years	1.5 – 3 years	1.5 – 3 years	
	3 – 5 years	3 – 5 years	3 – 5 years	3 – 5 years
	5 – 10 years		5 – 10 years	5 – 10 years

• Results

A summary of results can be found in the MDB EWKR Vegetation Theme summary report (the document to which this is an appendix).

Communication of results can also be found in the following appendices within the MDB EWKR Vegetation Theme summary report.

- Presentation: Campbell et al. 2018. *From the four corners of the Basin: assessing vegetation responses to flow regimes*. Ecological Society of Australia conference, Brisbane, 25-29 November 2018. (Appendix V3.2)
- Paper: Campbell et al. (draft). *Vulnerability of resilient systems to the Anthropocene* (target journal Global Change Biology) (Appendix V3.3)

Outputs also include two datasets: Dataset 1: Extant field site assessment and Dataset 2: Seed bank germination (refer Appendix V5.1, Theme data inventory) and results presented in this document.

Results are only presented here where they are additional to results presented in Campbell et al. (draft) *Vulnerability of resilient systems to the Anthropocene* (Appendix 3.3).

Non-woody extant understorey vegetation and comparisons with seed bank data

Extant understorey vegetation data

In the extant field surveys 407 species were recorded in total (Figure 1), across both years (2017 and 2018), and in both point-intercept surveys and records of incidental species in quadrats. Species recorded ranged from terrestrial herbs and shrubs to flow-responsive wetland species (Appendix 1).

There was an overwhelming influence of location on both the extant vegetation and seed bank communities, with each of the four locations having quite distinct assemblages (Figure 2, extant vegetation). Across all four locations there are no clear patterns in terms of vegetation class or flow category (Figure 3, extant vegetation).

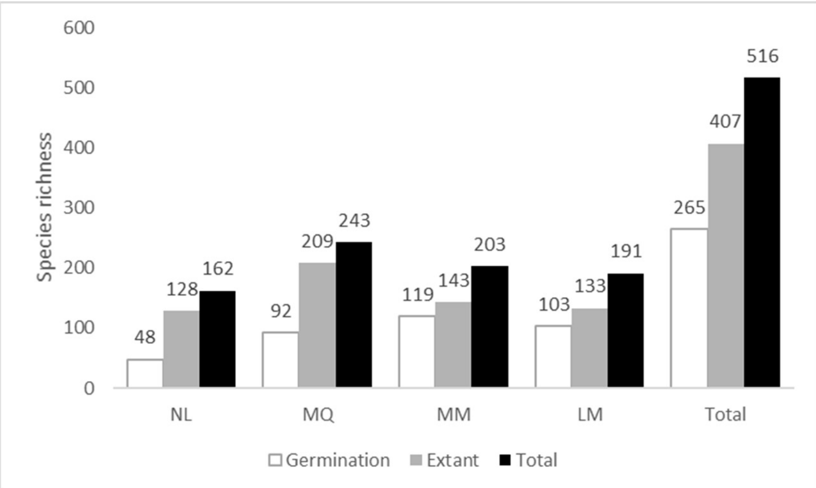

Figure 1: Plant species richness recorded in germination trials, extant field surveys and in total (combined) at the four locations (NL = Narran Lakes, MQ = Macquarie Marshes, MM = Mid-Murray, LM = Lower Murray). NB. Different numbers of strata were surveyed at each location which in turn reflects the number of strata germinated in seed bank trials (see Table 1 and Figure 2).

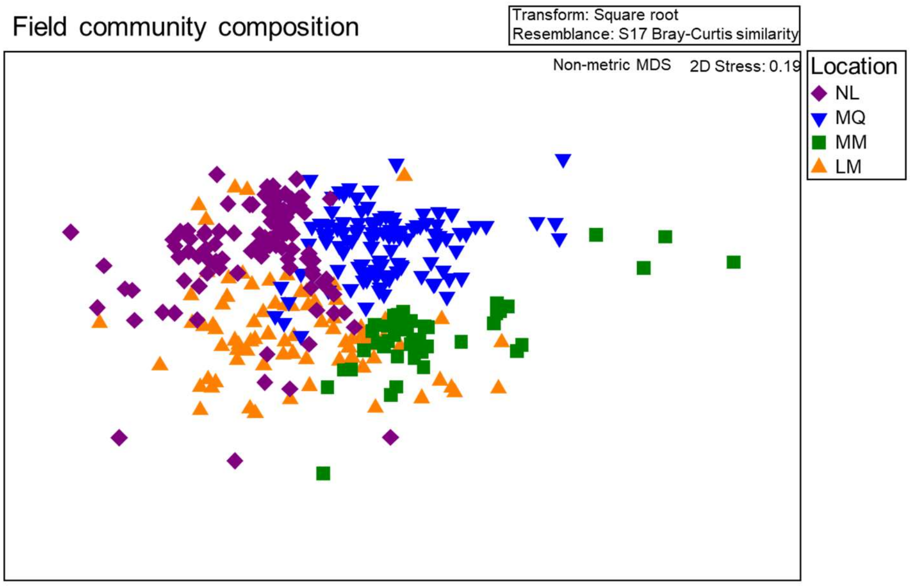

Figure 2: nMDS displaying differences in community composition based on extant understorey data at four locations (NL = Narran Lakes, MQ = Macquarie Marshes, MM = Mid-Murray, LM = Lower Murray). NB. 11 outliers have been removed to enable display of the data, four from LM, five from MM, and one each from MQ and NL. All points were from the Non-woody wetlands vegetation class and contained one recorded species (7 points) or very few recorded species (4 points).

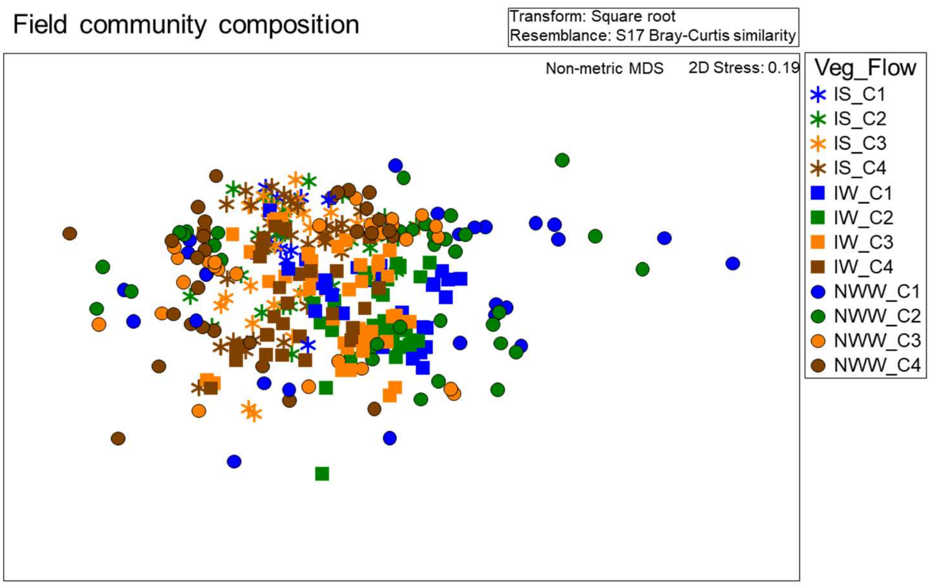

Figure 3: nMDS displaying differences in community composition based on extant understorey data stratified according to four locations (NL = Narran Lakes, MQ = Macquarie Marshes, MM = Mid-Murray, LM = Lower Murray), three vegetation classes (NWW = Non-woody wetlands, IS = Inland Shrubland and IW = Inland Woodland) and four flow categories (C1 = near annual, C2 = 1 flow in 1.5 to 3 years, C3 = 1 flow in 3 to 5 years, C4 = 1 flow in 5 to 10 years). NB. 11 outliers have been removed to enable display of the data, four from LM, five from MM, and one each from MQ and NL. All points were from the Non-woody wetlands vegetation class and contained one recorded species (7 points) or very few recorded species (4 points).

Within each location, there were also different influences on the understory vegetation response. For example, there was a strong relationship with flood frequency and extant vegetation communities at the Macquarie Marshes, a strong-moderate relationship at the Mid Murray and only weak relationships at Narran Lakes and the Lower Murray (as evidenced by gradients in colour, Figure 4). In relation to vegetation structure and extant vegetation communities, there were strong relationships at Narran Lakes and the Mid Murray but only weak relationships at the Macquarie Marshes and Lower Murray (as evidenced by separation of symbols, Figure 4).

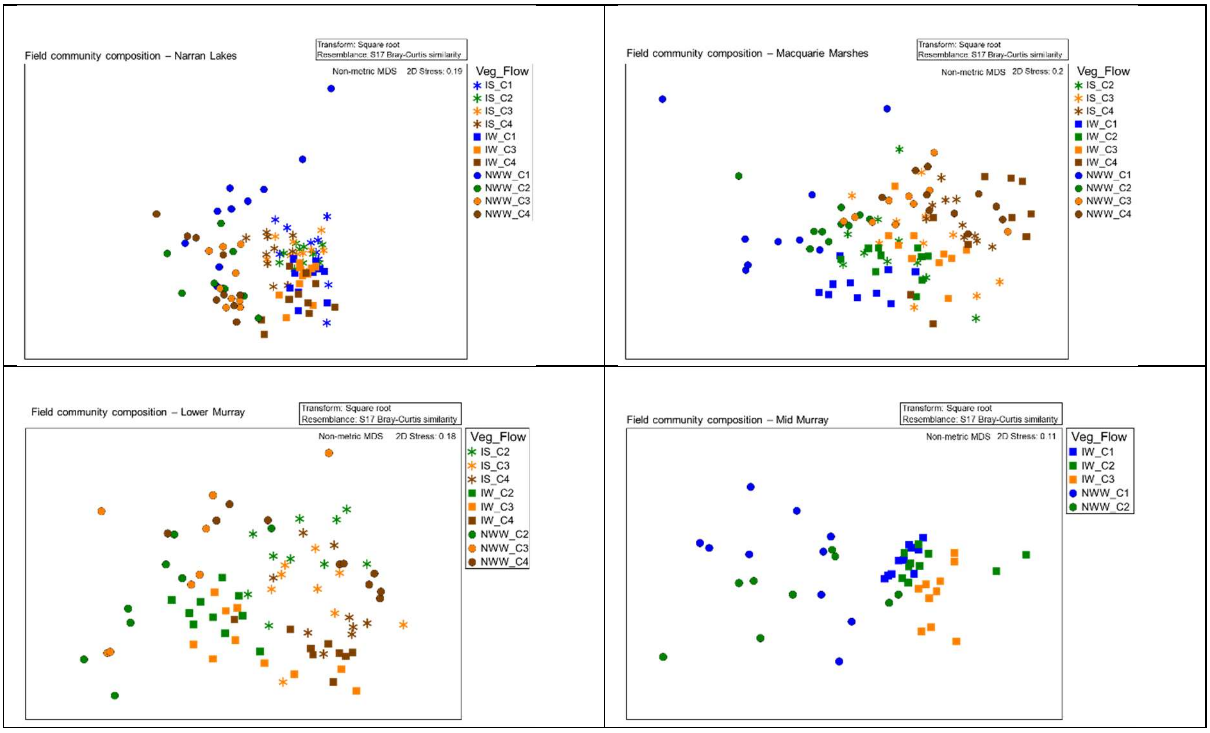

Figure 4: nMDS plots from each of four locations (NL = Narran Lakes, MQ = Macquarie Marshes, MM = Mid-Murray, LM = Lower Murray), displaying differences in community composition based on extant understorey data stratified according to three vegetation classes (NWW = Non-woody wetlands, IS = Inland Shrubland and IW = Inland Woodland) and four flow categories (C1 = near annual, C2 = 1 flow in 1.5 to 3 years, C3 = 1 flow in 3 to 5 years, C4 = 1 flow in 5 to 10 years). NB. To display the data 4 outliers have been removed from the Lower Murray and one from Narran Lakes.

Species characteristic of each of the strata at each of the locations are given in Appendix 2.

Comparisons between extant understorey vegetation data and seed bank data

Across both the extant and germination studies there was relatively high species richness with more than 500 species recorded in total (Figure 1.). There were few shared species between the extant field surveys and the germination trials, with only ~30% of species recorded in both.

There was also relatively limited recorded spatial occurrence of species with ~30% of species only recorded from a single location-vegetation-flow strata (e.g. Macquarie Marshes-Non-woody wetlands-Flow category 3 is a single strata, with five replicate sites). Species richness varied between location, vegetation class and flow category (Figure 5).

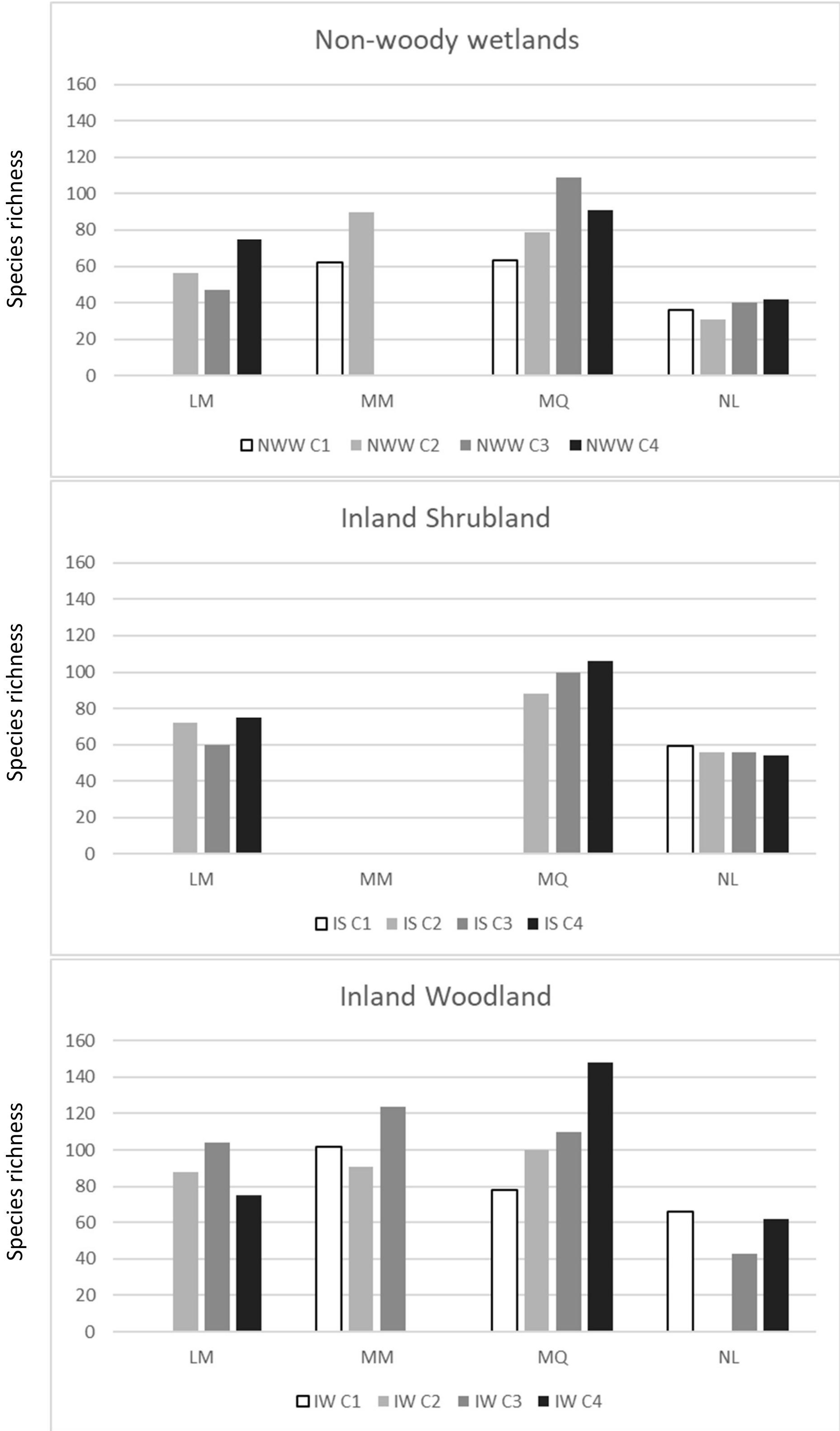

Figure 5: Plant species richness recorded in both germination trials and extant field surveys at four locations (NL = Narran Lakes, MQ = Macquarie Marshes, MM = Mid-Murray, LM = Lower Murray), in three different vegetation classes (Non-woody wetlands, Inland Shrubland and Inland Woodland) and in four different flow categories (C1 = near annual, C2 = 1 flow in 1.5 to 3 years, C3 = 1 flow in 3 to 5 years, C4 = 1 flow in 5 to 10 years) . NB. There are five replicate sites in each strata. Not all strata are represented within each Location (see Table 1).

Five species commonly recorded in the germination trials were *Cyperus difformis*, *Ammannia multiflora, Centipeda cunninghamii, Alternanthera denticulata* and *Elatine gratioloides*. Of these both *Alternantherna deticulata* and *Centipeda cunninghamii* were relatively common in the field (based on counts of occurrence of sites, not abundance). *Cyperus difformis* had patchy occurrence in the field and was mostly recorded at the Macquarie Marshes, however this species was present at all four locations in the germination trials and particularly common at the Lower Murray and Narran Lakes. *Ammannia multiflora* and *Elatine gratioloides* had limited occurrence in the field, however both species, particularly *Ammannia multiflora*, were common in germination trials.

A number of flood responsive species were only recorded in the germination trials and not in the extant field surveys. These included *Bergia ammonioides, Callitriche sonderi, Eleocharis pallens, Isolepis australiensis, Lipocarpha microcephala* and *Myosurus australis*.

Interesting finds

Of interest was the record of *Schoenoplectiella dissachantha* from a non-woody wetland site, flow category C4 (1 flow in 5 to 10 years), in the Lower Murray (Chowilla Floodplain, SA) (Figure 6). This species (a single individual) was recorded in the germination trials in a submerged treatment. The extant community at this location was dominated by chenopods such as *Sclerolaena tricuspis, S. brachyptera* and *Atriplex lindleyi*. The last recorded occurrence of *Schoenoplectiella dissachantha* in the Southern Basin was in 1994 from Lyrup in South Australia (on flooded ground) (ALA 2019).

Figure 6: (a) *Schoenoplectiella dissachantha* recorded from a submerged treatment in the germination trials and (b) imagine of the non-woody wetland at Chowilla Floodplain in the Lower Murray (LM_NWW_C4_4) where the soil for the germination trials was collected (J. Nicol, SARDI, April 2017)

Woody seedlings

Recruitment of woody species was relatively sparse and patchy across the four locations. In terms of woody seedling recruitment (defined here as less than 20 cm high), the number of seedlings recorded at an individual site, for all woody tree species, ranged from 0 to 180. Average seedling recruitment varied between location, vegetation class and flow category (Figure 7). Only one woody seedling was recorded at Macquarie Marshes. The average number of seedlings recorded in veg-flow strata in the Mid-Murray ranged from 0 to 1.8 seedlings. Averages were slightly higher at both Narran Lakes and the Lower Murray with average seedling numbers per strata ranging from 0 to 18.2 and 0 to 26.1 respectively. Seedling numbers were greatest in Inland Woodland vegetation categories, however seedlings were recorded from all three vegetation classes. Seedlings were also recorded from all four flow categories. There is no clear pattern in terms of seedling recruitment and veg-flow strata. Woody seedlings (<20cm) were recorded for seven different species (*Eucalyptus camaldulensis, E. largiflorens, E. coolabah, E. melliodora, Acacia stenophylla, Duma florulenta,* and *Exocarpos strictus*).

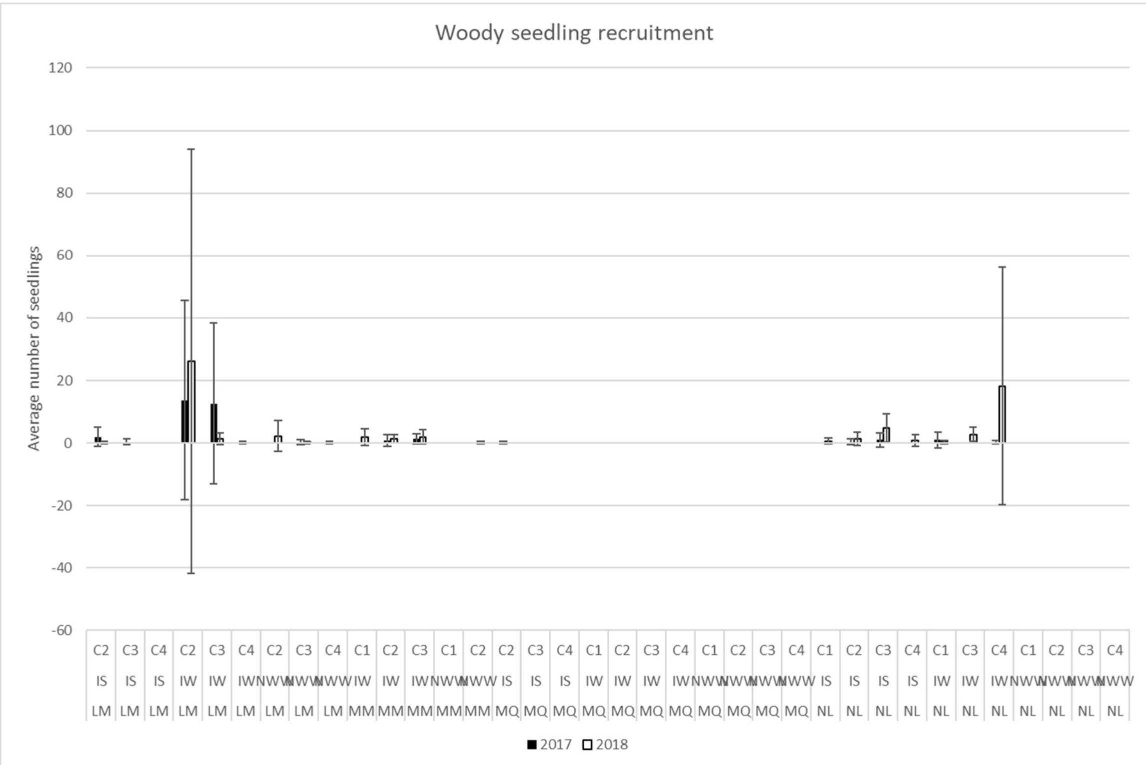

Figure 7: Mean number of woody seedlings (± s.d.) recorded in 2017 and 2018 at four locations (NL = Narran Lakes, MQ = Macquarie Marshes, MM = Mid-Murray, LM = Lower Murray), in three different vegetation classes (NWW = Non-woody wetlands, IS = Inland Shrubland and IW = Inland Woodland) and in four different flow categories (C1 = near annual, C2 = 1 flow in 1.5 to 3 years, C3 = 1 flow in 3 to 5 years, C4 = 1 flow in 5 to 10 years) . NB. There are five replicate sites in each strata. Not all strata are represented within each Location (see Table 1).

Lignum structure

As only scarce lignum individuals were detected in the Mid-Murray and all Non-woody wetland categories, data are displayed for the Lower Murray, Macquarie Marshes and Narran Lakes for Inland Shrublands and Inland Woodlands.

The most frequently inundated lignum (flow category C1) had the greatest volume at both Macquarie Marshes (in Inland Woodlands) and Narran Lakes (in Inland Shrubland) (Figure 8). At Narran Lakes there is a monotonic decrease in lignum volume with a decrease in flood return frequency at both Inland Shubland and Inland Woodland sites. There is also a humped relationship with lignum density and flood return frequency. Fewer clumps are present in the mostly frequently inundated sites (C1) and in the less frequently inundated sites (C4), while greater numbers of clumps are present in flow categories C2 and C3. Combined with the monotonic decrease in lignum volume, this means there are a smaller number of very large clumps in the frequently inundated sites (C1), greater numbers of small clumps in the moderately inundated sites (C2 and C3), and smaller numbers of small clumps in the less frequently inundated sites (C4). A slightly similar relationship exists at Macquarie Marshes, with a decline in volume with decreasing flood return frequency for sites in Inland Woodland. Average volume is relatively similar across flow categories for Inland Shrubland sites. Lignum density displays a similar humped relationship for Inland Shrubland sites at Macquarie Marshes. However, there is a general decline in the number of lignum clumps with declining flood return frequency for Inland Woodland sites at Macquarie Marshes.

In contrast, volume of lignum is detectably lower at the Lower Murray sites (Figure 8). At the Lower Murray, there is a decline in the number of clumps with declining flood return frequency, coupled with generally much lower volume across all flow categories (noting no sites were sampled in the C1 category).

The width and height of some of the recorded lignum clumps greatly exceeds the size mentioned in existing literature. Roberts and Marston (2011) refer to lignum clumps as being up to 3m in diameter and 2 to 3m tall. The largest recorded clump in our dataset was 4m high and 20m wide. Height values ranged up to 6.3m tall, with more than 60 clumps recorded as 3m tall or higher. Sixteen clumps were recorded as having width's of 10m or more.

Of the 2,705 clumps of lignum recorded across the four locations, 394 clumps (14.5%) scored a 0 for lignum condition index (LCI) (i.e. 0 for viability and 0 for colour). As lignum can re-grow from root stock, defining a plant as dead is problematic (see Freestone et al. 2017), despite this an LCI of 0 infers death, dormancy or extremely poor condition. Of the 394 clumps of lignum with an LCI score of 0, one (0.3%) was from Macquarie Marshes (IS_C3), 49 (12.4%) were from Narran Lakes (the majority in drier flow categories, C3 and C4) and 344 (87.3%) were from the Lower Murray.

Seed bank germination responses

Seed bank responses have been reported in Campbell et al. (draft) *Vulnerability of resilient systems to the Anthropocene* (Appendix V3.3) and in the presentation given by Dr Sam Capon, *Vulnerability of dryland wetland vegetation soil seed banks to altered water regimes and canopy structure* (Appendix V3.5)

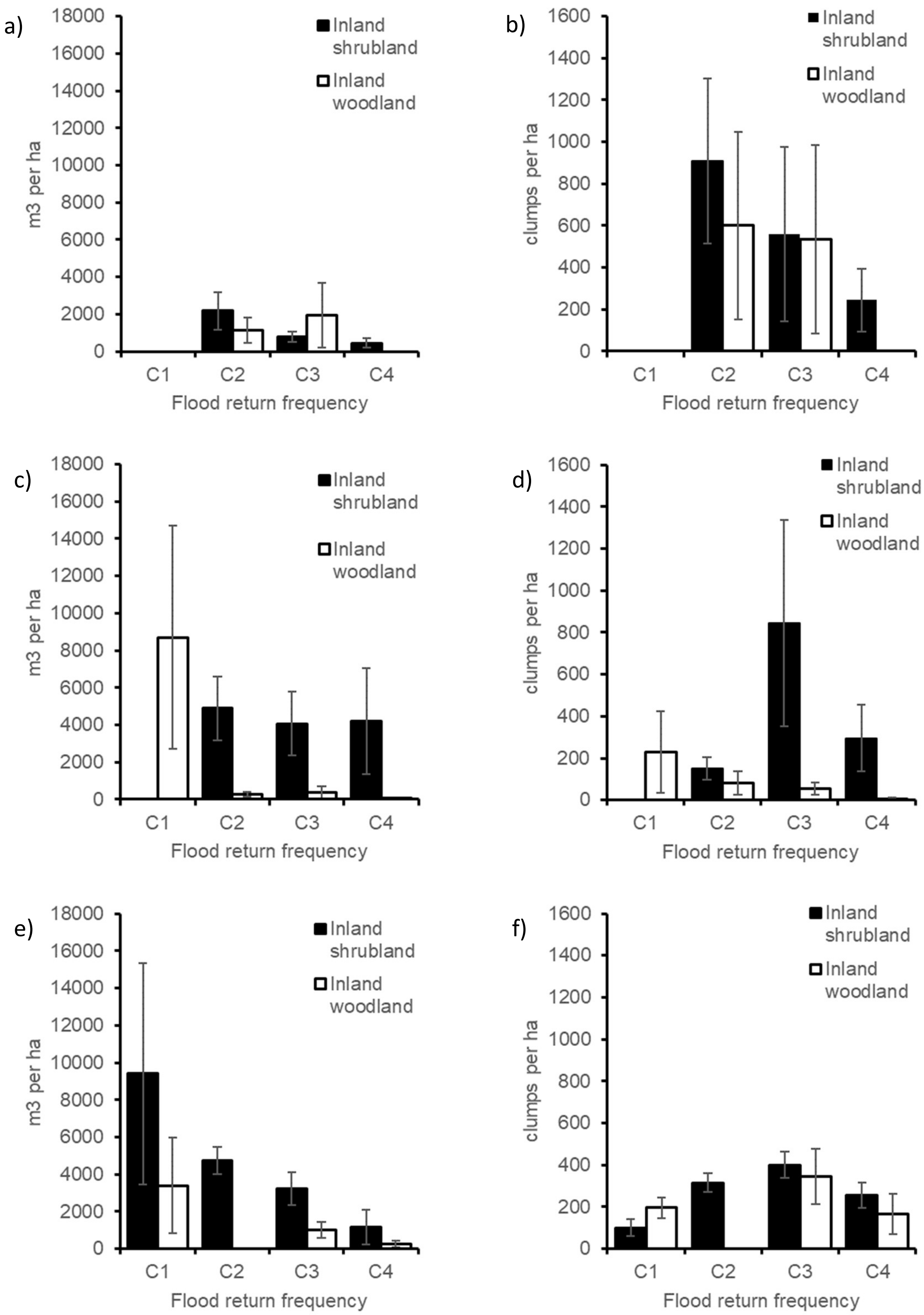

Figure 8. Average lignum volume per hectare (a, c, e) (± s.e.) and average clumps per hectare (b, d, f) (± s.e.) for Lower Murray (a, b), Macquarie Marshes (c, d) and Narran Lakes (e, f). Flood return frequency; C1 = near annual; C2 = 1 flow in 1.5-3 years; C3 = 1 flow in 3-5 years; C4 = 1 flow in 5-10 years. C1 sites are not present at the Lower Murray, C1 sites in Inland Shrubland are not present at Macquarie Marshes and C2 Inland Woodland sites are not present at Narran Lakes (see Table 1). C4 Inland Woodland sites were surveyed at the Lower Murray however no lignum was recorded.

• Discussion / applications

Outcomes from this component inform how environmental watering events might be undertaken, including considerations such as what are the key components of the flow regime (e.g. flooding frequency) or how should non-flow drivers (e.g. vegetation structure) be considered to achieve target responses. These outcomes can be used to better predict responses to environmental watering events and use those predictions to help plan or prioritise watering actions.

Non-woody understory vegetation

The overwhelming influence of location highlights the diversity of understorey communities in space and time at a landscape scale. This has implications for water management decisions in terms of prioritising areas for inundation to maximise the potential diversity at a Basin-scale. There will inevitably still be trade-off questions that arise in long-term planning such as should we maximise the extent of inundation to potentially maximise diversity spatially or should we build up resilience and temporal diversity at a more limited suite of locations? Basin-scale management should aim to be equitable and representative of a large number of vegetation types in a range of areas over time (cumulative spatial representativeness across multiple years), while retaining the flexibility to build resilience and temporal diversity at identified locations (targeted, multi-year waterings). The key is to balance outcomes over time.

Our results also suggest that within wetland complexes there are different influences, potentially linked to recent conditions. For example, at the Macquarie Marshes, where there was complete inundation of surveyed sites in the recent history, there is a strong influence of flood frequency on understory vegetation communities, but only a weak influence of vegetation structure. Conversely, at Narran Lakes, where there was no recent flooding prior to surveys in 2017, there is a strong influence of vegetation structure on understory vegetation communities, and only a weak influence of flood frequency. While in the Lower Murray there were only weak relationships with both flood frequency and vegetation structure, suggesting other influences dominate.

Woody seedlings

Given the variability in woody seedling responses, specific, targeted surveys, including in neighbouring open patches, need to be undertaken where seedling recruitment is a key response outcome. No clear relationship with flood history suggests that other drivers are influential. Consideration should then be given to what might be limiting the success of recruitment, from extent of flowering and seed viability, to germination cues and influences on seedling establishment such as soil moisture, grazing pressure, light and litter levels.

Lignum structure

Maintaining lignum with structural qualities to support processes such as waterbird breeding and fledging is likely to require particular flow regime characteristics, including flood-return-frequency in the range of 1 flow in every 1 – 3 years. It is likely that waterbirds prefer lignum clumps to be of a particular height and width (the assumption is larger is better). Larger lignum clumps are maintained with a more frequent inundation regime. Further analysis of lignum structural responses with inundation mapping and additional hydrology metrics, such as depth and duration, will aid refinement of the characteristics required. Further consideration of where these flow characteristics don't support desirable structural qualities (such as certain sites within the Lower Murray) will help to identify potential non-flow drivers limiting responses.

Actual volumes per hectare of lignum are very high and further refining of these estimates should be made. Generalising clumps as perfect cylinders does not account for irregularities in shape and air space contained within the clumps. The estimates from Narran Lakes and Macquarie Marshes in the most frequently inundated category are over to 8000 m^3/ha. In North America, Douglas-fir, Redwood and Fir-Spruce forests may contain nearly 2000 m^3/ha, which is comprised of live and standing dead trees. It is extremely unlikely that the volume of biomass of lignum is so much higher than that of softwood forests (Smith et al. 2003). While the absolute volumes of biomass are greatly overestimated, the relative patterns between locations and strata and very informative. Counting of individual lignum clumps is also problematic and can vary greatly as it is extremely difficult to define an 'individual' clump. At some sites the numbers of clumps varied greatly between years.

Conclusions / further work

From this research and other components (e.g. modelling undertaken in V2 DISC) we are starting to determine community assembly rules: i) identify what the significant filters are; ii) determine their relative importance; and iii) understand their interactions.

Some of the key outcomes to emerge from this work include:

- Location is the most important predictor of community composition
- Other factors, such as medium to long term flow regimes or vegetation structure may interact to modify responses:
  - E.g. if a wetland has been dry over the medium term, around 3-10 years, then vegetation structure becomes a key predictor of wetland community, or
  - E.g. if a wetland has been wet over the medium term, around 3-10 years, then the medium to long term flow regime becomes a key predictor of wetland community

By understanding what the significant filters are, their relative importance and how they interact, we are improving our capacity to predict expected outcomes to environmental watering events and can use those predictions to help plan or prioritise watering actions.

Further work

A number of opportunities for further work have been identified.

Data from the field and germination component highlighted the overwhelming influence of location on community composition. There are, however, other drivers within locations and within vegetation / flood inundation categories. Covariate data was collected in the field that has not been analysed in full (e.g. the potential influence of tree density and condition, litter cover, site disturbance etc.) as well as data that would enable structural responses to be assessed (e.g. individual plant height data). Further analysis of the existing data would enable additional drivers of vegetation responses to be determined.

- Additional data analysis:
  - Determine relationships between response metrics and explanatory variables
  - Analyse according to different response metrics
    - Define, develop and analyse different vegetation response metrics to incorporate structural and process responses or responses at different levels of ecological organisation (e.g. seedling recruitment, strata, communities)

In the future, re-assessment of field sites, including soil seed banks, could be undertaken to collect additional vegetation response data to different environmental conditions, for example surveys in a different season or specifically following inundation.

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Appendix 1: Extant species list

Table A1.1: Plant species recorded in both 2017 and 2018 at each stata at each location. NL = Narran Lakes, MQ = Macquarie Marshes, MM = Mid-Murray, LM = Lower Murray, NWW = Non-woody wetlands, IS = Inland Shrubland, IW = Inland Woodland, C1 = near annual, C2 = 1 flow in 1.5 to 3 years, C3 = 1 flow in 3 to 5 years, C4 = 1 flow in 5 to 10 years.

Location-Veg-Flow	LM	LM	LM	LM	LM	LM	LM	LM	LM	MM	MM	MM	MM	MM	MQ	MQ	MQ	MQ	MQ	MQ	MQ	MQ	MQ	MQ	MQ	NL	NL	NL	NL	NL	NL	NL	NL	NL	NL	NL	Count across strata
	IS	IS	IS	IW	IW	IW	NWW	NWW	NWW	IW	IW	IW	NWW	NWW	IS	IS	IS	IW	IW	IW	IW	NWW	NWW	NWW	NWW	IS	IS	IS	IS	IW	IW	IW	NWW	NWW	NWW	NWW	
Species	C2	C3	C4	C2	C3	C4	C2	C3	C4	C1	C2	C3	C1	C2	C2	C3	C4	C1	C2	C3	C4	C1	C2	C3	C4	C1	C2	C3	C4	C1	C3	C4	C1	C2	C3	C4	
Abitulon fraseri																													X			X	X				3
Abutilon otocarpum																				X		X															2
Abutilon oxycarpum															X							X			X												3
Abutilon theophrasti									X						X																						2
Acacia salicina																					X																1
Acacia sp.																				X																	1
Acacia stenophylla	X	X		X	X	X	X	X	X						X	X		X	X		X					X	X	X	X	X	X	X	X				21
Aeschynomene indica															X			X		X		X	X		X												6
Aira cupaniana																				X																	1
Alternanthera denticulata	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X												24
Alternanthera nana																									X												1
Alternanthera nodiflora	X	X		X	X																					X				X							6
Alternanthera pungens																					X																1
Alternanthera sp. 1												X																									1
Amaranthaceae										X																											1
Amaranthus macrocarpus															X	X	X			X	X			X	X	X											8
Ammannia multiflora				X			X	X	X						X	X		X		X			X														9
Amphibromus nervosus													X																								1
Aristida contorta																																			X		1
Arthropodium minus											X																										1
Aster subulatus															X	X		X	X	X	X	X	X	X													9
Asteraceae										X		X				X	X				X								X								6
Astrebla elymoides															X									X													2
Astrebla lappacea																																	X	X	X		3
Atriplex eardleyae					X																																1
Atriplex leptocarpa	X	X	X	X	X	X															X			X													8
Atriplex lindleyi	X	X	X	X	X	X			X												X																8
Atriplex lindleyi subsp. inflata		X	X	X	X	X																															5
Atriplex lindleyi subsp. conduplicata			X																																		1
Atriplex macropterocarpa																																			X		1
Atriplex microcarpa																														X							1
Atriplex nummularia				X	X	X																				X	X			X			X	X	X	X	10
Atriplex semibaccata			X	X								X			X	X	X		X	X	X		X	X	X												12
Atriplex sp.	X		X	X		X			X		X	X																									7
Atriplex spongiosa																														X						X	2
Atriplex stipitata		X		X					X																												3
Atriplex suberecta	X		X	X	X	X								X																							6
Atriplex tridentata																											X										1
Atriplex turbinata																										X	X								X	X	4
Atriplex vesicaria			X																		X						X										3
Austrobryonia micrantha	X	X	X	X	X		X																														6
Avena fatua																	X				X																2
Azolla filiculoides							X						X	X	X			X	X			X	X														8
Azolla pinnata													X																								1
Azolla sp.													X	X																							2
Bassia decurrens																														X							1

Location-Veg-Flow	LM	LM	LM	LM	LM	LM	LM	LM	LM	MM	MM	MM	MM	MM	MQ	MQ	MQ	MQ	MQ	MQ	MQ	MQ	MQ	MQ	MQ	NL	NL	NL	NL	NL	NL	NL	NL	NL	NL	NL	Count across strata
	IS	IS	IS	IW	IW	IW	NWW	NWW	NWW	IW	IW	IW	NWW	NWW	IS	IS	IS	IW	IW	IW	IW	NWW	NWW	NWW	NWW	IS	IS	IS	IS	IW	IW	IW	NWW	NWW	NWW	NWW	
Species	C2	C3	C4	C2	C3	C4	C2	C3	C4	C1	C2	C3	C1	C2	C2	C3	C4	C1	C2	C3	C4	C1	C2	C3	C4	C1	C2	C3	C4	C1	C3	C4	C1	C2	C3	C4	
Bassia paradoxa																														X							1
Bassia tricuspis																												X	X	X	X	X		X			6
Bidens pilosa																					X																1
Boerhavia dominii																	X			X	X			X	X												5
Bolboschoenus fluviatilis																						X	X														2
Brachyscome basaltica					X																																1
Brachyscome basaltica var. gracilis																X				X			X														3
Brachyscome ciliaris																																	X				1
Brachyscome curvicarpa																												X	X								2
Brachyscome dentata																	X							X	X												3
Brachyscome paludicola					X							X				X				X			X														5
Brassicaceae																											X										1
Callitriche stagnalis														X																							1
Calotis cuneifolia		X		X	X			X																													4
Calotis hispidula	X	X			X				X		X																			X							6
Calotis scabiosifolia																		X		X																	2
Calotis scapigera					X						X															X	X			X	X		X				7
Calotis sp.					X																											X					2
Carex appressa																																X					1
Carex inversa											X	X																									2
Carex tereticaulis										X	X																										2
Carrichtera annua			X		X	X																															3
Carthamus lanatus																								X													1
Centaurea melitensis																	X				X				X												3
Centaurea solstitialis																													X								1
Centaurium tenuiflorum	X																							X													2
Centipeda cunninghamii	X	X		X	X	X	X	X	X	X	X	X	X	X				X	X														X				16
Centipeda minima	X			X	X			X	X	X					X			X		X					X												10
Centipeda sp.					X		X																														2
Centipeda thespidioides																										X											1
Ceratophyllum demersum																						X															1
Chamaesyce drummondii															X	X	X				X			X	X												6
Charophyte													X																								1
Chenopodiaceae											X	X				X					X	X															5
Chenopodium album																											X										1
Chenopodium auricomum																										X		X	X	X							4
Chenopodium desertorum																																			X		1
Chenopodium pseudomicrophyllum																										X	X	X	X	X	X	X			X		8
Chenopodium sp.																														X							1
Chenopodium nitrariaceum		X	X	X	X	X																															5
Chloris truncata											X										X																2
Cirsium vulgare										X	X	X			X	X	X	X	X	X	X	X	X	X	X	X	X						X		X		18
Citrullus lanatus																									X												1
Convolvulus erubescens																X	X				X			X	X												5
Conyza albida																					X																1
Conyza bonariensis															X			X			X		X			X		X		X							7
Conyza sp.										X						X	X	X	X		X				X												7
Conyza sumatrensis																			X																		1
Cotula coronopifolia																										X	X	X	X	X	X	X		X			8
Cotula sp.																										X											1
Crassula helmsii														X																							1

Location-Veg-Flow	LM	LM	LM	LM	LM	LM	LM	LM	LM	MM	MM	MM	MM	MM	MQ	MQ	MQ	MQ	MQ	MQ	MQ	MQ	MQ	MQ	MQ	NL	NL	NL	NL	NL	NL	NL	NL	NL	NL	NL	Count across strata
	IS	IS	IS	IWI	IWI	IWI	NWW	NWW	NWW	IWI	IWI	IWI	NWW	NWW	IS	IS	IS	IWI	IWI	IWI	IWI	NWW	NWW	NWW	NWW	IS	IS	IS	IS	IWI	IWI	IWI	NWW	NWW	NWW	NWW	
Species	C2	C3	C4	C2	C3	C4	C2	C3	C4	C1	C2	C3	C1	C2	C2	C3	C4	C1	C2	C3	C4	C1	C2	C3	C4	C1	C2	C3	C4	C1	C3	C4	C1	C2	C3	C4	
Cressa australis	X	X	X			X																															4
Cucumis myriocarpus subsp. leptodermis	X			X	X	X				X	X		X				X		X	X	X			X	X										X		14
Cullen cinereum															X	X	X			X				X		X				X			X				8
Cuscuta campestris				X	X		X								X	X		X		X			X		X	X	X	X	X	X	X	X	X				17
Cynodon dactylon	X	X	X		X		X			X	X	X								X					X												10
Cyperaceae															X				X			X															3
Cyperus difformis															X	X		X	X			X	X	X	X						X	X					10
Cyperus eragrostis										X																											1
Cyperus exaltatus																X		X																			2
Cyperus gilesii				X																																	1
Cyperus gymnocaulos				X	X		X	X	X																												5
Cyperus sp.																X	X	X	X	X	X			X	X												8
Dactyloctenium radulans																					X																1
Damasonium minus															X			X	X	X		X	X														6
Dichondra repens										X																											1
Disphyma crassifolium subsp. clavellatum			X			X			X																												3
Dittrichia graveolens					X				X			X																									3
Duma horrida									X																												1
Duma florulenta	X	X	X	X	X	X			X	X	X				X	X	X	X	X	X	X					X	X	X	X	X	X	X		X	X		25
Dysphania platycarpa					X																																1
Dysphania pumilio				X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X		X		X		X					25
Echinochloa colona															X				X																		2
Echinochloa crus-galli																					X	X															2
Echinochloa inundata																			X	X																	2
Echium plantagineum											X	X												X													3
Eclipta platyglossa				X	X					X		X			X	X	X	X	X	X	X		X	X	X												14
Einadia nutans		X	X	X	X	X				X	X	X		X	X	X	X		X	X	X	X		X		X	X	X	X	X	X	X		X	X	X	27
Einadia nutans subsp. linifolia																X	X		X	X	X																5
Einadia nutans subsp. nutans															X	X	X	X	X	X	X			X	X												9
Einadia polygonoides												X																									1
Einadia trigonos																				X																	1
Elatine gratioloides														X																							1
Eleocharis acuta	X			X									X																	X		X					5
Eleocharis plana															X	X	X	X	X	X	X	X	X	X	X												11
Eleocharis sp.				X						X	X		X																								4
Enchylaena tomentosa	X		X	X	X	X	X				X	X					X				X				X	X	X			X	X	X			X		17
Enteropogon acicularis																X					X																2
Epilobium hirtigerum												X																									1
Eragrostis australasica									X																												1
Eragrostis dielsii				X																																	1
Eragrostis lanicaulis																					X																1
Eragrostis leptocarpa															X						X																2
Eragrostis parviflora																		X																			1
Eragrostis sp.																		X										X									2
Eremophila bignoniiflora																												X		X	X	X					4
Eremophila divaricata subsp. divaricata			X																																		1
Erigeron bonariensis							X	X		X	X	X																									5
Erigeron sp.										X	X	X																									3
Eriochloa crebra			X																														X	X			3
Eriochloa procera																X	X							X													3
Eriochloa pseudoacrotricha																					X																1

Location-Veg-Flow	LM	LM	LM	LM	LM	LM	LM	LM	LM	MM	MM	MM	MM	MM	MQ	MQ	MQ	MQ	MQ	MQ	MQ	MQ	MQ	MQ	MQ	NL	NL	NL	NL	NL	NL	NL	NL	NL	NL	NL	Count across strata
	IS	IS	IS	IWI	IWI	IWI	NWW	NWW	NWW	IWI	IWI	IWI	NWW	NWW	IS	IS	IS	IWI	IWI	IWI	IWI	NWW	NWW	NWW	NWW	IS	IS	IS	IS	IWI	IWI	IWI	NWW	NWW	NWW	NWW	
Species	C2	C3	C4	C2	C3	C4	C2	C3	C4	C1	C2	C3	C1	C2	C2	C3	C4	C1	C2	C3	C4	C1	C2	C3	C4	C1	C2	C3	C4	C1	C3	C4	C1	C2	C3	C4	
Erodium crinitum																					X				X												2
Eucalyptus camaldulensis	X			X	X	X	X	X		X	X	X	X	X	X	X		X	X	X	X									X	X	X					20
Eucalyptus coolabah																				X	X						X	X	X	X	X	X					8
Eucalyptus largiflorens	X		X		X	X					X	X																									6
Eucalyptus melliodora												X																									1
Eucalyptus microcarpa										X																											1
Eucalyptus populnea subsp. bimbil																					X																1
Eucalyptus sp.												X																									1
Euchiton sphaericus										X																											1
Euphorbia dallachyana	X	X	X	X	X	X		X		X	X	X							X						X												12
Euphorbia drummondii															X	X	X	X	X	X	X		X	X	X			X	X							X	13
Euphorbia planiticola																	X																				1
Euphorbia sp.																				X	X																2
Exocarpos strictus										X		X																									2
Fallopia convolvulus																												X									1
Frankenia pauciflora						X			X																												2
Galium murale					X																																1
Galium sp.					X																																1
Geijera parviflora																				X	X																2
Glinus lotoides	X	X		X	X	X	X	X	X			X	X			X	X		X		X	X	X	X	X	X				X			X				21
Glossostigma elatinoides														X																							1
Glyceria maxima																		X		X	X																3
Glycine clandestina																					X																1
Glycyrrhiza acanthocarpa	X			X			X	X	X						X																						6
Goodenia fascicularis																											X										1
Goodenia glabra																																	X	X	X	X	4
Goodenia glauca	X		X	X	X	X			X																												6
Goodenia heteromera			X																																		1
Goodenia sp.												X															X										2
Haloragis aspera				X	X				X																	X		X		X		X					7
Haloragis glauca																													X								1
Haloragis glauca f. glauca																X		X																			2
Haloragis sp.												X																									1
Heliotropium curassavicum				X	X		X	X	X																												5
Heliotropium europaeum			X		X	X			X			X		X												X											7
Heliotropium sp.	X																																				1
Heliotropium supinum	X	X	X	X	X	X	X	X	X			X			X		X	X	X			X	X	X	X	X	X										20
Hordeum leporinum																	X				X			X	X												4
Hordeum sp.																					X																1
Hydrocotyle sp.										X																											1
Hypericum perforatum												X																									1
Hypochaeris glabra												X																									1
Hypochaeris radicata												X																									1
Iseilema vaginiflorum																													X					X		X	3
Isoetopsis graminifolia							X	X	X																												3
Jasminum lineare																					X																1
Juncaceae											X																										1
Juncus aridicola															X	X		X	X	X		X	X	X													8
Juncus flavidus															X							X		X													3
Juncus holoschoenus																						X	X														2
Juncus ingens													X	X																							2

Location-Veg-Flow	LM	LM	LM	LM	LM	LM	LM	LM	LM	MM	MM	MM	MM	MM	MQ	MQ	MQ	MQ	MQ	MQ	MQ	MQ	MQ	MQ	MQ	NL	NL	NL	NL	NL	NL	NL	NL	NL	NL	NL	Count across strata
	IS	IS	IS	IW	IW	IW	NWW	NWW	NWW	IW	IW	IW	NWW	NWW	IS	IS	IS	IW	IW	IW	IW	NWW	NWW	NWW	NWW	IS	IS	IS	IS	IW	IW	IW	NWW	NWW	NWW	NWW	
Species	C2	C3	C4	C2	C3	C4	C2	C3	C4	C1	C2	C3	C1	C2	C2	C3	C4	C1	C2	C3	C4	C1	C2	C3	C4	C1	C2	C3	C4	C1	C3	C4	C1	C2	C3	C4	
Juncus sp.										X	X	X			X			X	X	X			X	X	X												10
Juncus subsecundus										X	X	X																									3
Kickxia elatin												X																									1
Lachnagrostis filiformis			X							X	X	X			X	X	X	X	X	X	X	X	X	X	X												15
Lactuca serriola										X	X	X			X	X	X	X		X	X			X													10
Lemna disperma													X	X	X			X	X			X	X														7
Lepidium africanum																	X																				1
Lepidium bonariense																											X							X	X	X	4
Lepidium fasciculatum																					X			X	X												3
Lepidium pseudohyssopifolium				X	X							X									X																4
Lepidium sp.																	X																				1
Limosella australis														X																							1
Lobelia concolor					X					X		X																									3
Ludwigia octovalvis															X	X		X	X				X														5
Ludwigia peploides subsp. montevidensis							X			X			X	X	X	X		X	X	X		X	X	X													12
Lysiana exocarpi					X																					X											2
Lythrum hyssopifolia							X					X			X	X		X				X	X														7
Lythrum wilsonii															X			X																			2
Maireana appressa		X				X			X																	X	X	X	X			X	X	X	X	X	12
Maireana brevifolia					X																X						X	X	X	X		X	X	X	X	X	11
Maireana decalvans		X	X		X							X					X				X				X												7
Maireana enchylaenoides																					X		X		X												3
Maireana pentagona			X																																		1
Maireana pyramidata						X																															1
Maireana sp.			X		X	X	X		X	X		X																									7
Malacocera tricornis			X			X																															2
Malva parviflora																		X		X	X	X	X	X	X							X					8
Malva preissiana																										X											1
Malvaceae																	X																				1
Malvastrum americanum															X		X			X	X	X		X					X						X	X	9
Marrubium vulgare										X		X									X																3
Marsilea drummondii	X		X			X			X		X	X			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X					24
Medicago polymorpha															X		X	X	X		X		X	X	X												8
Medicago sp.								X	X													X															3
Melilotus indicus																X																					1
Mentha pulegium										X																											1
Mesembryanthemum nodiflorum			X		X				X																												3
Mimulus gracilis																			X		X																2
Mimulus repens		X							X																												2
Morgania floribunda																																				X	1
Mukia sp.				X					X																												2
Myoporum montanum																					X																1
Myoporum parvifolium	X				X		X																														3
Myriophyllum caput-medusae													X																								1
Myriophyllum crispatum													X	X																							2
Myriophyllum papillosum													X		X	X		X	X			X	X	X													8
Myriophyllum sp.														X																							1
Myriophyllum verrucosum										X				X																							2
Nicotiana megalosiphon subsp. megalosiphon																			X	X	X																3
Nicotiana suaveolens																												X	X		X	X					4

Location-Veg-Flow	LM	LM	LM	LM	LM	LM	LM	LM	LM	MM	MM	MM	MM	MM	MQ	MQ	MQ	MQ	MQ	MQ	MQ	MQ	MQ	MQ	MQ	NL	NL	NL	NL	NL	NL	NL	NL	NL	NL	NL	Count across strata
	IS	IS	IS	IWI	IWI	IWI	NWW	NWW	NWW	IWI	IWI	IWI	NWW	NWW	IS	IS	IS	IWI	IWI	IWI	IWI	NWW	NWW	NWW	NWW	IS	IS	IS	IS	IWI	IWI	IWI	NWW	NWW	NWW	NWW	
Species	C2	C3	C4	C2	C3	C4	C2	C3	C4	C1	C2	C3	C1	C2	C2	C3	C4	C1	C2	C3	C4	C1	C2	C3	C4	C1	C2	C3	C4	C1	C3	C4	C1	C2	C3	C4	
Nymphoides crenata													X					X	X			X	X														5
Onopordum acanthium																									X	X				X	X	X	X				6
Osteocarpum acropterum						X																															1
Ottelia ovalifolia													X																								1
Oxalis chnoodes																				X	X																2
Oxalis corniculata												X					X		X								X	X	X								6
Oxalis perennans															X	X					X			X	X		X			X	X	X					9
Oxalis sp.					X							X					X				X								X			X					6
Pachycornia tenuis																												X									1
Panicum decompositum																X											X	X									3
Paspalidium constrictum																					X																1
Paspalidium distans																					X				X												2
Paspalidium globoideum																					X			X	X												3
Paspalidium jubiflorum	X	X	X	X	X	X				X	X	X				X	X	X	X	X	X		X	X	X												18
Paspalidium sp.																	X									X	X	X	X		X	X					7
Paspalum dilatatum																			X		X	X	X														4
Paspalum distichum															X		X	X	X			X	X	X													7
Persicaria attenuata																		X	X																		2
Persicaria decipiens				X			X			X			X	X	X	X		X	X	X		X	X	X													13
Persicaria hydropiper										X			X	X																							3
Persicaria lapathifolia													X																								1
Persicaria orientalis																			X			X															2
persicaria prostrata				X						X			X	X			X																				5
Persicaria sp.							X			X	X			X									X	X													6
Phragmites australis													X	X		X						X	X	X		X											7
Phyla canescens					X										X	X		X	X	X	X		X	X	X		X		X				X			X	14
Phyla nodiflora																											X						X			X	3
Phyllanthus lacunarius	X	X	X	X	X	X	X																			X											8
Phyllanthus sp.																													X								1
Phyllanthus virgatus																											X	X			X						3
Physalis lanceifolia															X	X		X						X													4
Physalis minima																			X																		1
Plantago cunninghamii																	X							X	X												3
Plantago turrifera																										X	X	X	X		X	X		X		X	8
Poa pratensis			X		X					X		X	X																								5
Poaceae				X				X		X	X	X				X					X	X			X	X					X						11
Polygonaceae										X				X																							2
Polygonum arenastrum																					X				X												2
Polygonum aviculare										X		X			X	X	X		X	X	X	X	X	X	X												12
Polygonum plebeium				X			X	X		X			X	X			X									X							X				9
Polypogon monspeliensis															X	X				X																	3
Portulaca filifolia																												X	X							X	3
Portulaca oleracea																X	X		X	X	X			X	X				X			X					9
Potamogeton sulcatus													X	X																							2
Pratia concolor															X	X		X	X	X	X	X	X	X	X												10
Pseudognaphalium luteoalbum					X		X			X	X	X																									5
Pseudoraphis spinescens							X						X	X																							3
Psoralea eriantha																																	X				1
Ranunculus inundatus										X				X																							2
Ranunculus sp.														X																							1
Ranunculus undosus															X	X		X	X			X	X	X													7

Location-Veg-Flow	LM	LM	LM	LM	LM	LM	LM	LM	LM	MM	MM	MM	MM	MM	MQ	MQ	MQ	MQ	MQ	MQ	MQ	MQ	MQ	MQ	MQ	NL	NL	NL	NL	NL	NL	NL	NL	NL	NL	NL	Count across strata
	IS	IS	IS	IWI	IWI	IWI	NWW	NWW	NWW	IWI	IWI	IWI	NWW	NWW	IS	IS	IS	IWI	IWI	IWI	IWI	NWW	NWW	NWW	NWW	IS	IS	IS	IS	IWI	IWI	IWI	NWW	NWW	NWW	NWW	
Species	C2	C3	C4	C2	C3	C4	C2	C3	C4	C1	C2	C3	C1	C2	C2	C3	C4	C1	C2	C3	C4	C1	C2	C3	C4	C1	C2	C3	C4	C1	C3	C4	C1	C2	C3	C4	
Rapistrum rugosum																X																					1
Rapistrum sp.																																		X			1
Rhagodia parabolica																																		X		X	2
Rhagodia spinescens			X		X	X											X		X	X	X			X	X										X	X	11
Rorippa eustylis																			X																		1
Rosa rubiginosa												X																									1
Rosa sp.												X																									1
Rumex bidens				X			X																														2
Rumex brownii		X					X				X	X														X	X	X	X		X	X		X			11
Rumex conglomeratus																		X				X															2
Rumex crispus										X	X												X														3
Rumex sp.											X	X		X	X	X		X	X	X	X		X	X	X												12
Rumex tenax			X							X	X	X		X	X		X	X	X	X	X		X	X	X												14
Rytidosperma setaceum										X	X	X																									3
Salsola australis	X					X						X			X	X	X		X	X	X		X	X	X												12
Salsola kali var. kali																					X			X	X												3
Salvia verbenaca																										X											1
Schenkia australis											X																										1
Sclerolaena articulata																					X																1
Sclerolaena bicornis																				X	X			X					X						X		5
Sclerolaena birchii																			X	X	X											X					4
Sclerolaena brachyptera	X	X	X		X	X			X																												6
Sclerolaena calcarata																					X				X				X			X			X	X	6
Sclerolaena decurrens																														X		X					2
Sclerolaena diacantha					X	X																				X	X	X	X	X	X	X	X		X	X	12
Sclerolaena divaricata			X	X	X																								X			X					5
Sclerolaena intricata																																			X	X	2
Sclerolaena lanicuspis																																X					1
Sclerolaena muricata	X	X	X		X	X	X		X						X	X	X		X	X	X		X	X	X		X	X	X	X	X	X	X	X	X	X	26
Sclerolaena muricata var. muricata	X	X	X	X	X	X									X	X	X	X	X	X	X		X	X	X												16
Sclerolaena muricata var. semiglabra															X	X	X		X	X	X			X	X												8
Sclerolaena muricata var. villosa												X																									1
Sclerolaena obliquicuspis						X																								X							2
Sclerolaena patenticuspis					X	X																															2
Sclerolaena sp.																																			X		1
Sclerolaena stelligera			X			X			X																							X					4
Sclerolaena tricuspis	X	X	X	X	X	X			X			X					X			X	X							X		X		X	X		X	X	17
Scrophulariaceae												X																									1
Senecio cunninghamii var. cunninghamii				X	X		X			X																											4
Senecio glossanthus																					X																1
Senecio platylepis																													X								1
Senecio quadridentatus										X	X	X																		X							4
Senecio runcinifolius															X	X																					2
Senecio sp.														X	X		X							X				X		X							6
Sesbania cannabina var. cannabina															X			X	X				X										X				5
Sida ammophila												X																									1
Sida corrugata																										X		X	X	X	X	X	X	X	X	X	10
Sida cunninghamii																					X														X		2
Sida rhombifolia																														X							1
Sida sp.			X																		X											X					3
Sida trichopoda																	X				X																2

Location-Veg-Flow	LM	LM	LM	LM	LM	LM	LM	LM	LM	MM	MM	MM	MM	MM	MQ	MQ	MQ	MQ	MQ	MQ	MQ	MQ	MQ	MQ	MQ	NL	NL	NL	NL	NL	NL	NL	NL	NL	NL	NL	Count across strata
	IS	IS	IS	IW	IW	IW	NWW	NWW	NWW	IW	IW	IW	NWW	NWW	IS	IS	IS	IW	IW	IW	IW	NWW	NWW	NWW	NWW	IS	IS	IS	IS	IW	IW	IW	NWW	NWW	NWW	NWW	
Species	C2	C3	C4	C2	C3	C4	C2	C3	C4	C1	C2	C3	C1	C2	C2	C3	C4	C1	C2	C3	C4	C1	C2	C3	C4	C1	C2	C3	C4	C1	C3	C4	C1	C2	C3	C4	
Sigesbeckia orientalis subsp. orientalis										X	X	X									X					X											5
Silybum marianum																									X												1
Sisymbrium erysimoides					X							X																									2
Sisymbrium irio															X	X	X	X		X	X		X														7
Sisymbrium sp.																X	X		X																		3
Solanum aviculare										X																											1
Solanum esuriale															X	X	X		X	X	X			X	X				X			X					10
Solanum lacunarium	X	X	X						X																												4
Solanum nigrum		X		X	X	X				X	X	X			X			X	X	X	X																12
Solanum orientalis										X																											1
Solanum sp.											X																										1
Sonchus oleraceus										X					X			X	X	X	X		X														7
Sonchus sp.						X				X			X																								3
Spergularia marina														X																							1
Sphaeromorphaea littoralis	X			X	X	X	X	X	X																												7
Sporobolus caroli																					X						X	X				X					4
Sporobolus mitchellii	X	X		X	X			X	X								X			X	X			X		X	X		X				X	X	X	X	17
Stellaria angustifolia														X	X	X		X	X																		5
Stellaria angustifolia subsp. angustifolia															X	X		X	X	X			X	X	X												8
Stellaria angustifolia subsp. tenella										X																											1
Stemodia florulenta				X	X	X		X	X																												5
Tecticornia pergranulata							X																														1
Tetragonia moorei		X	X	X		X																															4
Tetragonia tetragonioides	X																X		X	X	X				X			X	X								8
Teucrium racemosum	X	X	X		X			X	X																											X	7
Trachymene sp.																																			X		1
Tragus australianus																					X																1
Trianthema sp.																													X								1
Trianthema triquetra																X	X		X	X	X			X	X												7
Tribulus sp.																					X																1
Tribulus terrestris																	X																				1
Triglochin procera																			X																		1
Triglochin sp.										X	X																										2
Trigonella suavissima																										X	X	X	X	X		X	X		X		8
Typha angustifolia													X																								1
Typha domingensis															X	X		X	X	X		X	X	X													8
Typha orientalis																							X														1
Typha sp.														X	X				X																		3
Verbena gaudichaudii															X	X	X	X	X	X	X			X													8
Verbena officinalis		X																	X																		2
Verbena supina		X	X	X	X		X																		X	X	X	X					X				10
Verbesina encelioides																					X																1
Vittadinia cervicularis												X																									1
Vittadinia cuneata var. cuneata																					X																1
Vittadinia dissecta												X																									1
Vittadinia gracilis												X																									1
Vittadinia sp.												X									X																2
Wahlenbergia fluminalis				X	X					X	X	X																									5
Walwhalleya proluta																	X																				1
Xanthium occidentale															X	X	X	X	X	X	X	X	X	X	X	X											12
Xanthium orientale				X	X																																2

Location-Veg-Flow	LM	LM	LM	LM	LM	LM	LM	LM	LM	MM	MM	MM	MM	MM	MQ	MQ	MQ	MQ	MQ	MQ	MQ	MQ	MQ	MQ	MQ	NL	NL	NL	NL	NL	NL	NL	NL	NL	NL	NL	Count across strata
	IS	IS	IS	IW	IW	IW	NWW	NWW	NWW	IW	IW	IW	NWW	NWW	IS	IS	IS	IW	IW	IW	IW	NWW	NWW	NWW	NWW	IS	IS	IS	IS	IW	IW	IW	NWW	NWW	NWW	NWW	
Species	C2	C3	C4	C2	C3	C4	C2	C3	C4	C1	C2	C3	C1	C2	C2	C3	C4	C1	C2	C3	C4	C1	C2	C3	C4	C1	C2	C3	C4	C1	C3	C4	C1	C2	C3	C4	
Xanthium spinosum					X					X		X		X	X		X		X	X	X	X	X	X	X												13
Xanthium strumarium				X	X			X																													3
Xerochrysum bracteatum											X	X																									2
Zaleya galericulata																					X																1
Zygophyllum sp.																										X		X	X			X					4
Species richness in strata	40	36	49	59	77	49	36	24	42	62	47	76	31	38	75	70	68	64	78	75	116	44	58	72	68	44	38	39	43	41	25	45	28	19	30	26	

Appendix 2: SIMPER analysis

Table A2.1: Plant species characteristic of each stata at each location. NB. Species identified using SIMPER analysis in Primer V7, displaying all the species accounting for ~70% of the cumulative explanation of similarity. Not all strata are represented within each Location (see Table 1). Light grey shaded cells indicate strata which are not represented.

Veg	Flow	Lower Murray	Mid Murray	Macquarie Marshes	Narran Lakes
NWW	C1		*Juncus ingens* *Myriophyllum papillosum* *Pseudoraphis spinescens*	*Ludwigia peploides ssp. montevidensis* *Paspalum distichum* *Phragmites australis* *Persicaria decipiens*	*Sporobolus mitchellii* *Cullen cinereum* *Glinus lotoides* *Sclerolaena muricata*
	C2	*Eucalyptus camaldulensis* *Tecticornia pergranulata* *Heliotropium supinum*	*Eucalyptus camaldulensis* *Juncus ingens* *Persicaria hydropiper* *Ludwigia peploides ssp. montevidensis* *Phragmites australis* *Azolla sp.*	*Paspalum distichum* *Ludwigia peploides ssp. montevidensis* *Eleocharis plana* *Xanthium occidentale* *Pratia concolor* *Ranunculus undosus* *Persicaria decipiens* *Marsilea drummondii*	*Sporobolus mitchellii* *Sclerolaena muricata*
	C3	*Eucalyptus camaldulensis* *Sphaeromorphaea littoralis* *Glycyrrhiza acanthocarpa* *Sporobolus mitchellii* *Stemodia florulenta*		*Eleocharis plana* *Eclipta platyglossa* *Xanthium occidentale* *Marsilea drummondii* *Dysphania pumilio* *Xanthium spinosum* *Paspalum distichum* *Pratia concolor* *Euphorbia drummondii* *Alternanthera denticulata*	*Sporobolus mitchellii* *Sclerolaena muricata* *Sclerolaena diacantha* *Einadia nutans*
	C4	*Sclerolaena tricuspis* *Atriplex lindleyi* *Sclerolaena stelligera* *Sporobolus mitchellii*		*Sclerolaena muricata* *Eleocharis plana* *Xanthium spinosum* *Euphorbia drummondii*	*Sclerolaena diacantha* *Maireana appressa* *Sporobolus mitchellii*

Veg	Flow	Lower Murray	Mid Murray	Macquarie Marshes	Narran Lakes
		Sclerolaena muricata *Maireana sp.* *Sclerolaena brachyptera*		*Portulaca oleracea* *Boerhavia dominii* *Eclipta platyglossa* *Xanthium occidentale* *Marsilea drummondii* *Salsola australis* *Dysphania pumilio*	
IS	C1				*Duma florulenta* *Einadia nutans*
	C2	*Duma florulenta* *Sporobolus mitchellii* *Sclerolaena muricata* *Sclerolaena tricuspis*		*Duma florulenta* *Xanthium occidentale* *Eleocharis plana* *Dysphania pumilio* *Eclipta platyglossa* *Acacia stenophylla*	*Duma florulenta*
	C3	*Chenopodium nitrariaceum* *Sclerolaena muricata* *Duma florulenta* *Solanum lacunarium* *Calotis hispidula* *Sclerolaena tricuspis*		*Duma florulenta* *Eleocharis plana* *Marsilea drummondii* *Xanthium occidentale* *Alternanthera denticulata* *Euphorbia drummondii* *Sclerolaena muricata*	*Duma florulenta* *Sclerolaena muricata*
	C4	*Atriplex lindleyi ssp. inflata* *Sclerolaena tricuspis* *Chenopodium nitrariaceum* *Sclerolaena muricata* *Sclerolaena brachyptera* *Duma florulenta* *Enchylaena tomentosa*		*Duma florulenta* *Sclerolaena muricata* *Marsilea drummondii* *Xanthium occidentale* *Eclipta platyglossa* *Euphorbia drummondii* *Paspalidium jubiflorum* *Salsola australis*	*Duma florulenta* *Portulaca oleracea* *Sclerolaena muricata*

Veg	Flow	Lower Murray	Mid Murray	Macquarie Marshes	Narran Lakes
IW	C1		*Eucalyptus camaldulensis* *Centipeda cunninghamii* *Alternanthera denticulata*	*Eucalyptus camaldulensis* *Xanthium occidentale* *Ludwigia peploides ssp. montevidensis* *Persicaria decipiens* *Alternanthera denticulata* *Marsilea drummondii* *Duma florulenta* *Pratia concolor*	*Duma florulenta* *Eucalyptus camaldulensis* *Einadia nutans* *Acacia stenophylla*
	C2	*Eucalyptus camaldulensis* *Acacia stenophylla* *Alternanthera denticulata* *Centipeda cunninghamii* *Solanum nigrum* *Sphaeromorphaea littoralis* *Duma florulenta*	*Eucalyptus camaldulensis* *Paspalidium jubiflorum* *Centipeda cunninghamii* *Alternanthera denticulata*	*Eucalyptus camaldulensis* *Eleocharis plana* *Alternanthera denticulata* *Dysphania pumilio* *Marsilea drummondii* *Xanthium occidentale* *Eclipta platyglossa* *Duma florulenta* *Euphorbia drummondii*	
	C3	*Eucalyptus camaldulensis* *Eucalyptus largiflorens* *Acacia stenophylla* *Duma florulenta* *Enchylaena tomentosa* *Stemodia florulenta* *Einadia nutans* *Chenopodium nitrariaceum*	*Eucalyptus camaldulensis* *Einadia nutans* *Alternanthera denticulata* *Vittadinia gracilis* *Rytidosperma setaceum* *Solanum nigrum* *Euphorbia dallachyana* *Eucalyptus largiflorens*	*Eclipta platyglossa* *Eucalyptus camaldulensis* *Marsilea drummondii* *Xanthium occidentale* *Duma florulenta* *Alternanthera denticulata* *Eleocharis plana* *Phyla canescens* *Solanum esuriale* *Eucalyptus coolabah*	*Eucalyptus coolabah* *Duma florulenta* *Einadia nutans*
	C4	*Eucalyptus largiflorens* *Rhagodia spinescens* *Chenopodium nitrariaceum* *Enchylaena tomentosa*		*Eucalyptus coolabah* *Sclerolaena muricata* *Rhagodia spinescens* *Einadia nutans* *Salsola australis*	*Acacia stenophylla* *Einadia nutans* *Eucalyptus coolabah* *Duma florulenta* *Sclerolaena muricata*

Veg	Flow	Lower Murray	Mid Murray	Macquarie Marshes	Narran Lakes
				Solanum esuriale *Enchylaena tomentosa* *Boerhavia dominii* *Portulaca oleracea* *Xanthium occidentale* *Cirsium vulgare* *Eucalyptus camaldulensis* *Geijera parviflora* *Marsilea drummondii* *Sclerolaena bicornis*	

Recruitment of long-lived floodplain vegetation:

Literature review

Prepared by: Rebecca Durant, Fiona Freestone, Danielle Linklater, Christine Reid and Cherie Campbell.

November 2016

Final Report

MDFRC Publication 136

Recruitment of long-lived floodplain vegetation: Literature review

Final Report prepared for the Department of Environment and Energy by The Murray–Darling Freshwater Research Centre.

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This report was prepared by The Murray–Darling Freshwater Research Centre (MDFRC). The aim of the MDFRC is to provide the scientific knowledge necessary for the management and sustained utilisation of the Murray–Darling Basin water resources. The MDFRC is a joint venture between La Trobe University and CSIRO. Additional investment is provided through the University of Canberra.

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Executive summary

The 'Recruitment of Long-lived Floodplain Vegetation' component of the MDB EWKR Vegetation Theme aims to increase our understanding of how recruitment and seedling establishment occurs in response to varying watering regimes. Four woody floodplain species, River Red Gum, Black Box, Tangled Lignum and Coolibah, were identified as key target species within the Murray–Darling Basin to investigate the drivers of sustainable populations within four field-based research sites, Upper Murray, Lower Murray, Macquarie Marshes and the Lower Balonne floodplain.

Species-specific literature reviews where undertaken to assess and collate existing information available about the recruitment and establishment of seedlings. This review focused on understanding how flow and non-flow drivers influence recruitment and seedling establishment responses and the response to water regimes over multi-year timeframes for the key target species.

For the four key species identified, the aims of this report were to:

- summarise existing knowledge of seedling establishment requirements
- conceptualise the processes that lead to successful seedling establishment
- identify the key flow and non-flow drivers influencing successful establishment
- highlight knowledge gaps that can be addressed through mesocosm experiments
- develop appropriate experimental designs for prioritised mesocosm experiments to address the knowledge gaps identified.

A number of knowledge gaps where identified in relation to the four key species' recruitment and seedling establishment phases:

- Depth of inundation –
    - How do rates of rise and fall of floodwater affect soil moisture?
    - What are ideal and maximum flood depths?

- Duration of inundation –
    - What are the effects on germination success?
    - What are ideal and maximum flood durations?

- Sequence of and/or consecutive inundation –
    - Are there seasonality effects?
    - Are effects site specific?
    - Do multiple small and/or follow-up flooding benefit seedling establishments?

- Timing/season of inundation –
    - What are the effects in response to soil moisture persistence?
    - How are reproduction and seedling establishment, including survival and growth affected?

- Water stress
    - Are effects site specific?

- Soil salinity
    - Effects are unknown

In addition to the species-specific literature review, a review on the methods and experimental approaches conducted on woody floodplain species was undertaken to aid in the design of a mesocosm experiment. The review on experimental designs and use of mesocosm methods has highlighted possible designs/methods that can be used to address the key questions of long-lived vegetation, such as:

1. What is the relationship between soil moisture and seedling survival and root development?
2. What is the relationship between flow parameters such as duration and frequency (sequential, multi-year) and seedling survival and root development?
3. What is the critical time period between germination and successful seedling establishment and, therefore, what sequence of multi-year watering may be required to facilitate successful establishment?
4. How do stressors and threats (e.g. soil type, salinity, grazing pressure) modify the expected recruitment outcomes to flow regimes?

Understanding seedling root growth development and the drivers of population structure across multiple scales is fundamental to successful recruitment and seedling establishment. There is a critical time period between germination and seedling establishment, and so it is important to determine at what point seedling establishment is successful.

1 Introduction

1.1 Project background

The Vegetation Theme was established in the Murray–Darling Basin Environmental Water Knowledge Research (MDB EWKR) project to enable effective exploration of the effect of flow on aquatic and floodplain plants and an understanding of how stressors (e.g. land use, grazing, salinity and climate change) influence predicted outcomes from the use of environmental water for both understorey plant communities and long-lived woody vegetation (MDFRC 2015a). Aquatic and floodplain plants are critical components of floodplain and wetland ecosystems in that they provide refuge, breeding habitat and an important food source for a wide range of organisms, contribute to ecosystem services such as, nutrient and carbon cycling, water and sediment oxygenation, and have an intrinsic biodiversity value (MDFRC 2015a). Across the Murray–Darling Basin (MDB), the maintenance or improvement in the 'health' of aquatic and floodplain vegetation is a priority objective of the majority of management plans through the delivery of environmental water (MDFRC 2015a).

The Priority Research Question agreed to by the Steering Committee (Burns & Gawne 2014) for the Vegetation Theme will aim to address:

> *'What are the drivers of sustainable populations and diverse communities of water-dependent vegetation?'*

This question seeks to explore the key functional processes that drive outcomes for water-dependent vegetation populations and communities, as well as the situations under which each of these processes become limiting (Burns & Gawne 2014). From this process, seedling establishment was identified as being a priority for water managers, and recent literature reviews have identified successful establishment as a knowledge gap (Casanova 2015). It was felt that datasets looking specifically at establishment responses were likely to be limited (pers. comm. EWKR Vegetation Theme Leadership Group), and that focusing studies on seedling responses was an appropriate way to ensure this priority research question was addressed.

1.2 MDB EWKR research sites

The MDB EWKR project identified four sites (Table 1) for the focus of research activities, noting non-field-based activities are not defined by the boundaries of the four sites (Burns & Gawne 2014). The sites needed to be able to provide opportunities to address priority questions at both the area and basin scales, yet not all priority research topics need to be explored at all four sites. For more information relating to the selection and description of research sites, refer to Burns and Gawne (2014) and MDFRC (2015b).

Table 1. MDB EWKR field research sites.

Basin region	Research site	Incorporating area
Southern Basin	Upper Murray	Centred around Barmah–Millewa Forest and potentially including lower reaches of adjacent tributaries (Goulburn and Campaspe) and parts of the Edward–Wakool system
	Lower Murray	Centred around the Chowilla–Lindsay–Wallpolla Floodplain and potentially including the Riverland Ramsar site and adjacent floodplain systems and river reaches
Central Basin	Macquarie Marshes	Focusing on the floodplain wetlands of the Macquarie Marshes, which form north of Marebone Weir on the Macquarie River, including parts of the Macquarie Marshes Ramsar site and Nature Reserve
Northern Basin	Lower Balonne floodplain	Focusing on the Narran Lakes ecosystem, which includes the Narran Lakes Nature Reserve in the north, Narran Lake in the south and the surrounding floodplain in-between.

1.3 Key species

The recruitment and establishment of long-lived woody vegetation seedlings was specifically identified as a priority in most workshops with managers. Four key woody floodplain species were identified as target species, consisting of three eucalypt tree species, River Red Gum (*Eucalyptus camaldulensis* Dehnh.), Black Box (*Eucalyptus largiflorens* F.Muell.) and Coolibah (*Eucalyptus coolabah* Blakely & Jacobs), and one native floodplain shrub species, Tangled Lignum (*Duma florulenta* Meissner) (Burns & Gawne 2014). This is due to their significant role as major structural components of floodplain communities in the MDB and because expected outcomes for these species are specifically mentioned in the Murray–Darling Basin Authority's (MDBA) Basin-Wide Environmental Watering Strategy (BEWS) (MDBA 2014).

River Red Gum has been well studied in the southern MDB, and its ecology is better known than any other riparian tree in Australia (Roberts & Marston 2011). However, flow regime requirements and the influence of flow on seedling recruitment and establishment are less well understood for Black Box, Coolibah and Lignum. As such, it is anticipated that more effort will be expended on the latter three species. These four species occur throughout the MDB, although populations are not consistent at all of the MDB EWKR research sites, and thus reviews and mesocosm experimental designs will reflect this distribution (Table 2).

Table 2. Species distribution within the Murray-Darling Basin, based on the Atlas of Living Australia (X = present in MDB EWKR research site).

Sites Key species	Upper Murray	Lower Murray	Macquarie Marshes	Lower Balonne floodplain
River Red Gum	X	X	X	X
Black Box	X	X	X	X
Coolibah			X	X
Tangled lignum	X	X	X	X

While adult population survival and condition are among the better understood aspects of vegetation ecology (certainly in relation to River Red Gum and Black Box), seedling establishment and recruitment are not as well understood, yet these remain central to environmental watering decisions. Major knowledge gaps identified in relation to the four key species recruitment and seedling establishment phases include:

- How do vegetation responses vary with water regimes and among sites across the Basin?
- How do required water regimes vary with plant condition or age?
- How do required water regimes vary between years (e.g. with respect to antecedent conditions, benefits of cumulative events)?

1.4 Aims and key research questions

The Vegetation Theme aims to improve the capacity to predict vegetation outcomes in response to the delivery of environmental water through an enhanced understanding of how flow and non-flow variables influence vegetation responses (Burns & Gawne 2014). Specifically, the proposed research questions are:

1. What flow regimes best support recruitment within populations of long-lived floodplain vegetation species?
    - o How significant are the individual drivers (habitat availability, connectivity – dispersal) on recruitment?
    - o How do key drivers interact to influence outcomes?
    - o How should flows be managed to enhance drivers and thereby recruitment?
    - o How do the characteristics of sites (soil type, climate etc.) influence these flow requirements?
2. How do threats impact on the drivers and recruitment outcomes?

The aim of this population recruitment priority research topic is to understand the drivers of population recruitment and seedling establishment for key species across multiple scales (Burns & Gawne 2014). Priority threats identified in Burns and Gawne (2014) as most significant in terms of their potential impact on vegetation outcomes, include:

<u>Flow independent threats</u>
- Invasive species
- Grazing
- Habitat loss/land use

<u>Flow related threats</u>
- Climate change
- Groundwater/salinization
- Water quality

For the four key species identified, the aim of this report was to:

- summarise existing knowledge of seedling establishment requirements
- conceptualise the processes that lead to successful seedling establishment
- identify the key flow and non-flow drivers influencing successful establishment
- highlight knowledge gaps which can be addressed through mesocosm experiments
- develop appropriate experimental designs for prioritised mesocosm experiments to address the knowledge gaps identified.

1.5 Purpose and approach

There is general consensus that the flow regime requirements of seedling establishment and recruitment differ to the requirements of adult survival and condition, and are not as well understood (MDFRC 2015a). Seedling establishment is reliant on the availability of viable seeds, appropriate conditions for germination, and ongoing suitable conditions for the growth and establishment of seedlings (MDFRC 2015a). Species-specific literature reviews were undertaken to assess and collate the existing information available about the recruitment of seedlings of the four key species. This brief review acknowledges the recent work of others and draws heavily on their findings to avoid duplication (Casanova 2015; Johns *et al.* 2009; Roberts & Marston 2011; Rogers & Ralph 2011). The review also includes an assessment of experimental techniques that have been used to assess seedling responses, to ensure that the techniques applied in the MDB EWKR project build on the knowledge of previous work.

This literature review will aid in the development of a pilot study to test techniques for establishing seedlings, including seedling root development in mesocosm tanks, and to test techniques to apply different flow and non-flow variables to undertake these mesocosm experiments. An experimental design will be finalised, based on the results of the pilot study, to determine flow and non-flow variables, levels of manipulation, interactions between variables and required replication. Assessment of seedling responses will include seedling survival, root development, above and below-ground biomass in relation to soil moisture and linked flow parameters (duration, frequency, sequencing of events) and stressors (grazing pressure, soil salinity, soil type).

Outputs from this component will be used to inform water regimes and complementary management of tree and Lignum seedlings through:

- a literature review report summarising the current knowledge of seedling recruitment
- an experimental design report.

1.6 What is a seedling?

The germinant-seedling stage is possibly the most vulnerable life-history stage for most plant species (Capon 2012; Holloway *et al.* 2013; Johns *et al.* 2009), with root growth regarded as an important factor in seedling survival (Schütz *et al.* 2002). Despite the importance in understanding seedling development and establishment, there is no consensus on what constitutes a seedling or when it ceases to be a seedling. Studies have defined seedlings as; 1) a young plant that has developed from a seed (Fenner 1987), 2) is formed following the radicle that grows into the soil as the root and the plumule that grows away from the soil towards light as a shoot (Shivanna & Tandon 2014) or 3) is a non-reproductive plant (Hanley *et al.* 2004).

A plant is no longer considered to be a seedling when; 1) it has emerged from the soil surface until the end of its exponential growth (Sattin & Sartorato 1997); 2) when seed reserves are exhausted (Hanley *et al.* 2004); 3) when the seedling changes its dependence from seed resources to external resources (Soriano *et al.* 2013); or 4) based on seedling height (Shivanna & Tandon 2014). The seedling establishment phase for eucalypt species defined by Johns *et al.* (2009) is the period of growth from production of the first true leaves until sapling stage, when the root systems has developed sufficiently to access moisture from sources other than surface flows, including deep soil moisture and/or groundwater resources.

The definition of what constitutes a seedling for floodplain shrubs differs to that for floodplain trees, and the height or age of when Lignum reaches maturity or ceases to become a seedling is unknown. Lignum has the ability to develop from a seed or vegetatively reproduce (Casanova 2015; Holloway *et al.* 2013), with vegetative reproduction occurring more than sexual reproduction (Cale 2009; Capon *et al.* 2009) and new plants striking from nodes on roots or on branches once they come into contact with the soil (Jensen 2008). Consequently, determining seedling morphology is problematic.

For the purpose of this review, a seedling has been defined as a young plant, starting from the production of the first true leaves and ceasing based on the seedling height of 0.6–1.3 m tall (Capon 2012; Fox *et al.* 2004; George 2004; Johns *et al.* 2009).

1.7 Conceptualisation

Conceptual models focusing on flow and non-flow variables of the four key species in relation to recruitment (i.e. germination) and seedling establishment identify linkages between various flow regime components and species responses. Single models for recruitment (Figure 1) and seedling establishment (Figure 2), each, are sufficient to describe the linkages for these species.

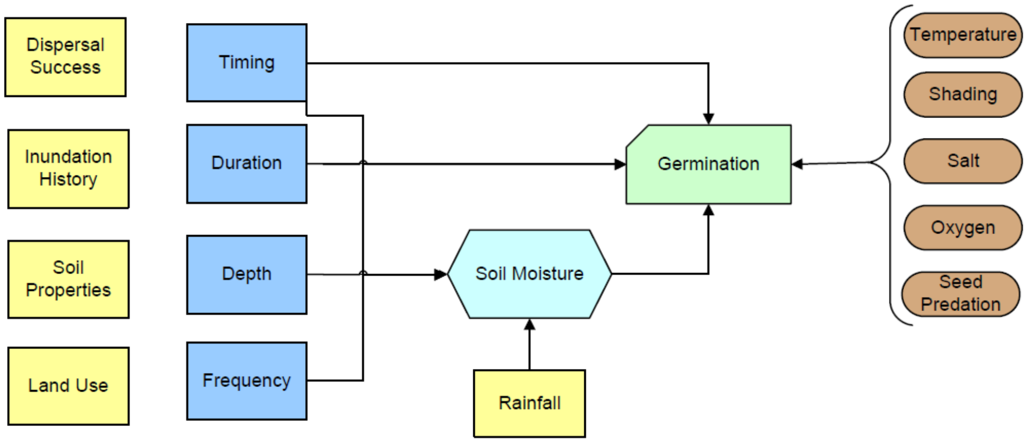

Figure 1. Conceptual model summarising the main relationships between flow and non-flow variables and germination. Blue boxes indicate flow variables, hexagons are primary controls, yellow boxes are modifying factors, green boxes are response components and brown ovals are non-flow (potential stressor) variables (Johns *et al.* 2009).

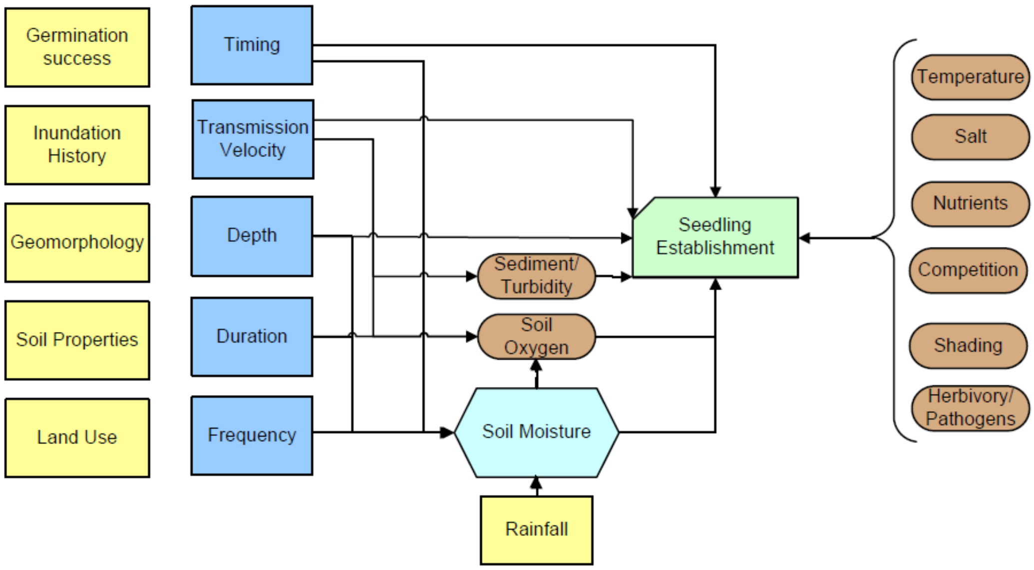

Figure 2. Conceptual model summarising the main relationships between flow and non-flow variables and seedling establishment. Blue boxes indicate flow variables, hexagons are primary controls, yellow boxes are modifying factors, green boxes are response components and brown ovals are non-flow (potential stressor) variables (Johns *et al.* 2009).

2 Black Box: *Eucalyptus largiflorens*

2.1 Introduction

Black Box is a small to medium tree, 10–20 m tall, with a large spreading crown and drooping branches that forms open woodlands on floodplains and on the fringes of ephemeral lakes and water courses (Cunningham *et al.* 1992). It is one of the dominant floodplain tree species throughout the MDB, although its distribution is largely confined to the MDB (Atlas of Living Australia as cited in Casanova 2015). This species typically occurs at higher elevations on the floodplain and displays a low tolerance to waterlogging (Roberts & Marston 2011). It can withstand long periods without floods by shedding leaves, reducing canopy transpiration rates and lowering stomatal conductance (Jolly & Walker 1996).

Black Box trees are important components of riparian zones, providing habitat as well as carbon and nutrient inputs via litter fall (Bogenhuber & Linklater 2012). These ecosystem engineers (sensu Jones *et al.* (1994)) also stabilise banks and regulate water movement through the soil (Bramley *et al.* 2003; Colloff & Baldwin 2010). The condition or health of riparian trees, therefore, influences floodplain function, and it has been suggested that tree condition, population structure and recruitment may be a useful substitute for ecosystem resilience (Colloff & Baldwin 2010).

Growth, flowering and germination in Black Box tend to occur in pulses in response to flooding (Casanova 2015; Roberts & Marston 2011). Reductions in flood frequency are, therefore, likely to reduce growth rates, flowering frequency and recruitment, and subsequently lead to declines in community viability (SKM & Roberts 2003). Although the species is drought hardy and salt tolerant, recent work has drawn attention to reductions in the crown condition and community viability (i.e. insufficient recruitment) of Black Box along the lower Murray River floodplain (George *et al.* 2005; Henderson *et al.* 2010; Lane & Associates 2005; Wallace 2009). These changes have been attributed to increases in soil salinity associated with shallower water tables and reductions in flood frequency (MDBC 2002; Slavich *et al.* 1999).

It is generally accepted that flooding is necessary for Black Box trees to maintain condition, and probably to recruit (Roberts & Marston 2011). Black box also responds to wet conditions (high rainfall and/or flooding) through improvement in tree condition and germination (Bogenhuber *et al.* 2013; Henderson *et al.* 2014a, b; Jensen 2008; Treloar 1959).

2.2 Water requirements for seedling establishment

Black Box seed germination and seedling establishment is reliant on natural flooding events (Holland *et al.* 2013), and subsequent local flooding or rainfall (Dexter 1967 as citied in (George *et al.* 2005; Jensen *et al.* 2008). Maintaining a constant soil moisture level of 10–25% is critical for seedling survival (Jensen 2008). Germination and, therefore, seedling establishment generally occur in a belt along the flood high-water line (Cunningham *et al.* 1992). With germination cued by sunlight and a temperature range of 15–35°C, pre-regulation spring floods would have provided Black Box seedlings with moist growing conditions into summer (Rogers & Ralph 2011).

However, flood duration and drawdown rates are critical. Black Box seeds are able to germinate underwater, but seedlings do not tolerating waterlogging and are unlikely to survive complete immersion unless it is only for a brief duration (Cunningham *et al.* 1992; Jensen 2008). Establishing seedlings experience slow growth when flooded to a depth of 5 cm (Heinrich 1990 as cited in Johns *et al.* (2009)).

Seedling establishment in the first year of growth is largely inhibited by competition for soil moisture, or drought and grazing pressure (Duncan *et al.* 2007; Llewelyn *et al.* 2014; Treloar 1959). Grazing, particularly by sheep rather than cattle, along with burrowing of rabbits distributing the soil profile have been observed to restrict Black Box regeneration (Victoria. 1990).

Seedling establishment to sapling stage or to develop a sinker root takes up to two years, under optimal conditions (George 2004). Newly germinated seedlings are susceptible to frost and heat injury (Johns *et al.* 2009). However, Morris (1984) showed that irrigated two-year-old Black Box seedlings displayed higher tolerance to frost damage than other trees and shrubs. Black Box saplings and mature trees have a relatively high tolerance to saline ground water (<40 dS/m); however, highly saline groundwater reduces the ability of roots to take up water, consequently reducing growth rates (Akeroyd *et al.* 1998; Jolly & Walker 1996). A study on irrigated Black Box seedlings noted high mortalities in the first two years at a site with sodic soils overlying highly saline groundwater within two metres of the surface (Morris 1984). Follow-up flooding, shortly after germination, may be required to provide sufficient soil moisture and nutrients for seedling establishment (George 2004).

2.3 Knowledge gaps

A number of studies and extensive reviews have been conducted on Black Box, covering aspects of life history attributes including recruitment and seedling establishment in relation to water requirements. While Black Box are considered to recruit episodically with floods, this is not the sole reason for regeneration and studies have shown trees can tolerate a range of wet-dry and fresh-saline conditions (Roberts & Marston 2011). Thus, further research questions regarding the specific effects that flooding and/or local rainfall has on soil moisture and the timing/seasonality of these effects, are worthy of consideration:

- If flooding in winter–late spring with water receding into early summer provides optimal moist conditions for germination and seedling establishment, what depth of inundation is required to maintain 10–25% soil moisture and avoid a prolonged drawdown rate causing waterlogging or complete immersion?
- Alternatively, is it better to have multiple short, shallow inundation periods?

- At what age/height or length of time can a seedling tolerate waterlogging or does age/height not matter?

3 Coolibah: *Eucalyptus coolabah*

3.1 Introduction

Coolibah are among the most common trees in arid riverine environments in the north-west of the MDB (Roberts & Marston 2011; Rogers & Ralph 2011). They dominate infrequently inundated floodplains of northern rivers such as the Darling and Gwydir (Roberts & Marston 2011; Rogers & Ralph 2011). Coolibah are medium sized trees, 15–20 metres tall, that vary in shape from erect to spreading (Cunningham *et al.* 1992; Harden 2002; Roberts & Marston 2011). They provide important habitat and shelter for animals on the floodplain and have cultural and heritage significance (Roberts & Marston 2011).

There have been very few ecological studies of Coolibah, and the majority of the studies undertaken to date have been conducted in the Lake Eyre Basin (Roberts & Marston 2011); e.g. regeneration and growth (Roberts 1993) and water sources (Costelloe *et al.* 2008). There are three sub-species of Coolibah in the MDB: *E. coolabah* ssp. *coolabah*, *E. coolabah* ssp. *exerata* and *E. coolabah* ssp. *arida* (Roberts & Marston 2011). For the purposes of this literature review, Coolibah is considered only at the species level, as ecological differences between sub-species are not well established (Roberts & Marston 2011).

3.2 Water requirements for seedling establishment

Flooding is likely to be important for reproduction and seedling establishment of Coolibah trees (Roberts & Marston 2011). A sequence of floods, or flood and wet years, may be necessary to ensure seedlings are well established (Li & Wang 2003; Roberts & Marston 2011; Tuomela *et al.* 2001). Coolibah trees occur on different soil types and on different parts of the floodplain with great variability in flood frequency among sites (Rogers & Ralph 2011). Coolibah trees on the Gwydir are flooded on average every 10–20 years, while Coolibah trees on the Cooper Creek have reportedly been flooded one in every five to six years (Rogers & Ralph 2011). It is likely that successful large-scale regeneration events are dependent on floods and are, therefore, episodic (Roberts & Marston 2011). In New South Wales, it is possible that only six major regeneration events have occurred in the last 105 years (Kerle 2005). The ideal and maximum flood duration and depth for reproduction and regeneration of Coolibah trees are unknown (Rogers & Ralph 2011).

Flowering times vary between regions and across years, and reproductive efforts may be lowered by stressors such as soil salinity and water stress (Roberts 1993; Roberts & Marston 2011). Germination requirements are not well understood, although it has been suggested that Coolibah trees are adapted to regenerate after late summer flooding, based on temperatures required for germination (Roberts & Marston 2011). The effect of season and flood timing on reproduction and seedling establishment are unknown. Successful recruitment may require protection from grazing (Roberts 1993).

3.3 Knowledge gaps

Coolibah trees are quite salt-tolerant (Costelloe *et al.* 2008; Roberts & Marston 2011). On the floodplain of the Diamantina River, mature Coolibahs are growing and using groundwater at salinities of at least 20 000–30 000 mg chloride (Costelloe *et al.* 2008). It is not known if Coolibahs in the MDB have similar tolerances (Roberts & Marston 2011). While mature Coolibahs are able to utilise saline groundwater, higher soil salinities (e.g. ≥ 0.2 dSm^{-1}) may lower their reproductive output (Roberts 1993). The effect of (soil) salinity on seedling establishment is unknown and should be investigated. It is also unknown at what stage (and over what timeframe) Coolibah seedlings transition from reliance on surface and sub-surface soil moisture to developing roots that are able to access and utilise groundwater, including saline groundwater.

In general, further research into the flood responses and water requirements of Coolibah at a range of sites and in all subspecies is required (Roberts & Marston 2011; Rogers & Ralph 2011). Very little is known about seedling establishment requirements of Coolibah, and thus, many research questions need to be addressed, including:

- Is the timing, season or duration of a flood important for seedling establishment?
- Are multiple small floods required (i.e. to trigger flowering, provide sufficient soil moisture for germination and follow up shallow flooding to promote seedling establishment)?
- What are the soil moisture requirements for seedling establishment?
- How critical are depth and duration of flooding for seedling establishment?
- What are the depth and duration limits that seedlings can tolerate?
- What impact does grazing have on seedling establishment?
- What impact does soil salinity have on seedling establishment and how does it affect the flow requirements for establishment?
- What is the critical time period between germination, seedling development and successful establishment?
- At what stage (and over what timeframe) do seedlings go from relying on surface and sub-surface soil moisture to developing roots that are able to access and utilise groundwater, including saline groundwater?

4 River Red Gum: *Eucalyptus camaldulensis*

4.1 Introduction

River Red Gum is the most widely distributed eucalypt in Australia (Brooker *et al.* 2002; Colloff 2014; Roberts & Marston 2011; Rogers 2011; Romanowski 2013), occurring across an area of approximately 5 million km^2 (Boland *et al.* 2006; Butcher *et al.* 2009) that encompasses most climatic zones (McDonald *et al.* 2009). This iconic eucalypt grows along thousands of kilometres of waterways and in intermittently flooded areas, such as on floodplains, and is particularly common around billabongs and other floodplain wetlands (Roberts & Marston 2011; Romanowski 2013). There are seven subspecies currently recognised, with three of these occurring in the MDB: *E. camaldulensis* ssp. *camaldulensis, E. camaldulensis* ssp. *actua,* and *E. camaldulensis* ssp*. arida* (Roberts & Marston 2011). This review concentrates on subspecies *camaldulensis* due to its occurrence mainly in and rarely outside the MDB, and disregards the other two subspecies due to their sporadic occurrences and apparent rarity within the Basin (Casanova 2015; Roberts & Marston 2011).

In the MDB, River Red Gums are dependent on flooding for recruitment and maintenance (Roberts & Marston 2011). The construction of dams, weirs and levees, and increases in water diversions/extractions has reduced the magnitude and duration of mid-range flows required to flood River Red Gums (Bren 1988; Kingsford 2000; Maheshwari *et al.* 1995; Walker 1985). River regulation-induced changes in flow regime are not uniform throughout the Basin. For example, changes in flow duration are minimal at Albury and become more pronounced further downstream, whereas changes in the seasonal distribution of monthly flows (i.e. winter–spring flows reduced and summer–autumn flows increased) are more pronounced at Albury than further downstream where inflows from tributaries augment winter–spring flows (Maheshwari *et al.* 1995). The implications of river regulation for River Red Gums are therefore site dependent and may differ markedly throughout the Basin.

The majority of studies have been done in the southern regions of the Basin on floodplain forests (Roberts & Marston 2011). Many of the early studies into the relationships between river flows and life-history processes of River Red Gum were done as part of the silviculture industry in the mid-Murray region (Dexter 1967, 1970, 1978). Due to the value of River Red Gum as a commercial resource, it has been well studied and its ecology is better known than for any other riparian tree in Australia (Roberts & Marston 2011). However, the knowledge generated in one part of the Basin may lack relevance or applicability elsewhere.

4.2 Water requirements for seedling establishment

Seedling survival in the first year after germination is a critical stage in River Red Gum stands, with the main factors affecting initial survival and establishment being soil moisture and seedbed conditions (Dexter 1967). Low density 'maintenance' seedling establishment can occur in response to above-average (>300 mm) annual rainfall on the lower Murray River floodplain (George 2004; Jensen *et al.* 2008). However, higher density establishment usually occurs in response to medium to large flood events, which are likely to recharge soil moisture reserves for some time afterward (George 2004; Jensen *et al.* 2008).

Seedlings are vulnerable to moisture stress; therefore, moisture must be maintained in the upper levels of the soil profile until seedlings produce sinker roots, allowing access to deeper soil moisture, and then groundwater (George 2004; Jensen *et al.* 2008). In a recent pot experiment, 10–20% soil moisture (volumetric moisture content) was found to be the minimum necessary to sustain seedling growth, with seedlings wilting and dying rapidly once soil moisture fell below 10% (Jensen 2008). During early establishment, River Red Gum seedlings invest more resources into developing roots than other riparian species (Chong *et al.* 2007), so when 23 cm tall, they can produce roots approximately four times plant height (Dexter 1978; Roberts & Marston 2011). Seedlings also develop resilience to stress at a relatively early stage; seedlings only 15 cm were able to shed leaves under stress and recover from axillary buds (Roberts & Marston 2011). Competition for moisture by other understorey vegetation and/or by overstorey trees can influence seedling survival (Roberts & Marston 2011).

River Red Gum seedlings are vulnerable to the effects of flooding and do not tolerate prolonged immersion (Roberts & Marston 2011). However, seedlings do possess some adaptations that allow them to cope with periods of anoxia associated with waterlogging, including adventitious root production and aerenchymatous tissue (Roberts & Marston 2011). Soil moisture is the most important factor for seedling establishment (Johns *et al.* 2009). Tolerance to drying increases as seedlings become established, root systems extend and sapling height increases (Roberts & Marston 2011). Two-month-old seedlings can survive in waterlogged soils for one month without obvious effects on leaf number and height (Marcar 1993; Roberts & Marston 2011). Seedlings 50–60 cm in height can survive extended flooding of 4–6 months, and complete submergence for several weeks, by shedding leaves (Roberts & Marston 2011).

Seedling establishment times for River Red Gum vary according to growing conditions. Seedlings may establish within one year at temperate sites (George 2004). Seedlings are thought to transition to juveniles somewhere between size weeks and 22 months after germination (Roberts & Marston 2011), however, at Banrock Station, on the lower River Murray floodplain, seedlings were not considered fully established until they were 2–3 years of age and >1.3 m in height (George 2004). Drought, lack of flooding and high soil and groundwater salinity at this semi-arid site contributed to extremely high mortality rates in the 2–3 years after germination (George 2004).

Winter floods receding in spring–early summer provide ideal conditions for River Red Gum seedling establishment (Dexter 1967; 1978, cited in Roberts and Marston 2011). Flooding at this time avoids exposure of seedlings to extreme temperatures, and ensures that surface moisture is available to support seedlings during initial root development (Dexter 1967; 1978, cited in Roberts and Marston 2011). Ideally, adequate water to support seedlings through the first summer should be applied before germination (Roberts & Marston 2011). Flooding after germination may lead to seedling mortality due to burial, dislodgement or excessive immersion periods (Johns *et al.* 2009).

Optimum watering frequency will vary between sites according to rainfall, inundation periods and other factors, but should be sufficient to maintain soil moisture levels above a minimum of 10–20% in the top 10 cm during the first summer after germination (Jensen 2008). A follow-up watering may be required one year later to maintain seedlings while root systems develop further (George 2004).

Tolerance to waterlogging increases with seedling height — seedlings 50–60 cm high can survive waterlogging (but not complete immersion) for 4–6 months (Roberts & Marston 2011). Complete immersion of seedlings should be avoided (Roberts & Marston 2011). Seedlings 50–60 cm high ceased growing and shed their leaves after 1–3 weeks of immersion (Roberts & Marston 2011). A rapid drawdown rate is preferable owing to the inability of seedlings to tolerate prolonged periods of immersion (Roberts & Marston 2011). However, soil moisture content should be maintained above 10% within the seedling root depth range (Jensen 2008). Flowing water may lead to dislodgement or burial of establishing seedlings (Johns *et al.* 2009). Seedlings can tolerate experimental waterlogging (surface inundation, not groundwater) with saline solutions equivalent to 1700 Na Cl (Roberts & Marston 2011).

Grazing by sheep, cattle and kangaroos have been noted to severely restrict River Red Gum regeneration, with cattle less destructive compared to sheep and rabbits (Victoria. 1990). This observation was based on the regeneration of River Red Gums occurring in protected areas (e.g. islands, reed beds or dense patches of Lignum) rather than in grazed areas (Victoria. 1990).

4.3 Knowledge gaps

Of the four floodplain species under consideration in this document, more is known about the requirements of River Red Gum seedlings. However, information is required to determine the effects of floodwater retention or flow enhancement on the habitat requirements for River Red Gum seedlings. Quantitative information on how the depth, duration and frequency of flood events affect soil moisture and groundwater levels and quality affect seedling growth and health, is currently limited.

Seedling establishment, rather than germination, is the critical stage in stand regeneration (ANBG 2004). The effects of environmental watering on the water quality and sediment type, may affect the health and growth of seedlings. From this, there may be site-specific requirements for seedlings based on the differences in the habitat of River Red Gum across the Murray–Darling Basin, and this may influence the way environmental water is delivered in different areas of the Basin.

5 Tangled Lignum: *Duma florulenta*

5.1 Introduction

Tangled Lignum (*Duma florulenta* (Meisn.) T.M. Schust; formerly known as *Muehlenbeckia florulenta* Meisn.) is considered one of the most ecologically significant floodplain shrubs in arid and semi-arid regions of Australia (Roberts & Marston 2011; Rogers & Ralph 2011). It dominates large areas of arid and semi-arid floodplain and is particularly common in the Murray–Darling and Lake Eyre basins (Campbell 1973; Capon 2005; Roberts & Marston 2011). Following favourable conditions such as flooding, Lignum can grow to three metres in diameter and form dense thickets (Cunningham *et al.* 1992; Jensen *et al.* 2006; Sainty & Jacobs 1981) and attain 1–3 m in height with persistent rootstock at least 2–3 m deep (Craig *et al.* 1991). This structure is significant as breeding habitat for many colonially nesting waterbirds (Maher & Braithwait 1992; Roberts & Marston 2011), including threatened species (Braithwaite 1976; Frith 1967; Rogers *et al.* 2004), and provides shelter for fish and aquatic invertebrates (Roberts & Marston 2011; Young 2001). During dry periods, the structure of Lignum facilitates the growth of floodplain understorey herbs (Roberts & Marston 2011).

Despite its recognised ecological significance, Lignum is an understudied species (Capon *et al.* 2009). The limited published literature has considered Lignum with respect to: flooding and soil correlations (Craig *et al.* 1991), seed banks (Chong & Walker 2005), germination and growth (Jensen 2008), seedling response to water regimes (Capon *et al.* 2009), gender distribution (Lynch 2006), or as part of broad vegetation community studies (Capon 2005).

5.2 Water requirements for seedling establishment

Lignum seedling establishment across the MDB is variable and not well understood (Roberts & Marston 2011). It has been suggested that initial seedling development may require consecutive floods; one to promote flowering and seed set, then one to promote germination (Rogers & Ralph 2011). Floodplain wet and dry phases do appear to provide important cues for Lignum. The wet phase (i.e. flood inundation) promotes vigorous growth in mature Lignum plants (Campbell 1973; Craig *et al.* 1991; Jensen 2008) and generates seed setting and germination in water or on wet mud (Campbell 1973; Chong & Walker 2005). Damp conditions associated with the drying phase (i.e. following floodwater recession) are fundamental in facilitating seedling growth (Capon *et al.* 2009).

In laboratory experiments, Lignum seedlings demonstrated considerable tolerance to a range of hydrological conditions (Capon *et al.* 2009; Lynch 2006), with damp conditions promoting the greatest growth (Capon *et al.* 2009). Root depth of seedlings under damp and drying conditions grew rapidly, almost tripling in length after 2–4 months, whereas flooding, waterlogging and dry conditions significantly impeded seedling growth (Capon *et al.* 2009). Successful root depth may have important implications for the survival of mature Lignum plants. Mature Lignum plants are estimated to have roots to more than three metres deep, which could enable them access to groundwater during times of low soil moisture or drought (Craig *et al.* 1991).

Although Lignum seedlings demonstrated tolerance of both flooding and drying, Capon *et al.* (2009) noted that these stressors on seedling establishment in the field are likely to be exacerbated by additional pressures such as grazing. Young leaves found on Lignum seedlings are considerably more palatable than mature plants and the authors suggest that this could partially explain the rarity of Lignum seedlings in the field. Lynch (2006) commented on the lack of seedlings recorded in her field surveys investigating growth responses to soil moisture. Jensen (2008) recorded seedlings at only one of three sites in her two and a half year study looking at the role of seed banks and soil moisture in Lignum recruitment. The seedlings were subject to grazing by kangaroos, but survived with stunted growth (Jensen 2008). Further investigation into the effects of grazing on Lignum seedlings would be beneficial to inform management of their survival and maintenance in the field.

5.3 Knowledge gaps

The importance of soil moisture on seedling growth has been identified in Capon *et al.* (2009) for Lignum seedlings from Narran Lakes in the northern MDB. It would be beneficial to determine if the soil moisture requirements for Lignum seedling growth and establishment were similar in other parts of the Basin. It would also be beneficial to assess how identified stressors such as grazing and salinity affect the soil moisture requirements for growth and establishment. While it is known that soil moisture is important for early seedling growth, it is unknown how flood depth and duration affect seedling survival and establishment. Seedling establishment requirements should be given priority for investigation due to the potential ecological consequences of shifts in floodplain vegetation communities (e.g. where seedlings will or will not establish as a result of altered hydrological regimes) (Capon *et al.* 2009).

Rogers and Ralph (2011) suggest that the ideal flood timing for reproduction and regeneration is spring/summer, increasing soil moisture conditions throughout the warmer summer months when growth of (mature) Lignum is at its greatest. It would be beneficial to determine if seedling growth rates are affected by temperature and flood timing (e.g. season). This knowledge would improve management practices (e.g. delivery of water) for seedling survival and growth.

Flooding may also be important in distributing genetic material (Roberts & Marston 2011) and for the (re)colonisation of habitats. Lignum is a dioecious plant and connection of the floodplain to wetlands and rivers during flood may play an important role in gender distribution. Lynch (2006) and Jensen (2008) investigated gender distribution; however, their studies were inconclusive and further investigation is required.

Given changes in flooding regimes throughout the MDB (e.g. as a result of river regulation and climate change), seedling establishment requirements should be given priority for investigation (Capon *et al.* 2009; Jensen 2008). The potential changes to where seedlings can or cannot establish as a result of altered flow regimes may shift floodplain vegetation communities, resulting in significant ecological consequences (Capon *et al.* 2009).

Lignum seedlings can survive inundation; however, inundation may delay seedling development, which could then hamper growth when conditions become favourable (e.g. damp soil as floodwater recedes) (Capon *et al.* 2009). The effect of flooding depth and duration on early seedling establishment requires further investigation. Lynch (2006) suggests that experimenting with varying levels of soil moisture for different periods of time would improve understanding of the factors that promote or inhibit Lignum growth response.

In addition to soil moisture, other factors worthy of investigation that could impact seedling establishment are salinity, soil nutrients, grazing and flood timing (i.e. season). The successful development of root systems is seen as important for the long term survival of Lignum and should be investigated further. Consideration should also be given to how long it takes for seedlings to reach maturity, as well as trends associated with soil moisture and plant gender.

6 Summary on key species

The key research questions relate to flow regimes, sustainable populations, stresses and threats on woody floodplain species in relation to recruitment and seedling establishment. The reviewed studies have concentrated on species in specific parts of the Basin and do not necessarily compare between populations. For example, the literature states that in the southern MDB, River Red Gum seedlings can withstand complete immersion during flooding for several weeks and waterlogging for two months, and that winter flooding maintains soil moisture to minimise the effects of extreme temperature stress during the spring–summer drawdown (Roberts & Marston 2011); however, it is unknown if similar effects occur in the northern MDB populations. While in the northern MDB, studies have identified that Lignum growth is impeded by waterlogging and dry conditions (Capon *et al.* 2009), yet the species can survive grazing pressure even with stunted growth (Jensen 2008). Again, it is unknown if these results are site specific or if similar situations occur in the southern MDB.

The influence of flow and non-flow variables on recruitment and seedling establishment vary between the four key species. Current knowledge and corresponding knowledge gaps are summarised in Table 3.

Table 3. Summary of germination and seedling establishment attributes, watering requirements and identified knowledge gaps. Blue is for refereed scientific literature; Red is for reviews and books; Grey is for published reports, proceedings and theses (grey literature).

Key species	Process	Description: Recruitment (germination)	Description: Seedling establishment	Knowledge gap
Black Box	Depth of inundation	• Most likely on moist–wet soils (Johns *et al.* 2009; Holloway et al 2013) • No direct impact on depth as seeds will germinate while floating or underwater (Jensen 2008; Johns *et al.* 2009) • High rainfall and/or flooding increases germination (Jensen 2008)	• Not tolerate waterlogging, unlikely to survive prolonged immersion (Jensen 2008; Johns *et al.* 2009) • Slower growth when flooded to 5 cm (Johns *et al.* 2009; Casanova 2015) • Recommended flood depth 4 cm (Casanova 2015) • Ideal depth less than total seedling height (Johns *et al.* 2009)	• Rates of rise and fall of floodwater that affect seed settlement are unknown (Johns *et al.* 2009) • Survival of seedlings underwater • Limited and/or unpublished data on flood depth effects on soil moisture (Johns *et al.* 2009)
	Duration of inundation	• No direct impact on duration, but unlikely to survive prolonged immersion (Johns *et al.* 2009) • Seeds die if submerged for >10 days (Casanova 2015)	• Ideal <30 days, maximum 30–60 days depending on seedling size (Johns *et al.* 2009) • Two-month-old plants can tolerate waterlogging for 1 month (Johns *et al.* 2009; Casanova 2015) • Signs of stress from waterlogging after 70 days at 22 months of age (Johns *et al.* 2009) • Duration should be sufficient to ensure maintenance of soil moisture (Johns *et al.* 2009) • Flood duration 4 weeks after 2 months of age (Casanova 2015)	• Limited and/or unpublished data on flood duration effects on germination (Johns *et al.* 2009)
	Sequence of and/or consecutive inundation events	• Requires follow up water (Casanova 2015)	• Follow up watering, whether rainfall or shallow inundation in the first or second year expected to improve establishment (Holloway *et al*. 2013). • Summer after germination (or local rainfall) (Casanova 2015)	• Unknown flood seasonality effects on seed fall and reproduction (Johns *et al.* 2009)

			• Frequency of inundation variable depending on a site's soil properties, evaporation rates and rainfall (Johns *et al.* 2009)	
	Water stress	• Requires flooding and/or local rainfall (Casanova 2015)	• Intolerant of waterlogging or complete immersion (Johns *et al.* 2009) • Soil moisture of 10–25% is critical (Jensen 2008) • Intolerant of drought (Casanova 2015) • Slow drawdown rates are detrimental to establishment as seedlings do not tolerate extended periods of waterlogging (Johns *et al.* 2009) • Flowing water may lead to dislodgement or burial (Johns *et al.* 2009) • Artificial flood not so useful (Casanova 2015)	
	Timing/season of inundation	• Requirements 15–35°C for germination (Rogers & Ralph 2011; Casanova 2015) • Inundation receding in spring–early summer provides moist conditions (Johns *et al.* 2009; Rogers & Ralph 2011; Holloway *et al.* 2013) • Local rainfall in spring–summer (Casanova 2015)	• Floods in winter–late spring optimal (Johns *et al.* 2009) • Flood recession in spring to summer to provide moist conditions (Holloway *et al.* 2013; Casanova 2015), or local rainfall (Casanova 2015) • Grow in summer after shedding old leaves and bark (Casanova 2015) • Newly germinated seedlings susceptible to frost and heat injury (Johns *et al.* 2009) • Follow-up inundation in same season as germination or following season (Holloway *et al.* 2013) • Timing should be sufficient to ensure maintenance of soil moisture in the first summer after germination (Johns *et al.* 2009)	• Limited and/or unpublished data on flood seasonality effects on soil moisture persistence (Johns *et al.* 2009)
	Grazing pressure	• Vulnerable (Casanova 2015)	• Vulnerable, seedlings are grazed (Casanova 2015) • Grazing, particularly by sheep (and more so than cattle and rabbit burrowing), restricts	

			establishment and impacts soil structure (Victoria. 1990)	
	Soil salinity		• Salinity tolerant (related to ground and surface water) (Casanova 2015) • Sodic soils overlying highly saline groundwater cause high mortality in first 2 years (Morris 1984)	• Impact of soil salinity
Coolibah	Depth of inundation	• Most likely occur on wet soils following floods or rainfall (Roberts & Marston 2011) • Not critical to seed germination (Holloway *et al.* 2013) • Requires moist soil (Holloway *et al.* 2013)		• What are the ideal and/or maximum flood depth requirements (Rogers & Ralph 2011)
	Duration of inundation		• Longer flood = fewer seedlings (Casanova 2015)	• What are the ideal and/or maximum flood duration requirements (Rogers & Ralph 2011)
	Sequence of and/or consecutive inundation events	• Follow-up floods in summer of first year thought to increase recruitment rates (Roberts & Marston 2011)	• Regular rainfall required for establishment (but saturated soil following inundation might be adequate) (Casanova 2015) • Follow-up rainfall or shallow inundation in summer of first year (or second year) thought to increase seedling recruitment rates (Roberts & Marston 2011) • Sequence of floods or flood and wet years may be necessary (Li & Wang 2003, Tuomela *et al.* 2001)	• Are multiple small floods required to provide sufficient soil moisture for germination and is follow-up shallow flooding needed to promote seedling establishment?
	Water stress	• Seeds take two weeks to germinate (Casanova 2015)		• Soil moisture requirements for seedling establishment
	Timing/season of inundation	• Fluctuating temperature 15–30°C for germination, vulnerable to frost, adapted to regeneration after late summer flooding (Capon *et al.* 2009).	• Shade or protection from summer heat required (Casanova 2015) • Flood recession in spring to provide warm and moist conditions (Holloway *et al.* 2013)	• Effects of season and flood timing on reproduction and seedling establishment are unknown

		• Flood recession in spring to provide warm and moist conditions (Holloway *et al.* 2013) • Timing not critical (Holloway *et al.* 2013)	• Flood summer–late summer (but other factors important e.g. rainfall) (Casanova 2015)	
	Grazing pressure	• Successful recruitment may require protection from grazing (Roberts 1993)	• Seedlings die from herbivory (Casanova 2015) • Grazing, seasonal conditions and competition from grass effects (not so important) (Casanova 2015)	
	Soil salinity	• Reproductive effort may be lowered by soil salinity (Roberts 1993; Roberts & Marston 2011)	• Are salt-tolerant in the Diamantina River region i.e. utilise saline groundwater (Costelloe *et al.* 2008; Roberts & Marston 2011)	• Impact of soil salinity • Is this site specific?
River Red Gum	Depth of inundation	• Moist soils required (Johns *et al.* 2009) • Germination success primarily controlled by seed availability and moisture availability after seed dispersal — most seeds germinate within 10 days of watering (Johns *et al.* 2009) • No direct impact on depth as seeds can germinate while floating (Johns *et al.* 2009) • Flooding after germination may lead to mortality (burial, dislodgement or immersion periods) (Johns *et al.* 2009)	• In southern MDB: not tolerate waterlogging and complete or prolonged immersion (Roberts & Marston 2011) • Depth will affect subsequent seedlings' survival and establishment (Johns *et al.* 2009) • On moist soil following flood recession • Tolerance to waterlogging increases with seedling height • Shallow flooding (20–30 cm) preferable to avoid over topping seedlings in first year (Holloway *et al.* 2013)	• Rates of rise and fall of floodwater that affect seed settlement are unknown (Johns *et al.* 2009) • Limited and/or unpublished data on flood depth effects on soil moisture (Johns *et al.* 2009)
	Duration of inundation	• No direct impact (Johns et al 2009) • Seeds die after 10 days of immersion (Casanova 2015)	• In southern MDB: 2-month-old plants can withstand waterlogging for 1 month; 50–60 cm plants can survive flooding for 4–6 months, but only for several weeks if completely submerged (Roberts & Marston 2011) • Maximum duration 1–6 months depending on seedling size (Holloway *et al.* 2013) • Susceptible to prolonged flooding (Roberts & Marston 2011)	• Limited and/or unpublished data on flood duration effects on germination (Johns *et al.* 2009)

			• Four-to-six weeks is adequate, but longer can be tolerated depending on age and if totally submerged (Holloway *et al.* 2013)	
	Sequence of and/or consecutive inundation events		• In southern MDB: follow-up watering 1 year after germination (George 2004) • Requires watering 1–2 months after spring rain or small flood (Casanova 2015) • Sufficient to maintain soil surface moisture during first year and needs adequate moisture in the second season (Johns *et al.* 2009) • Follow-up flood to recharge soil moisture is desirable in same year as germination or following year (Holloway *et al.* 2015)	
	Water stress	• Germinate within 5 days given adequate moisture (Holloway *et al.* 2013) • Soil moisture required >10% (Holloway *et al.* 2013) • Seeds require imbibing (saturation) and light to break dormancy (Casanova 2015)	• In southern MDB: soil moisture levels 10–25% in top 10 cm ideal (Jensen 2008; Holloway *et al.* 2013). • In southern MDB: low density response to above-average (>300 mm) annual rainfall, with higher establishment occurring in response to medium-to-large flood events — recharges soil moisture (George 2004; Jenson *et al.* 2008) • Inhibited by drought conditions, develops adventitious roots in response to flooding (Casanova 2015) • Rapid drawdown rate preferable as intolerant of prolonged periods of immersion (Roberts & Marston 2011) • Competition for moisture by other understorey and/or overstorey vegetation • Maintenance of soil moisture within first year is critical (Holloway *et al.* 2013) • Seedlings wilt and die rapidly once soil moisture falls below 10% (Jensen 2008)	Is this site specific?

	Timing/season of inundation	• Flood receding in spring–early summer preferred (Johns *et al.* 2009) • Rates limited by low temperatures and light availability (Holloway *et al.* 2013) • Require adequate moisture and day time temperature >30°C for germination (Holloway *et al* 2013) • Optimal temperature 35 °C (11–34 °C) (Casanova 2015) • Adequate water applied before germination (Roberts & Marston 2011)	• In southern MDB: winter flood receding in spring/early summer maintains soil moisture and avoids extreme temperatures for seedling survival (Roberts & Marston 2011) • Sensitive to frost • Flooding after germination may lead to seedling mortality due to burial, dislodgement or excessive immersion periods (Roberts & Marston 2011) • Recession spring/early summer (or sufficient rainfall), artificial watering to extend effect (Casanova 2015)	• Limited and/or unpublished data on flood seasonality effects on soil moisture persistence (Johns *et al.* 2009)
	Grazing pressure		• Seed predation varies through the year, lowest under sheep grazing, highest in ungrazed conditions, high under cattle grazing (Casanova 2015). • Compete with reeds and weeds (Casanova 2015) • Increased during flood (cattle, kangaroos, rabbits) (Casanova 2015) • Grazed more during drought (Casanova 2015) • Grazing (sheep, cattle and kangaroos) severely restrict regeneration (cattle are less destructive) (Victoria. 1990)	
	Soil salinity			• Impact of soil salinity
Tangled Lignum	Depth of inundation	• In water (while floating) or wet mud, occurs after flooding (Campbell 1973; Chong & Walker 2005; Holloway *et al.* 2013)	• Damp conditions promote growth; flooding, waterlogged and dry conditions impede growth (Capon *et al.* 2009) • Damp conditions associated with drying phase facilitate growth (Capon *et al.* 2009) • Depth of flood seedling establishment < 15 cm (Casanova 2015)	• Impact of depth?

	Duration of inundation		• In northern MDB: 2–4 months rapid growth in damp and drying conditions; flooding, waterlogged and dry conditions impede growth (Capon *et al.* 2009)	• Is this site specific?
	Sequence of and/or consecutive inundation events	• May require consecutive floods, one to promote flowering and seed set and one to promote germination (Rogers & Ralph 2011)	• Needs floods once in 12–18 months of 5–15 cm depth for 4–6 weeks in late spring–summer (Casanova 2015) • Spreads predominantly via vegetative growth, particularly in more frequently flooded areas (Casanova 2015) • Follow-up flood 9–12 months after germination (Casanova 2015)	
	Water stress	• Germination occurs within 14 days of dispersal (6–12 days) (Casanova 2015).	• Soil moisture known for northern MDB; damp conditions, can survive flooding (Capon *et al.* 2009). • More tolerant of drying than flooding (Capon *et al.* 2009) • Opportunistic and rapid under optimal experimental conditions (Holloway *et al.* 2013)	• Is this site specific?
	Timing/season of inundation	• Flood timing spring–summer preference (Rogers & Ralph 2011) • Rates are temperature dependent (Holloway *et al.* 2013) • Season appears to be critical for germination (late summer to autumn) (Casanova 2015). • Appears to recruit continuously (Casanova 2015)		• Temperature and flood timing (season) effects on seedling survival and growth
	Grazing pressure		• Can survive grazing, but growth is stunted (Jensen 2008) • Vulnerable to grazing (Capon *et al.* 2009) • Grazing and competition pressure unknown (Casanova 2015)	
	Soil salinity			• Impacts on soil salinity

7 Experimental designs and methods

7.1 Introduction

Mesocosms have been used as research tools for multiple experimental designs, because the physical dimensions, particularly length or shape, can be manipulated to provide researchers with a characterisation of the complexity or simplicity in the system they are trying to simulate or represent. Mesocosm studies provide a powerful means of quantifying causal relationships in a controlled environment and have the ability to focus specific variables, giving them the potential to be replicated in future research.

The focus of the review was on the four key species, River Red Gum, Black Box, Lignum and Coolibah, and on information relating to soil moisture, flow parameters (such as duration and frequency), stressors and threats to long-lived woody species. Information was compiled from various sources, a number of which do not relate to our key species, but that have a direct or indirect relationship to the key questions being asked in this MDB EWKR theme.

The most common reasons applied for conducting woody floodplain growth experiments in mesocosm designs relate to the ability to manipulate chosen variables (e.g. soil moisture or flooding), and to confirm predictions of effects on target-specific and/or single species. This review was undertaken to inform the selection of an appropriate mesocosm design to achieve the necessary answers in predicting the effects watering has on long-lived woody riparian species, specifically the four key species. The resulting analysis of literature was used to support the development of a pilot trial to test this design.

7.2 Quantitative design and facilities

Depending on the objectives of the studies reviewed, the number of replicated test systems and number of experimental treatment systems varied. Replication efforts were focused around increasing the assessment of variability, with the number of replicates ranging from two to 10, yet the more common approach was for four replicates. The experimental design considerations for the number of treatment units employed ranged from 1–5 for hydrological regimes, one or two soil/sediment types, and 1–6 months growth time with sampling events occurring daily, weekly, fortnightly, monthly, 3-monthly or only at the end of the experiment. The more commonly adopted treatment units approach was for three, four or five watering treatments, one sediment type (a mixture to represent natural soils at sample sites or floodplain soils) and approximately two growing months. The number of sampling events depended on the length of the experiment (growth time), yet the common approach with 2-monthly experiments was for observational monitoring weekly (e.g. number of stems, leafs, stem height) and harvesting/sampling at time of completion to record factors such as root depths and biomass.

The results of the review identified multiple structures and materials used to grow seedlings, ranging from ice cream containers (Jenson 2008) to more complexly designed apparatus’s consisting of water wells connected to soil tubes with automated pump systems (Hughes *et al.* 1997). The most common design approach consisted of PVC piping or plastic cylindrical pots, with size dependent on the target species and/or treatments applied (ranging from 9 to 75 cm in diameter and 0.3 to 1.25 m in length). Popular methods were to split the pipes or cylindrical pots lengthwise in half, and then seal them back together (e.g. with insulating tape or cooper wire) with one end open (for plants to grow up through) and one end closed off using nylon mesh, gauze, or fibreglass with drainage holes, or by lining the pipes with polythene bags. The benefit of splitting the PVC pipe in half appears to aid in the removal of the plant at the time of harvesting, thus providing opportunity’s to assess the root length and/or depth in the chamber whilst preventing overall damage to the specimen.

These pots/pipes were then deployed into systems that would maintain them in a vertical position to hold varying levels of water depending on the objective being tested, e.g. large buckets or galvanised tanks. The use of a glass or green house was preferred as it provided the ability to control environmental effects such as temperature and rainfall/watering. Outside facilities or field sites were also used; however, timing for the experiment was then dependant on and influenced by the season.

7.3 Soil moisture and flooding

A number of experiments have been conducted on soil moisture requirements for woody riparian species (Capon *et al.* 2009; Jensen 2008; Lynch 2006; Neave & Florence 1994), with up to five watering treatments over 10 to 24-week growth periods. In a laboratory experiment conducted by Capon *et al.* (2009), five watering treatments were applied to Lignum seedlings to investigate their response to flooding or drying, in two sediment types (clay and clay/sand). Root depth appeared to be the only variable affected by sediment type and this affect varied through time, and Lignum appeared to be more tolerant of drying then flooding.

Other experiments have looked at the effect of seedling growth from watering regimes of daily or weekly watering (Lynch 2006; Neave & Florence 1994) to determine the effects soil moisture has on root growth. Neave and Florence (1994) found that the treatment of drying sediment after a watering event produced eucalypt seedlings (including River Red Gum) with a larger root: shoot ratio then seedlings that were exposed to constant soil moisture. However, the root systems of seedlings in the constant soil moisture treatment were shallower than those in the dry treatment and contained more root weight in the upper part of the soil profile (Neave & Florence 1994). In comparison, Argus *et al.* (2015) study on *E. camaldulensis* subsp. *refulgens* states 32 days after flooding limits root growth, suggesting that early flood tolerance could be an adaptation to capitalise on scarce water resources, even though extensive adventitious roots developed in the seedlings in soil flooded for 88 days.

Li and Wang (2003) and Tuomela *et al.* (2001) investigated the growth of *E. microtheca* by subjecting seedlings to three water treatments, ranging from flooding to two levels of stress (soil retaining minimal moisture) over five months. When making comparisons between populations of seedlings, Tuomela *et al.* (2001) noted that root: shoot ratios were consistently higher in seedlings from seasonally dry sites compared to those from semi-arid sites, and Li and Wang (2003) similarly found that the morphological and physiological responses of *E. microtheca* to water availability differed among populations.

The timing or season is just as imperative when considering soil moisture and/or flooding. Jensen (2008) indicates eucalypt and Lignum seedlings, following germination on moist soil, may perish within 1–2 days of becoming water-stressed, which occurs when soil moisture drops below 10%. Thus, soil moisture-dependant species are unlikely to be able to survive predicted hot dry conditions that dry out the soil (Jensen 2008). Salazar *et al.* (2012) undertook a study in Brazil on woody species, and found that seedling establishment was low when covered with large quantities of litter, yet tree canopy cover actually facilitated seedling establishment by reducing stressful environmental conditions. Thus, shading effects on those hot dry days may facilitate establishment and recruitment.

Seedlings can be influenced directly or indirectly by flooding. Flooding of rivers, wetlands and floodplains has various effects on root development, by initiating a chain of reactions encompassing various physical, chemical and biological processes that lead to reduced soil conditions and implications for wetland and riparian species (Capon 2012; Pezeshki 2001). Moisture availability may also be a function of soil type, thus the germination and survival of seedlings may differ between soil types (Schütz *et al.* 2002).

Therefore, successful germination and/or establishment is not only connected to soil moisture (i.e. the duration (of flooding and drawdown), frequency or magnitude of flooding, rainfall availability or the occurrence of follow-up rain or flooding); rather, it also depends on the composition of the soil at each site, since this can influence the reactions each species may have to different treatments. These studies on the effects of soil moisture, flooding and populations on seedlings, highlight the importance of investigating site-specific requirements for species when climate, availability of water and soil types differ.

7.4 Multi-year watering

The literature acknowledges follow-up flooding or rainfall to maintain moisture in the soil as being important for seedling survival and germination success (George 2004; Jensen 2008). Awe *et al.* (1976) looked at root development of three eucalypt species (including River Red Gum) in a simulated design of prolonged drought and progressive drying out of the soil profile. This experiment was designed to simulate a natural situation of germinating seeds on saturated soil, followed by a drought where the soil moisture was allowed to progressively dry out. Results suggested that River Red Gum seedlings rapidly produce a massive root system when faced with a rapidly drying soil profile (Awe *et al.* 1976). However, this study saw seedlings watered for only three weeks with a total of 80 growth days (just under three months). Jensen (2008) showed that rainfall and flooding in the first year of establishment are essential, with subsequent follow-up rain/flood events over the next two years required for River Red Gum, Black Box and Lignum for successful germination and recruitment. However, Jensen's (2008) study on the response of the treatments (rainfall, flood, rainfall followed later by flooding, flooding followed later by rain, and constant dry to the germination of woody species) was only for a 12-week growth period (three months).

The literature revealed that experiments on seedling survival and root development have occurred over a six month period, and not over multi-year time scales. Statements have been made that in the first year of growing, seedlings require watering and/or follow-up rain or watering, but no studies were found to have extended longer than the first year of growth.

7.5 Stressors and threats

Stressors and threats to germination and establishment of woody species include (but are not limited to) grazing, salinity, soil type and climate change. From a physiological-ecology standpoint, the knowledge of the various soil compounds has critical implications for wetland plant functioning (Pezeshki 2001). The condition of the soil is influenced by nutrients received and lost through events such as in-channel and overbank flooding (Pezeshki 2001; Whitworth *et al.* 2012). The reduction of soil conditions, through droughts, or poorly timed or prolonged flood events, may lead to the inhibition of nutrient uptake and transport due to root dysfunction and/or death of plants (Pezeshki 2001).

Soil type has been looked at in relation to roots being able to penetrate heavy clays (Bell *et al.* 1993), the composition of the soil and effect it has on growth of eucalypt seedlings (Bennett *et al.* 1986), the soil's ability to maintain water (i.e. soil moisture) (Capon *et al.* 2009; Jensen 2008; Lynch 2006; Neave & Florence 1994), and the compaction of the soil as a result of grazing or heavy machinery use (Neave & Florence 1994). The sediment/soil type is just one part of the puzzle. The location of seedlings in the river/creek channel and the influence of drought, flooding and landform changes on the channel, will also affect woody riparian species' ability to survive, in relation to high water flows and the scouring of sediments (McBride & Strahan 1984). The location of species at specific elevations along a hydrological gradient generally reflect the water requirements and flood tolerance of that species and community, and thus, the soil moisture and water table depth that exists along the gradient determines the distribution of the vegetation community (Xu *et al.* 2015). Therefore, designing an experiment that considers the four key species residing within sites (i.e. MDB EWKR research site) at different elevations associated with different soil structures and nutrient deposition rates could be beneficial. In the absence of using sediment from the different sites and different elevations, simple chemical/physiological examination of the soil structure could be helpful.

Craig *et al.* (1991) compared Lignum plants under four levels of watering and four salinity levels over a 10-week period in plastic pots of native surface soil in a greenhouse, and found that Lignum cover was more strongly associated with soil hardiness (or compaction) and moisture rather than soil conductivity. Akilan *et al.* (1997) found that waterlogging River Red Gum plants with salt water over 16 weeks reduced shoot extension more than under freshwater waterlogged conditions; however, waterlogged freshwater plants produced adventitious roots just below the surface whereas no adventitious roots where formed in the salt water-affected plants.

Groundwater recharge or water table declines have been looked at internationally on other woody species (Horton & Clark 2001; Hughes *et al.* 1997; Mahoney & Rood 1991; Stella & Battles 2010). Even though these studies do not relate to the target species, the design may be beneficial if considering effects groundwater has on root development. Horton and Clark (2001) and Mahoney and Rood (1991) recorded that optimal growth and seedling survival in relation to groundwater decline was reached at ≤1 cm/day, while Hughes *et al.* (1997) measured highest growth rates in well-drained soils when water level decline rates were at 1 or 3 cm/day. Stella and Battles (2010) also considered water table declines as a stress on first year riparian seedlings (cotton wood and willows), by analysing the growth and below ground allocation response to water stress over 62 days. They found that water table recession had a strong negative influence on plant growth, with no evidence that plants increased below ground allocation in response to drawdown.

Shading effects require important considerations, as shading can impact or effect soil moisture and thus seedling establishment. A field study in Brazil investigating the importance of spatial variation in canopy cover and seasonal variation in the survival of seedlings of neotropical savannah woody species, indicated seedling establishment was low when covered with large quantities of litter (Salazar *et al.* 2012). Salazar *et al.* (2012) concluded that tree canopy cover reduces stressful environmental conditions, which, in turn, facilitates seedling establishment.

Grazing pressure on seedling establishments for the four key species is considered to be largely unknown. Cunningham *et al.* (1992) argued that the species are not readily grazed by stock except in times of drought and/or feed shortage. However, observational and anecdotal evidence suggests grazing on Lignum and River Red Gum seedlings does occur, by native animals (e.g. kangaroos) and domestic stock (e.g. sheep) (Capon *et al.* 2009; Jensen *et al.* 2008).

The re-sprouting ability of seedlings after a disturbance (e.g. grazing or flooding which results in loss of all stem and leaf material) has been investigated for northern Australian riparian tree species (Chong *et al.* 2007). To examine the disturbance, clipping was assigned to seedlings under six time treatments, and comparisons were made on seed size and seedling growth patterns and allocation to root mass and lateral root development. The results indicated that re-sprouting capacity was related to physiological and morphological specie traits rather than size or growth rates (Chong *et al.* 2007). In another grazing study that involved fenced, unfenced areas and cutting roots of woody species, the competition for resources between ground cover and woody vegetation, in the absence of grazing, was more evident (Smith *et al.* 2013).

The studies by Chong *et al.* (2007) and Smith *et al.* (2013) provide evidence of the indirect impacts grazing can have on establishment rates. Grazing can change/influence plant biomass (Reid *et al.* 2011), vegetation structure and community composition (Yates *et al.* 2000). Although in semi-arid regions, grazing has been shown to have a small influence on floodplain vegetation (excluding eucalypts) in comparison to flooding (Westbrooke *et al.* 2005). Cloven-hoofed livestock also affect the soil structure and soil regulatory processes, with soil compaction impeding root growth and thus the ability to provide plants with water and nutrients (Neave & Florence 1994; Yates *et al.* 2000). As a consequence, soil water availability may be limited in heavily grazed woodlands compared to in ungrazed woodlands, with implications for seedling establishment (Yates *et al.* 2000). Yates *et al.* (2000) concluded that livestock grazing in remnant *Eucalyptus salmonopholia* woodlands has impacted soil surface condition, and soil chemical, physical and hydrological properties, which subsequently have flow on effects to the restoration and establishment of plant species. Yates et al. (2000) found that heavily grazed woodland remnants were more susceptible to erosion due to loss of protective features such as perennial shrubs, woody debris and litter, and had higher concentrations of soil chemical properties (e.g. pH, nitrogen, EC, phosphorous, potassium) impacting nutrient status. Furthermore, rates of soil water infiltration were lower and soil temperatures were warmer when compared to remnants rarely grazed or ungrazed (Yates *et al.* 2000).

Looking beyond the seedling (and its potential root development and establishment rates) to the site-specific soil structure and associated impacts is just as important. Understanding impacts grazing has on the soil structure is also important to ensure soil water recharge, soil water storage and soil water availability are not declining in grazed areas to below-critical thresholds for seed germination and seedling establishment (Yates *et al.* 2000).

Conclusion

Understanding seedling root growth development is fundamental to successful seedling establishment. Environmental watering events should consider the successful establishment of seedlings that are able to withstand the next dry period. Putting energy and effort into the development of roots is likely to be the key way in which seedlings ensure they have access to soil moisture and potentially groundwater to enable them to survive between flows; however, very little is known about seedling root development. Understanding how different flow regimes affect seedling root development will inform the delivery of environmental watering events in terms of maintaining adequate soil moisture. Measuring the water regime parameters that affect soil moisture will help inform watering variables of frequency and duration of events. Understanding how certain non-flow drivers (e.g. grazing, soil type, soil salinity) affect the expected response of seedlings will allow water managers to alter watering events based on non-flow drivers of local relevance to their region and event, or to implement complementary management actions to mitigate these effects.

The literature highlighted a number of knowledge gaps in these areas for the establishment and recruitment of the four key species, including the lack of information between the northern and southern regions of the Basin (Table 3). The review on experimental designs and use of mesocosm methods has highlighted possible designs/methods (Table 4) that can be used to address the key questions of long-lived vegetation, such as:

1. What is the relationship between soil moisture and seedling survival and root development?
2. What is the relationship between flow parameters such as duration and frequency (sequential, multi-year) and seedling survival and root development?
3. What is the critical time period between germination and successful seedling establishment and, therefore, what sequence of multi-year watering may be required to facilitate successful establishment?
4. How do stressors and threats (e.g. soil type, salinity, grazing pressure) modify the expected recruitment outcomes to flow regimes?

Table 4. Identified knowledge gaps for recruitment and seedling establishment, based on this literature review of key species.

Process	Black Box	Coolibah	River Red Gum	Tangled Lignum
Depth of inundation	• Rates of rise and fall of floodwater • Flood depth effect on soil moisture	• Ideal and maximum flood depth	• Rates of rise and fall of floodwater • Flood depth effect on soil moisture • Site specific?	• Ideal and maximum flood depth
Duration of inundation	• Flood duration effects on germination	• Ideal and maximum flood duration	• Flood duration effects on germination • Site specific?	• Site specific? • Ideal and maximum flood duration
Sequence of and/or consecutive inundation events	• Flood seasonality effects on seed fall and reproduction	• Multiple small floods and/or follow-up shallow flooding for soil moisture effects on germination and seedling establishment	• Site specific?	
Timing/season of inundation	• Flood seasonality effects on soil moisture persistence	• Effect of season and flood timing on reproduction and seedling establishment	• Flood seasonality effects on soil moisture persistence • Site specific?	• Temperature and flood timing (season) effects on seedling survival and growth
Water stress		• Soil moisture requirements for seedling establishment	• Site specific?	• Site specific?
Soil salinity	• Unknown impact	• Unknown impact	• Unknown impact	• Unknown impact

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Recruitment of long-lived floodplain vegetation: Mesocosm study experimental design

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Recruitment of long-lived floodplain vegetation: Mesocosm study experimental design

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1 Introduction

Seedling recruitment was identified as being a priority for water managers and recent literature reviews have revealed a gap in the knowledge regarding recruitment success (Burns & Gawne 2014; Casanova 2015). It was felt that datasets looking specifically at recruitment responses were limited (pers. comm. Environmental Water Knowledge and Research (EWKR) Vegetation Theme Leadership Group), and that focusing studies on seedling responses was an appropriate way to ensure this priority research topic was addressed.

One way to investigate seedling establishment under controlled (or partially controlled) conditions is through mesocosm studies. Mesocosm studies provide a powerful means of quantifying causal relationships in a controlled environment. This study will focus on the responses of seedlings to a sequence of flooding and drying treatments. Work will be undertaken within experimental/laboratory set-ups, so there will be no specific work undertaken at MDB (Murray–Darling Basin) EWKR research sites.

Four woody floodplain species, River Red Gum (*Eucalyptus camaldulensis* Dehnh.), Black Box (*Eucalyptus largiflorens* F.Muell.) and Coolibah (*Eucalyptus coolabah* Blakely & Jacobs), and one native floodplain shrub species, Tangled Lignum (*Duma florulenta* Meissner), were identified as the key target species (Burns & Gawne 2014). Seedling-specific literature reviews were undertaken to assess and collate existing information about the recruitment of seedlings of the four key species (Durant *et al.* 2016). The information collated from the literature review, as well as expert discussions and input through workshops, teleconferences and emails, forms the basis of this experimental design.

The experiment will focus on addressing the primary question:

'What is the relationship between flow parameters such as duration, frequency and interflood-dry period (sequential, cumulative events) and establishment?'

It will also address the following secondary questions:

1. How important are patterns of root development to overall growth and survival in changing conditions?
2. How do sequential flooding and drying events affect seedling growth?
3. How does the initial condition of seedlings affect their response to a flooding/drying treatment?

Four key woody floodplain species were identified as target species, consisting of three eucalypt tree species, River Red Gum (*Eucalyptus camaldulensis* Dehnh.), Black Box (*Eucalyptus largiflorens* F.Muell.) and Coolibah (*Eucalyptus coolabah* Blakely & Jacobs), and one native floodplain shrub species, Tangled Lignum (*Duma florulenta* Meissner) (Burns & Gawne 2014).

These four species occur throughout the MDB, although populations are not consistent at all of the MDB EWKR research sites (Table 1). Due to logistical constraints, the same provenance will be used for individual species with no reference to the actual MDB EWKR research sites.

Table 1. Species distribution within the MDB based on the Atlas of Living Australia (X = present at the MDB EWKR research site).

Sites Key species	Upper Murray	Lower Murray	Macquarie Marshes	Lower Balonne floodplain
River Red Gum	X	X	X	X
Black box	X	X	X	X
Coolibah			X	X
Tangled Lignum	X	X	X	X

2 Summary of literature reviewed

A variety of literature sources were reviewed, relating directly or indirectly to the key questions and/or key species, and these have been used to form the basis of the mesocosm study experimental design. For more information about the literature reviewed on recruitment of long-lived floodplain vegetation and experimental designs, refer to Durant et al. (2016). Appendix A provides a summary of germination and seedling establishment attributes and watering requirements compiled from the literature review.

3 Experimental design

Appendix B shows a summarised version of experimental studies considered in the literature review, which forms the basis of designs and considerations in this experimental design. Knowledge gained from this literature review in combination with that regarding the current state of knowledge about the four key species, was applied to form the detailed experimental design methods described below.

3.1 Seeds source and germination

The seeds of the four species will be of known providence; however, they will not have been collected from the four MDB EWKR research sites. It is understood that in order to have an accurate representation of the key species, collection of seeds from the four MDB EWKR research sites would be preferential. However, the collection of seeds from the sites is an enormous task, and is dependent on variables such as the timing and availability of seed fall. It was determined that hydrology would be the focus of the experiment, and that the aims of this experiment would be best answered via the use of a seed source.

The germination of the four key species will be sub-contracted to a commercial nursery, who will source and grow the seedlings to the required height and/or age. Seeds will be germinated in standard horticultural germination media. Once the seedlings have grown approximately three leaves, they will be transplanted into the experimental pots (750 mm lengths of 90 mm diameter PVC pipe). After a maximum of six weeks, these pots will be deployed into the treatment tanks. Regular watering will be applied for a minimum period of two weeks to acclimatise seedlings.

3.2 Soil type

Site-specific soil characteristics play an important role in seedling establishment. Soil properties such as water holding capacity, particle size, porosity, nutrient status and organic matter will all influence plant productivity. This experiment is not investigating the effects of soil on plant production. As a result, a standard floodplain soil will be collected from the Murray River floodplain near Albury. Soil particle size (% sand and % clay), water holding capacity and the total carbon content will be determined prior to the commencement of the experiment.

The soil will not be sterilised prior to the seedling being transplanted. The main reason for soil sterilisation is to remove the seed bank of plants that may germinate during the experiment and compete with the target plant. The process of soil sterilisation can change the chemical and physical makeup of the soil. It also destroys much, if not all, of the soil micro flora and fauna, and thus may have implications for nutrient cycling. For the purpose of this study, it has been decided that pots will have non-target plants removed on a weekly basis rather than soil sterilisation.

3.3 Experimental pots

The design for the experimental pots is based on the common design approaches identified in the literature review (Appendix B), with one plant per pot. A pot is 75 cm in length and 90 mm diameter PVC pipe cut in half and taped back together. One end of the pipe will remain open (for the plant to grow up through) while the other end will be closed with a lid that has drainage holes. The benefit of splitting the PVC pipe in half is it aids in the removal of the plant at the time of sacrificial harvesting, providing an opportunity to distinguish the root length and/or depth in the chamber and preventing overall damage to the specimen. These pots/pipes will then be deployed vertically into ~1 m high outdoor circular fibre glass tanks at Wonga Wetlands, Albury. Temperature and rainfall at the site will be recorded.

3.4 Hydrological treatments

Four hydrological treatments (and potentially five treatments) (Table 2) have been identified to answer the questions:

- How do sequential flooding and drying events affect seedling growth?
- How does the initial condition of seedlings affect their response to a flooding/drying treatment?

Treatment 5 will only be added if there are sufficient plants available/alive after the establishment phase.

Table 2. Sequences of flooding/drying identified for the mesocosm study. Green indicates a drying treatment (where pots will be watered with 5 cm of water in the bottom of tanks), while blue indicates shallow flooding (2–3 cm) above the top of the pot. Red lines indicate sacrificial and observational harvesting, and the thick black lines indicate observational measurements.

	Time (Weeks)																						
Trea-tments	Establi-shment		Early Phase										Late Phase										
Week no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1																							
2																							
3																							
4																							
5																							

The experiment will be divided into three stages: establishment, and early and late development. Once seeds have germinated and reached a set age (≤ 6 weeks), seedlings will be deployed into the treatment tanks, and water levels will be maintained in the tanks 15 cm from the top of each pot for a minimum period of 2 weeks to acclimatise seedlings (establishment phase) after transportation from the commercial nursery. Treatments 1 and 2 will be flooded in the early phase for four weeks. Treatment 1 will be sequentially flooded in the late phase, while treatment 2 remains dry and treatment 5 remains constantly flooded (Table 2). Treatments 3 and 4 will remain dry in the early phase for 10 weeks. In the late phase, treatment 3 will be flooded, while treatment 4 continues to remain dry and treatment 5 continues to remain flooded (Table 2).

Floods will be imposed to a depth of 2–3 cm above the top of the pot. The length of a flood (4 weeks) has been based on the literature and known tolerance of each species (Table 3). In the absence of known tolerance levels, timing has been selected for ease of sampling based on information for species with known tolerance levels. Plants will be monitored on a weekly basis and the flooding duration may be shortened in response to high plant stress. If the flood duration is reduced, the duration of the early phase flood will become the duration of flood imposed in the late phase flooding events. The duration of flooding will be consistent across all species to allow for comparison.

Table 3. Optimal times each species can withstand flooding and/or waterlogging based on seedlings at ~2 months of age (Durant *et al.* 2016). ? equates to information unknown.

Species	Min. duration	Tolerance levels	Max. duration	Flood depth
Black Box	< 30 days	4 weeks	30–60 days	4 cm
Coolibah	?	?	?	?
River Red Gum	?	4 weeks	6 weeks	20–30 cm
Tangled Lignum	?	4–6 weeks	?	5–15 cm

To stimulate the drying period, a step down lowering rate will be applied. This step down method will be applied by lowering water in the holding tanks by approximately 15 cm a week over a 4 week period until 5 cm of water remains at the base of the pot, which will allow water movement through the soil via capillary action. If the plants become water stressed at a particular drawdown height, water will be maintained at that height and drawdown will cease, but no manual surface watering will be applied. Plants will be exposed to natural rainfall during the drying phase, as the experiment is being conducted in an outdoor facility.

3.5 Sampling and variables

Sampling time or length of experiment period in the literature ranged from 1–6 months growth time, with sampling events occurring daily, weekly, fortnightly, monthly, 3-monthly or at the end of the experiment. The length of the experiment influenced the number of sampling events required. Many different combinations of sampling events can be applied, but the inclusion of a greater number of sacrificial sampling events requires a greater monitoring effort and more seedlings.

A combination of sacrificial harvesting (three occasions) and observational monitoring (five occasions) will be undertaken through the experiment (Table 4). Sacrificial harvesting will occur at the end of the establishment phase and before the early phase, at the end of the early phase and before the late phase, and at the end of the late phase. Observational monitoring will be undertaken at the same time as sacrificial sampling and at every treatment change, as indicated by the bold black lines in Table 2. At each observation/harvest, 12 replicates (1 replicate = 1 pot = 1 plant) per species per treatment combination will be measured. As there are a different number of treatments during each phase of the experiment, there will be a different number of plants sampled at each sampling occasion, with a higher number of plants sampled during the late phase (Table 4). Treatment 5 will consist of a maximum of 16 plants per species. Based on both the literature and tolerance levels to waterlogging, it is not expected that these plants will survive past the early phase. If plants are still alive at the end of the early phase, we will have to consider the sampling replication at the end of the early phase. Variables to be measured are shown in Table 5.

Table 4. Number of replicates required to be harvested or observed per species during each phase of the experiment. Numbers in brackets will be applied in the scenario that there are sufficient plants to include the fifth (flooded) treatment.

Phase	Number of treatments and treatment type	Replicates per treatment per species	Plants harvested or observed per species within each phase
Establishment	1 – Drying	12	12
Early	2 – Drying – Flooded – Flooded then drying	12	24 (36)
Late	3 – Drying followed by drying – Flooded followed by flooded (only 4 reps) – Flooded then drying followed by flooded then drying – Flooded then drying followed by drying – Drying followed by flooded then drying	12	48 (52)
		Total plants per species required	84 (100)

Table 5. Method of sampling and associated variables. √ represents the sampling method applicable to the variable being tested.

Variables	Observational monitoring	Sacrificial harvesting
Seedling height Measured to the shoot tip (±1 mm)	√	
Soil moisture Surface soil moisture content as % volume determined via use of a soil moisture probe (Lynch 2006)	√	
Leaf numbers Leaf number calculated on leaves with a minimum length of 1 cm (Mahoney & Rood 1991)	√	
Mortality Number of seedlings that die recorded by date/time	√	
Root depth/length On day of harvest soil root column is exposed and length of root system measured to its lowest point in the soil (Neave & Florence 1994)		√
Biomass of shoot, root and leaf components Separation of roots from stem/leaves at the root-shoot junction and weighed separately after being dried in oven at 70 °C temperature for 48 hours (Horton & Clark 2001)		√
Leaf area Place leaves on scanner and determine area via image analysis (e.g. Bioscan Image analyser; Capon at al. 2009)		√

3.6 Block design and allocation of plants/treatments to tanks

Due to logistical constraints (large pot size and associated plant weight), it is not possible to randomise the allocation of treatments among different experimental tanks. As a result, each tank will be assigned to a treatment for the duration of the experiment. The current design is for the four treatments. This may change slightly (by the addition of an extra three tanks) if there are sufficient plants alive at the end of the establishment phase to include a fifth treatment. Twelve tanks will be used for the duration of the experiment, with three tanks randomly allocated to each treatment (Figure 1).

At the commencement of the experiment, each tank will have 28 plants/pots (seven plants of each species) randomly allocated to each of the 12 tanks. All surplus plants will be randomly placed into holding tanks and treated the same way as the experimental tanks. If it is found that plants have died or are visibly unwell at the end of the establishment phase, they will be replaced with plants from the holding tanks. Plant mortality (if observed) is likely to occur as a result of transportation/shock from being moved from a nursery to the open air research facility. At this stage it will be determined if there are sufficient plants remaining to run the fifth treatment, the holding tanks will become the fifth treatment. Four plants will be harvested at the end of the establishment phase from each of the treatment tanks (excluding the holding tanks) (one plant of each species). Monitoring during and sampling at the end of the early phase, where there are two (or three) treatments, eight plants will be harvested from each tank (two plants of each species). Monitoring during and sampling at the end of the late phase, where there are four (or five) treatments, 16 plants (or all remaining for the flooded treatment) will be harvested from each tank (four plants of each species). The tank numbers will be recorded to allow statistical testing to determine if there is a tank effect.

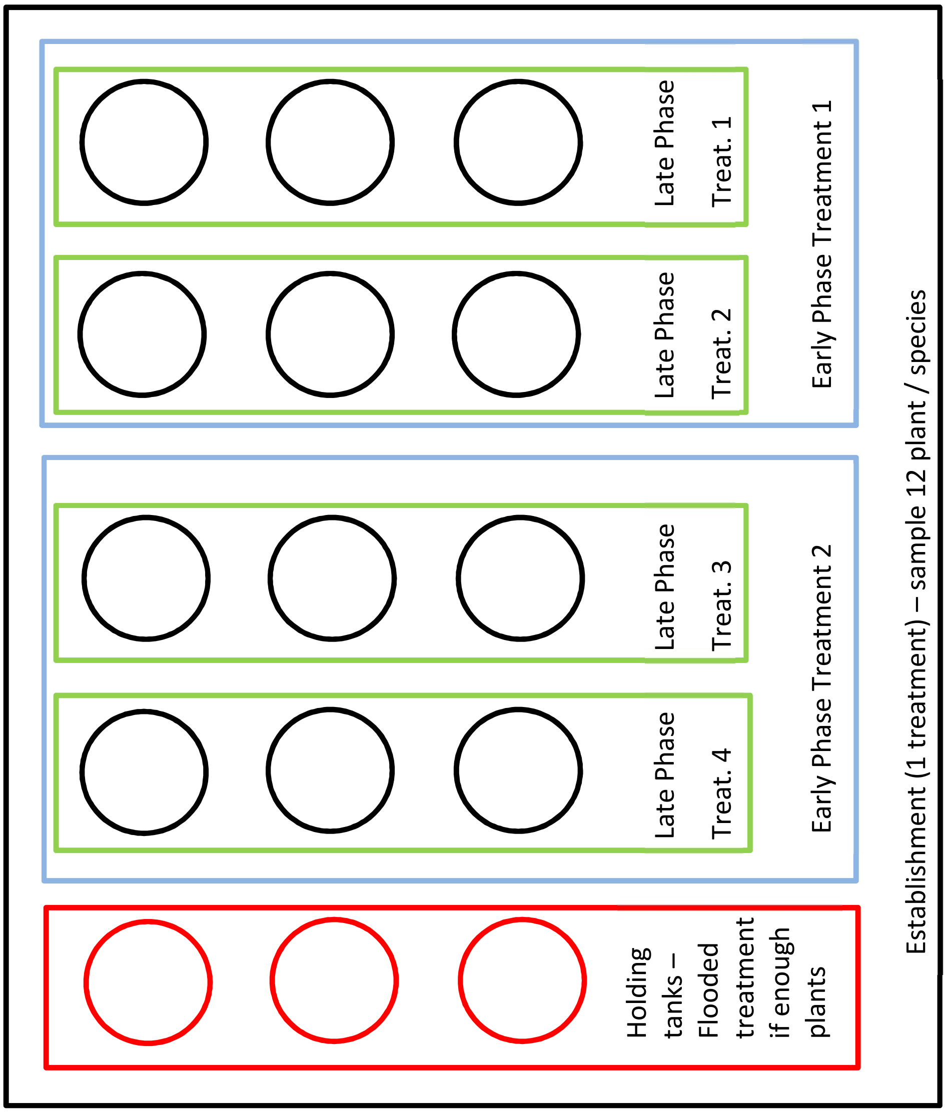

Figure 1 . Schematic diagram of experimental tanks and allocation of tanks to treatments.

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4 Appendix A: Summary of germination and seedling establishment attributes and watering requirements

Summary of germination and seedling establishment attributes and watering requirements. Blue is for refereed scientific literature; red is for reviews and books; grey is for published reports, proceedings and theses (grey literature).

Key Species	Process	Description	
		Recruitment (germination)	**Seedling establishment**
Black Box	Depth of inundation	• Most likely on moist–wet soils (Holloway *et al.* 2013; Johns *et al.* 2009) • No direct impact on depth as seeds will germinate while floating or underwater (Jensen 2008; Johns *et al.* 2009) • High rainfall and/or flooding increases germination (Jensen 2008)	• Not tolerate waterlogging, unlikely to survive prolonged immersion (Jensen 2008; Johns *et al.* 2009) • Slower growth when flooded to 5 cm (Johns *et al.* 2009; Casanova 2015) • Recommended flood depth 4 cm (Casanova 2015) • Ideal depth less than total seedling height (Johns *et al.* 2009)
	Duration of inundation	• No direct impact on duration, but unlikely to survive prolonged immersion (Johns *et al.* 2009) • Seeds die if submerged for >10 days (Casanova 2015).	• Ideal <30 days, maximum 30–60 days depending on seedling size (Johns *et al.* 2009) • Two-month-old plants can tolerate waterlogging for 1 month (Johns *et al.* 2009; Casanova 2015) • Signs of stress from waterlogging after 70 days at 22 months of age (Johns *et al.* 2009) • Duration should be sufficient to ensure maintenance of soil moisture (Johns *et al.* 2009) • Flood duration 4 weeks after 2 months of age (Casanova 2015)
	Sequence of and/or consecutive inundation	• Requires follow-up water (Casanova 2015)	• Follow-up watering, whether from rainfall or shallow inundation in the first or second year, is expected to improve establishment (Holloway *et al.* 2013). • Summer after germination (or local rainfall) (Casanova 2015) • Frequency of inundation variable depending on sites soil properties, evaporation rates and rainfall (Johns *et al.* 2009)

	Water stress	• Requires flooding and/or local rainfall (Casanova 2015)	• Not tolerate waterlogging or complete immersion (Johns *et al.* 2009) • Soil moisture of 10–25% is critical (Jensen 2008) • Intolerant of drought (Casanova 2015) • Slow drawdown rates detrimental to establishment as seedling do not tolerate extended periods of waterlogging (Johns *et al.* 2009) • Flowing water may lead to dislodgement or burial (Johns *et al.* 2009) • Artificial flood not so useful (Casanova 2015)
	Timing/season of inundation	• Requirements 15–35 °C for germination (Casanova 2015; Rogers & Ralph 2011) • Inundation receding in spring–early summer provides moist conditions (Johns *et al.* 2009; Rogers & Ralph 2011; Holloway *et al.* 2013) • Local rainfall in spring–summer (Casanova 2015)	• Floods in winter–late spring optimal (Johns *et al.* 2009) • Flood recession in spring to summer to provide moist conditions (Holloway *et al.* 2013; Casanova 2015) or local rainfall (Casanova 2015) • Grow in summer after shedding old leaves and bark (Casanova 2015) • Newly germinated seedlings susceptible to frost and heat injury (Johns *et al.* 2009) • Follow-up inundation in same season as germination or following season (Holloway *et al.* 2013) • Timing should be sufficient to ensure maintenance of soil moisture in the first summer after germination (Johns *et al.* 2009)
	Grazing pressure	• Vulnerable (Casanova 2015)	• Vulnerable, seedlings are grazed (Casanova 2015) • Grazing, particularly by sheep (and more so than cattle and rabbit burrowing), restricts establishment and impacts soil structure (Smith & Smith 1990)
	Soil salinity		• Salinity tolerant (related to ground and surface water) (Casanova 2015) • Sodic soils overlying highly saline groundwater causes high mortality in first 2 years (Morris 1984)

Coolibah	Depth of inundation	• Most likely to be influential on wet soils following floods or rainfall (Roberts & Marston 2011) • Not critical to seed germination (Holloway *et al.* 2013) • Requires moist soil (Holloway *et al.* 2013)	
	Duration of inundation		• Longer flood = fewer seedlings (Casanova 2015)
	Sequence of and/or consecutive inundation	• Follow-up floods in summer of first year thought to increase recruitment rates (Roberts & Marston 2011)	• Regular rainfall required for establishment (but saturated soil following inundation might be adequate) (Casanova 2015) • Follow-up rainfall or shallow inundation in summer of first year (or second year) thought to increase seedling recruitment rates (Roberts & Marston 2011) • Sequence of floods or flood and wet years may be necessary (Li & Wang 2003; Tuomela *et al.* 2001)
	Water stress	• Seeds take two weeks to germinate (Casanova 2015)	
	Timing/season of inundation	• Fluctuating temperature 15–30 °C for germination, vulnerable to frost, adapted to regeneration after late summer flooding (Capon *et al.* 2009). • Flood recession in spring to provide warm and moist conditions (Holloway *et al.* 2013) • Timing not critical (Holloway *et al.* 2013)	• Shade or protection from summer heat required (Casanova 2015) • Flood recession in spring to provide warm and moist conditions (Holloway *et al.* 2013) • Flood summer–late summer (but other factors important e.g. rainfall) (Casanova 2015)
	Grazing pressure	• Successful recruitment may require protection from grazing (Roberts 1993)	• Seedlings die from herbivory (Casanova 2015) • Grazing, seasonal conditions and competition from grass (not so important) effects (Casanova 2015)
	Soil salinity	• Reproductive effort may be lowered by soil salinity (Roberts 1993; Roberts & Marston 2011)	• Are salt-tolerant in the Diamantina River region i.e. utilise saline groundwater (Costelloe *et al.* 2008; Roberts & Marston 2011)

River Red Gum	Depth of inundation	• Moist soils required (Johns *et al.* 2009) • Germination success primarily controlled by seed availability and moisture availability after seed dispersal — most seeds germinate within 10 days of watering (Johns *et al.* 2009) • No direct impact on depth as seeds can germinate while floating (Johns *et al.* 2009) • Flooding after germination may lead to mortality (burial, dislodgement or immersion periods) (Johns *et al.* 2009)	• In southern MDB: do not tolerate waterlogging and complete or prolonged immersion (Roberts & Marston 2011) • Depth will affect subsequent seedlings survival and establishment (Johns *et al.* 2009) • On moist soil following flood recession • Tolerance to waterlogging increases with seedling height • Shallow flooding (20–30 cm) preferable to avoid over-topping seedlings in first year (Holloway *et al.* 2013)
	Duration of inundation	• No direct impact (Johns et al 2009). • Seeds die after 10 days of immersion (Casanova 2015)	• In southern MDB: 2-month-old plants can withstand waterlogging for 1 month, 50–60 cm plants can survive for 4–6 months flood, but only several weeks if completely submerged (Roberts & Marston 2011) • Maximum duration 1–6 months depending on seedling size (Holloway *et al.* 2013) • Susceptible to prolonged flooding (Roberts & Marston 2011) • Four-to-six weeks is adequate, but longer can be tolerated depending on age and if totally submerged (Holloway *et al.* 2013)
	Sequence of and/or consecutive inundation		• In southern MDB: follow-up watering 1 year after germination (George 2004) • Requires watering 1–2 months after spring rain or small flood (Casanova 2015) • Sufficient to maintain soil surface moisture during first year and needs adequate moisture in the second season (Johns *et al.* 2009) • Follow-up flood to recharge soil moisture is desirable in same year as germination or following year (Holloway *et al.* 2015)
	Water stress	• Germinate within 5 days given adequate moisture (Holloway *et al.* 2013) • Soil moisture required >10% (Holloway *et al.* 2013)	• In southern MDB: soil moisture levels 10–25% in top 10 cm ideal (Jensen 2008; Holloway *et al.* 2013) • In southern MDB: low density response to above-average (>300 mm) annual rainfall, with higher establishment

		• Seeds require imbibing (saturation) and light to break dormancy (Casanova 2015)	occurring in response to medium-to-large flood events — recharges soil moisture (George 2004; Jensen *et al.* 2008) • Inhibited by drought conditions, develops adventitious roots in response to flooding (Casanova 2015) • Rapid drawdown rate preferable as intolerant of prolonged periods of immersion (Roberts & Marston 2011) • Competition for moisture by other understorey and/or overstorey vegetation • Maintenance of soil moisture within first year is critical (Holloway *et al.* 2013) • Seedlings wilt and die rapidly once soil moisture falls below 10% (Jensen 2008)
	Timing/season of inundation	• Flood receding in spring–early summer preferred (Johns *et al.* 2009) • Rates limited by low temperatures and light availability (Holloway *et al*. 2013) • Require adequate moisture and day time temperature >30 °C for germination (Holloway *et al* 2013) • Optimal temperature 35 °C (11–34 °C) (Casanova 2015) • Adequate water applied before germination (Roberts & Marston 2011)	• In southern MDB: winter flood receding in spring/early summer maintains soil moisture and avoids extreme temperatures for seedling survival (Roberts & Marston 2011) • Sensitive to frost • Flooding after germination may lead to seedling mortality due to burial, dislodgement or excessive immersion periods (Roberts & Marston 2011) • Recession spring/early summer (or sufficient rainfall), artificial watering to extend effect (Casanova 2015)
	Grazing pressure (and competition)	• Seeds removed by ants (Casanova 2015)	• Seed predation varies through the year, lowest under sheep grazing, highest in ungrazed, high under cattle grazing (Casanova 2015) • Compete with reeds and weeds (Casanova 2015) • Increased during flood (cattle, kangaroos, rabbits) (Casanova 2015) • Grazed more during drought (Casanova 2015) • Grazing (sheep, cattle and kangaroos) severely restrict regeneration (cattle are less destructive) (Smith & Smith 1990)
	Soil salinity		

Tangled Lignum	Depth of inundation	• In water (while floating) or wet mud, occurs after flooding (Campbell 1973; Chong & Walker 2005; Holloway *et al.* 2013)	• Damp conditions promote growth, while flooding, waterlogged and dry conditions impede growth (Capon *et al.* 2009) • Damp conditions associated with drying phase facilitate growth (Capon *et al.* 2009) • Depth of flood seedling establishment < 15 cm (Casanova 2015)
	Duration of inundation		• In northern MDB: 2–4 months rapid growth in damp and drying, while flooding, waterlogging and dry conditions impede growth (Capon *et al.* 2009) • Flood duration 3 months (Casanova 2015)
	Sequence of and/or consecutive inundation	• May require consecutive floods, one to promote flowering and seed set and one to promote germination (Rogers & Ralph 2011)	• Needs flood once in 12–18 months of 5–15 cm for 4–6 weeks in late spring/summer (Casanova 2015) • Spreads predominantly via vegetative growth, particularly in more frequently flooded areas (Casanova 2015) • Follow up flood 9–12 months after germination (Casanova 2015)
	Water stress	• Germination occurs within 14 days of dispersal (6–12 days) (Casanova 2015)	• Soil moisture known for northern MDB; damp conditions, can survive flooding (Capon *et al.* 2009). • More tolerant of drying than flooding (Capon *et al.* 2009) • Opportunistic and rapid growth under optimal experimental conditions (Holloway *et al.* 2013)
	Timing/season of inundation	• Flood timing spring/summer preference (Rogers & Ralph 2011) • Rates are temperature dependent (Holloway *et al.* 2013) • Season appears to be critical for germination (late summer to autumn) (Casanova 2015) • Appears to recruit continuously (Casanova 2015)	
	Grazing pressure	• Vulnerable to ant predation (Casanova 2015)	• Can survive grazing but at stunted growth (Jensen 2008)

			• Vulnerable to grazing (Capon *et al.* 2009) • Grazing and competition pressure unknown (Casanova 2015)
	Soil salinity		

5 Appendix B: Summary of methods from relevant reviewed literature to aid in mesocosm designs

Summary of methods from the literature used to aid in the mesocosm design.

	Reference	Factors	Treatments	Sampling period	Method/design
Inundation/flow regime (including depth, duration, timing and sequence)	Li and Wang (2003)	Basal diameter, shoot height, stem cross sectional area. Leaf area. Biomass- leaves, stem and roots. Root length. Stomatal density and guard cell length. Leaf water potential. Carbon isotope on leaf. Evaporation of water loss from soil surface.	Three water treatments: 100 (well watered), 50 (water stress) and 25% (water stress) field capacity. One species: *E. microtheca*.	5 months.	POTS: pots enclosed in plastic bags. 5L pot. SOIL: sand with slow release fertiliser.
	Tuomela *et al.* (2001)	Total plant biomass. Allocation of dry matter to roots and shoots. Specific leaf area (SLA). Water use and long term water use efficiency. Carbon isotope. Temperature and relative humidity. Height, xylem diameter above root collar, total leaf area, total leaf shot and root dry weight.	Three watering treatments: field capacity, 50 and 20% field capacity.	5 months; Temperature and RH daily. Control field capacity watered every 2 days at dusk. Two-day cyclical watering regime for other two treatments. Invasive harvest at 3, 4 and 5 months — 4 reps.	POTS: 2 L pot. SOIL: commercial peat-sand mix with fertiliser. Fertilised twice daily. SET UP: naturally lit glasshouse minimum temperature 17 °C.
	Awe *et al.* (1976)	Seedling height, basal stem diameter, number of leaves on stem and on branches, number of branches and number of internodes. Length of roots, root and shoot dry weight.	Three watering treatments: prolonged drought, progressive drying out, continued moisture.	80 days: Observations made every 10 days.	POTS: PVC pipes 12 cm diameter, 1 m in length, open ended, pipes halved lengthwise then sealed back with insulating tape and copper wire. One open end enclosed with nylon mesh.

			Three plant species: *E. camaldulensis*, *E. saligna* and *E.. pilularis.*		SOIL: 3:1 mixture to represent natural riverbank of floodplain soil. SET UP: pots placed in small plastic buckets on coarse gravel, under controlled temperatures between 15 and 30 °C.
	George (2004)	Germination methods, seedling height, time growth stages, flowering, seed fall, abundance and diversity, tree aging.	Field survey — 127 plots along 21 transects. Two species — River Red Gum and Black Box.	Germination — 3 weeks.	Transects perpendicular to river. Diameter at Breast Height (DBH) for trees >1.3 m. Elevated seed traps to collect seeds. Germination via emergent method: CT room, 12 hour light/temperature. Temperatures 15 and 35 °C.
	Argus *et al.* (2015)	Root dry mass, above ground dry mass, root porosity, root anatomical measurements.	One species: *E. camaldulensis* subsp. *refulgens* Three treatments: flooding 2 cm, free draining, flooding 2 cm 88 days then free draining.	88 days and additional 35 days.	POTS: 4.25 L, 200 mm diameter. SOIL: coarse river sand. SET UP: glasshouse, temperature range of 15.9–27 °C.
	Maxwell *et al.* (2015)	Stem length, number leaves, biomass.	Two species: *Acacia stenophylla* and *Casurarina cunninhamiana.* 4 Treatments: control, flood then dried, fully submerged 15 cm & partially submerged 5 cm. 7-10 reps.	15, 30, 40, 50, 55, 70, 80 and 90 days.	POTS: 200 mm length, 50 mm diameter pipe with 90% shade cloth on bottom. SOIL: low grade coarse potting mix and potting sand ratio 10:1.

Water stress (i.e. soil moisture)	Jensen (2008)	Soil moisture, number of roots and shoots, length of shoots.	Three species: River Red Gum, Black Box and Lignum. Four watering treatments: rainfall, flood, rainfall followed later by flooding, flooding followed later by rain. Controls: constant dry, constant soil moisture. Five types of sediment. Five reps of each.	12 weeks	POTS: ice cream containers. SOIL: collected from floodplain sites. SET UP: glasshouse up to temperature 30 °C.
	Capon *et al.* (2009)	Seedling heights, root depths and leaf numbers. Total leaf area, biomass of shoot, root and leaf components.	Five watering treatments: deep flooding, shallow flooding, waterlogging, damp, drying. Two sediment types: clay and clay/river sand mix. One species: Lignum.	180 days (6 months). 4 harvest times: 30, 60, 120, 180 days.	POTS: PVC pipe 750 mm diameter, 30 cm in length. Open ended gauze at one end. SOIL: steam-sterilised clay collected from field site and mix of sterilised clay and river sand. SET UP: pipe sat in larger buckets. Temperatures averaged 17–37 °C.
	Capon (2012)	Number and type of seedlings, height, recruit type, distance to nearest adult plant, number of stems, % greenness, extant vegetation canopy, bare ground, leaf litter cover, soil composition	Four species: River Red Gum, Coolibah, River Cooba and Tangled Lignum. Transects: 50 m long perpendicular to river channel. Three monitoring trips.	6 months.	Transects perpendicular to river channel based on presence of seedling patches. Literature review — regeneration of plant species. Conceptual models generated.
	Neave and Florence (1994)	Biomass shoot and root. Length of root to lowest point in soil. Moisture content. Length of root through soil structure.	Soil structure divided into 3 lengths: top 20 cm, 20–40 cm and below 40 cm. Eight species: all eucalypts.	65 days.	POTS: PVC pipe 12 cm diameter, 1 m in length open ended. Pipe halved lengthwise and sealed back together, gauze at base.

			Two watering regimes: mechanically watering twice daily and bringing tubes to field capacity and then allowing them to dry. Four reps.		SOIL: sandy loam commercially purchased, fertiliser added.
	Lynch (2006)	Soil moisture, number of leaves, flowers.	Five readings of soil moisture: 0, 5, 10, 15 and 20 cm. Four watering treatments: 50, 500, 1000 ml and no water. One species: Lignum.	10 weeks. Non-invasive — weekly soil moisture, stem growth, number of leaves and flowers.	POTS: PVC pipe 90 mm diameter, 35 cm length. Bottoms covered with duct tape puncture for drainage. SOIL: 1:3:4 Couse sand: clay: potting mix. SET UP: glasshouse natural light.
	Jensen *et al.* (2008)	Soil moisture, wet and dry weights, surface soil salinity, pH and organic carbon.	Two species: River Red Gum and Black Box.		SET UP: in field within habitats.
	Florentine and Fox (2002)	Seedling height, number leaves, leaf dimensions, fresh and dry weight of root and shoot, leaf gas exchange	Three species: *E. victrix, E. terminalis, E. leucophloia.* Two water treatments: 1 cm deep, 15–20 mm deep (waterlogging).	32 weeks and 65 days (~9 weeks).	POTS: cylindrical 13 cm diameter, bottoms opening sealed with plastic draining tapes. SOIL: clay soil from site and red clay loan pH 7. SET UP: fibreglass tanks of 2.5 x 0.5 x 0.5 filled with rainwater to depth of 1 cm above pot soil level, outside in full sun.
	Schütz *et al.* (2002)	Root and hypocotyl lengths, fresh weight, soil moisture.	Five reps. Four species: all eucalypts. Two soil types: deep sand and lateritic loam.	14 days.	POTS: 80 mm diameter, 150 mm height with drainage holes, 700 ml soil material. SOIL: deep sand and lateritic loam.

			Two water treatments: full watering 250 ml, low watering 125 ml (below field capacity).		
Salinity (including soil, water and groundwater)	Akilan *et al.* (1997)	Shoot height. Leaf, steam and root dry weights, concentrations of Na and Cl in leaves and roots, water use, sap flow, stomatal conductance and net gas exchange.	Three treatments: freely drained control, waterlogging with fresh water, waterlogging with salt water. Two reps each. One species: River Red Gum, 2 clones.	16 weeks.	POTS: 255 mm diameter. Top of pot covered with black plastic. SOIL: composite mix peat/loam/sand with nutrients. SET UP: glasshouse.
	Craig *et al.* (1991)	Stem length.	Four water treatments: 10, 225, 450 and 900 ml. Four salinity treatments: 0, 250, 10 000 and 40 000 mgL^{-1}. Four reps. One species: Lignum.	10 weeks; weekly watering	POTS: 6L plastic pot SOIL: 3.6kg mixed native surface soil SET UP: glasshouse July - September Average temperature 13–27 °C.
	Horton and Clark (2001)	Mortality, plant height. Length of longest root, leaf area. Biomass of roots and shoots. Volumetric water content of substrate.	Four ground water decline rates: 0, 1, 2 and 4 cm/day. One sediment type: sand/gravel mix. Two species: *Salix* and *Tamarix*.	67 days. Non-invasive — twice weekly. One harvest.	POTS: PVC pipes see ref 13. SOIL: 3:1 volume of sand to river gravel mix (simulate natural river substrate). SET UP: 4 rhizopods (apparatus of PVC pipes and central reservoir). Consistent environmental conditions, 12 h light/dark cycle at night — 17 °C/30% RH, day — 24 °C/20% RH.

	Mahoney and Rood (1991)	Plaint height, leaf number. Length of major roots. Biomass of roots. Leaf area.	Five rates of water table decline: 0, 1, 2, 4 and 8 cm/day. Two species: Papules. One sediment type: sand/gravel mix.	46 days. Non-invasive — daily recording plant height and leaf number for 3 weeks, twice weekly thereafter. Each rhizopod water level adjusted 3 times daily.	POTS: 15 growth tubes (PVC pipe 8 cm diameter x 1.2 m long caped and sealed at bottom) around a central reservoir (PVC pipe 20 cm diameter x 1.2 m long sealed at bottom with fiberglass and fitted with drain valves and tygon tubes). Each growth tube connected to reservoir with tygon tubing. SOIL: base of each growth tube 10 cm coarse gravel. 1:2 mix of sand and gravel (simulate study area). SET UP: rhizopods — each rhizopod positioned in wooden frame.
	Hughes *et al.* (1997)	Shoot height, leaf number, leaf length and health. Leaf area, biomass shoot and root. Weight of nitrogen fixing nodules. Root length and depth.	Two sediment types: sandy silts, coarse sand/fine sand/gravel mix. Five water table drawdown rates: 3 cm/day, 3 cm/day + weekly rain application, 1 cm/day, 0.5 cm/day and 0 cm/day. One species: *Alnus incana*.	155 days (~5 months). Sampling at start, fortnightly for 14 weeks then 2 monthly intervals. Weekly watering.	POTS: rhizopods with terniometers to read soil suction. Sixteen growth tubes around central water well of 1.2 m height. SOIL: from river sites sandy silts and coarse/fine sand/gravel mix. Standard and uniform N-P-K soil nutrient prior to start applied. SET UP: pump system automatically applied to control drawdown rate. Five rhizopods in total. Greenhouse temp 15–30 °C.
	Stella and Battles (2010)	Root length, mortality, plant height, leaf length. Biomass of root, stem and leaf. Leaf tissue analyses — SLA, Carbon-	Three water table decline rates: 0, 1 and 3 cm/day.	62 days. Non-invasive daily monitoring of	POTS: PVC pipe 125 cm long x 3.2 cm diameter packed with sand and suspended in tanks.

		Nitrogen and stable carbon isotope ratio.	Three species: cottonwood and willows.	mortality, weekly height and length of longest leaf. Invasive sampling day 0, 18 and 49 when 4–8 live seedlings harvested.	SET UP: outside in late summer. Steel cylindrical tanks (125 cm deep x 61 cm diameter) with bottom drain and flexible discharge tube. Thirty-two to 41 seedlings. Tank water levels remained at 10 cm below soil surface for control and starting point for all other treatments.
Grazing (including shading and competition)	Salazar *et al.* (2012)	Light quantity and quality, soil nutrient availability, pH soil gravimetric water content and soil water potential. Quantify litter cover among vegetation types, effect of litter cover.	Three transects — 9 plots, 3 major vegetation types in field. Four monitoring times.	14 months.	In field: transects 1000 m length, 9 plots 20x20 m along each transect. Within each 20x20 m plot, 8 subplots of 1x1 m. All seedlings up to 30 cm tall tagged and identified.
	Chong *et al.* (2007)	Seed size, leaf developmental stage (number leaves, fresh shoot lengths, root length, number of lateral roots) biomass.	Three groups – clipping, biomass and control. Four species — *Melaleuca leucadendea, Asteromyrtus symphyocarpa, E. camaldulesis* var. *obtusa, tristaniopsis laurnia.* Six treatments: clipping at 5, 10, 15, 25, 40 and 60 days.	3 months.	POTS: seedling tubes (70x70x160 mm) SOIL: 6:3:2 steam sterilised sand/peat/perlite mix containing macro nutrients. SET UP: 5 PVC watering trays (1200x1200x150 mm) with 250 tubes per species (1000 seedling tubes).

	Good *et al.* (2014)	Biomass	Two treatments — without competition, with competition and clipping. Two species — *Paspalidium jubiflorum* and Coolibah. One water treatment — every 2 days with 500ml. Two clipping treatments to grass: clipped 5 cm above soil surface, clipped to maintain maximum growth of 30 cm.	2 months.	POTS; round — 20 cm diameter, 20 cm deep. SOIL: 2 cm sand at bottom, 2 cm from top of field soil.
	Smith *et al.* (2013)	Soil chemistry, groundcover.	Three treatments: grazing large herbivores, competition grazing exclusion. Habitat: woody encroachment with *E. populnea* subsp. *bimbil* and *E. intertexta* and Geijera, Dodonaea and Eremophila understorey.	Three, 16 and 30 months.	SET UP: in field, cut trenches recovered with soil surface.
Soil properties	Bennett *et al.* (1986)	Root and shoot biomass, root length and diameter. Plant height, number of leaves, and number of branches.	Two soil types: peat/sand 3:1 mix, forest soil from site. Two water treatments: 80% field capacity for forest soil, 100% for peat/sand mix. One species: Jarrah.	Four months; weekly sampling non-invasive of plant height. Sampling times at 0 then monthly, 5 reps each harvest.	POTS: undrained PVC pipes, 10.5 cm diameter and 40 cm deep, lined with polythene bags and about 50 ml of sand at bottom. SOIL: peat/sand 3:1 mix and forest soil from site. Soil surface covered with 30 g alkathene beans. Fertilised added twice weekly.

			Two cultures: seeds and micro-propagated plantlets.		SET UP: pH of soil adjusted to 6 and deionised water used. Pots placed in root cooling tank maintained at 20 °C in glasshouse with temperature range of 15–36 °C.
	Bell *et al.* (1993)	Plant height, diameter of trunk, leaf type, biomass of leaf and roots. Length and width area of leaf. Root length. Basic root shape, density, breadth.	Two soil treatments. One watering treatment. One species: River Red Gum. Three below ground root zones: upper 20 cm, 20–40 cm below, 40 cm below.	Six months.	POTS: in drums filled with sand, sand/clay mix — drums will drainage holes. SEEDS: had 5 months in jiffy pots before transferring to drums.

6 Appendix C: Proposed timing of experimental design

Proposed timeframe for experimental design

Week starting	**22/8**			**12/9**					**17/10**		**31/10**				**28/11**						**9/1**				**6/2**						**20/3**
Events: Week no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Seeds to nursery																															
Nursery time to grow seedlings																															
Pots cut/to nursery																															
Wonga clean up																															
Plant collection from nursery																															
Two-week establishment period																															
Establishment harvest																															
Change in treatment (observational)																															
Early phase harvest & change in treatment																															
Change in treatment (observational)																															
Late phase harvest																															

Key dates:

• Commencement of establishment period	17 October 2016
• Establishment harvest	31 October 2016
• Observational harvest/change in treatment	28 November 2016
• Early phase harvest and change in treatment	9 January 2017
• Observational harvest/change in treatment	6 February 2017
• Late phase harvest (end of experiment)	20 March 2017

EWKR
Environmental Water Knowledge & Research

LA TROBE UNIVERSITY

Early, late or constant – what are long-lived woody floodplain seedlings looking for?

Rebecca Durant, Cherie Campbell, Sam Capon, Susan Gehrig, Nerissa Haby, Cassie James, Kay Morris, Jason Nicol, Daryl Nielsen, Rachel Thomas and Jess Wilson

58th Australian Freshwater Sciences Society Congress
Adelaide, 23-28 September 2018

MDB EWKR is a 5 year, $10 million research project funded by the Commonwealth Environmental Water Office

The project is a collaboration between the MDFRC as lead together with 12 other research organisations

Aim to improve science to support environmental water planning and management

Address gaps in environmental watering information on waterbirds, vegetation, fish and food webs

Background

- Watering regimes
- Environmental watering
- Floodplain ecosystems

Background

- Floodplain vegetation

- Long-lived woody vegetation

Three Eucalypt species

Black Box (*Eucalyptus largiflorens* F.Muell.)

River Red Gum (*Eucalyptus camaldulensis* Dehnh.)

Coolibah (*Eucalyptus coolabah* Blakely & Jacobs)

Research

- Adult population survival and condition
- Early studies as part of silviculture industry
- Flow regime requirements and influence



Understanding – why?

- Importance of Root development
- Maintaining soil moisture



Aim

To determine the relationship between flow parameters such as duration, frequency and interflood-dry period (sequential, cumulative events) and establishment

1. How do sequential flooding and drying events affect seedling growth of long-lived species?
2. How important are patterns of root development to overall growth and survival in changing conditions?
3. How does the initial condition of seedlings (Phase 1) affect their response to a subsequent flooding / drying treatment (Phase 2)?

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3. How does the initial condition of seedlings (Phase 1) affect their response to a subsequent flooding / drying treatment (Phase 2)?

Seedling establishment

- Response to flooding
- Not germination


Mesocosm

Watering treatments

	Time (Weeks)																						
Treat-ments	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
SF																							

Sequential flooding (SF)

Watering treatments

	Time (Weeks)																						
Treat-ments	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
SF																							
EF																							

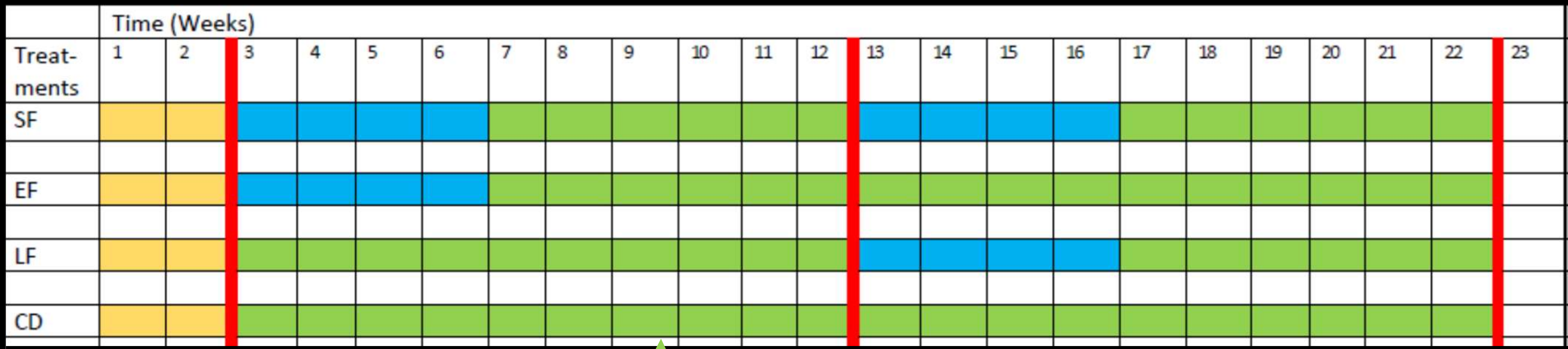
Early flood
(EF)

Watering treatments

	Time (Weeks)																						
Treat-ments	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
SF																							
EF																							
LF																							

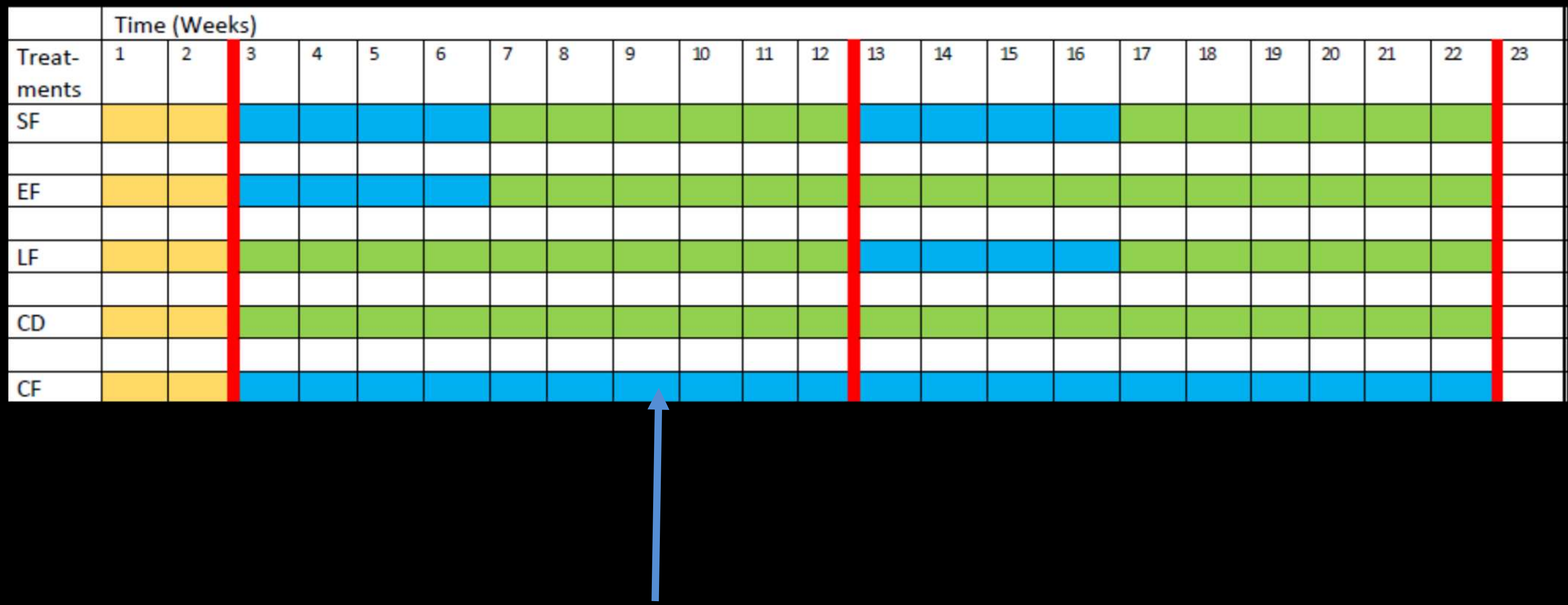
Late flood
(LF)

Watering treatments



Constant Dry (CD)

Watering treatments

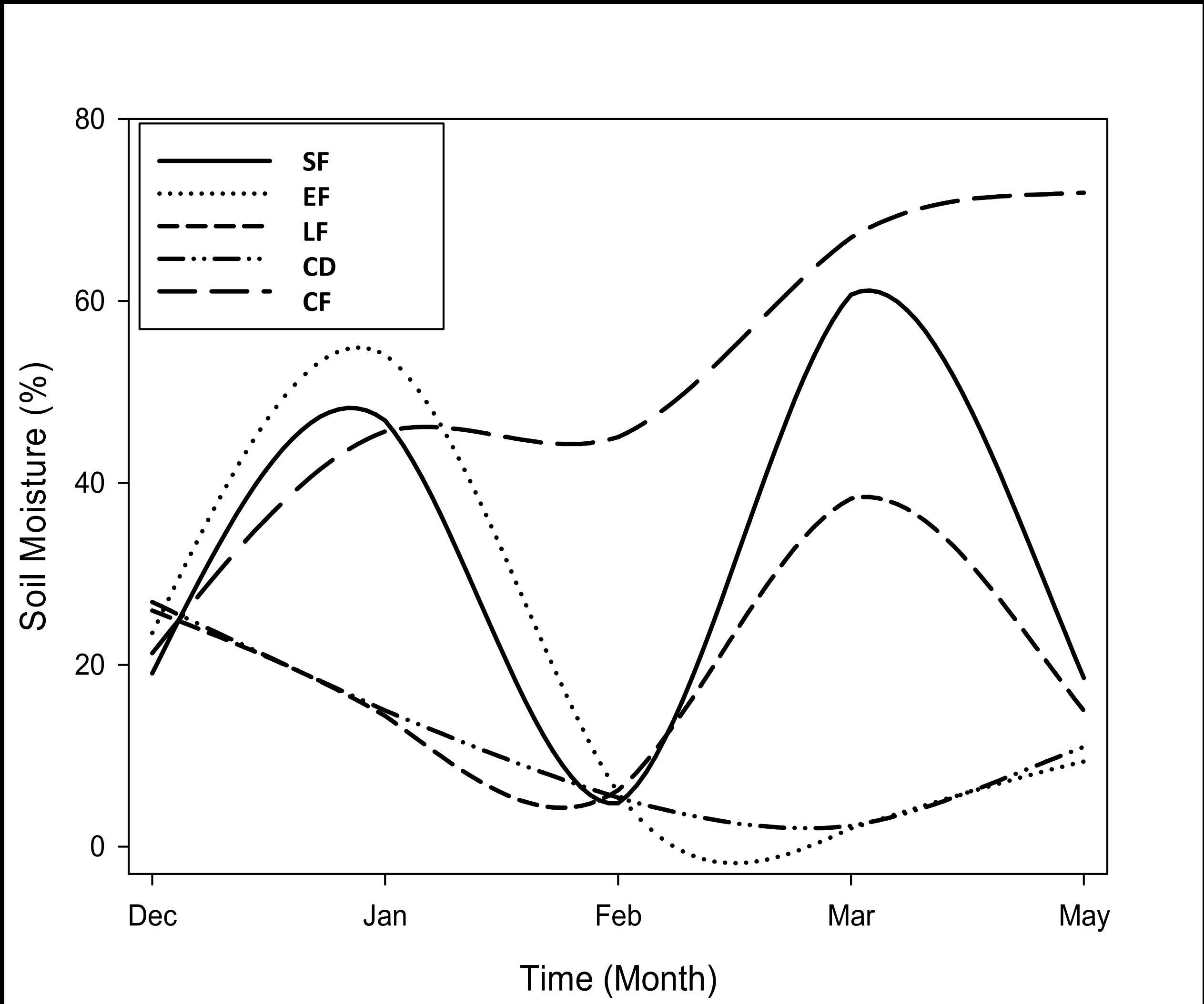


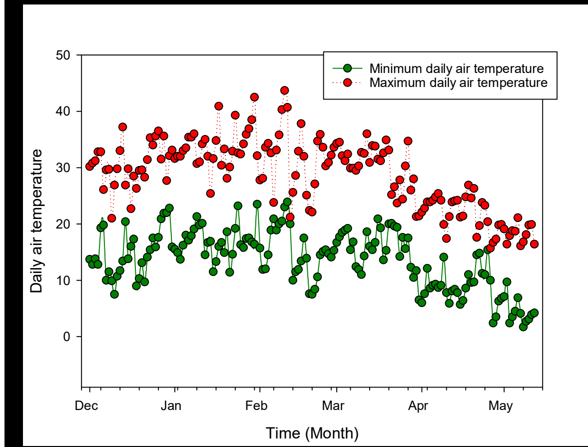
Constant Flood (CF)

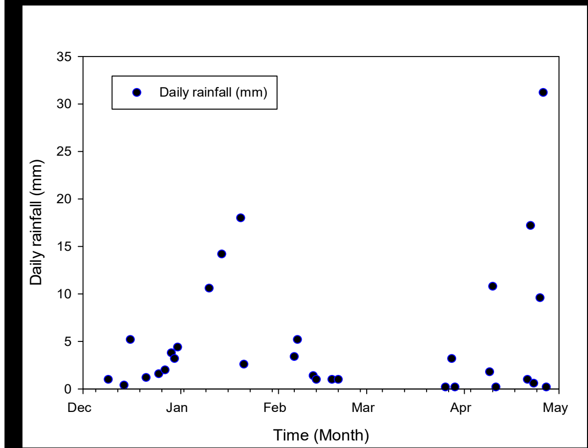
Depth

Soil moisture

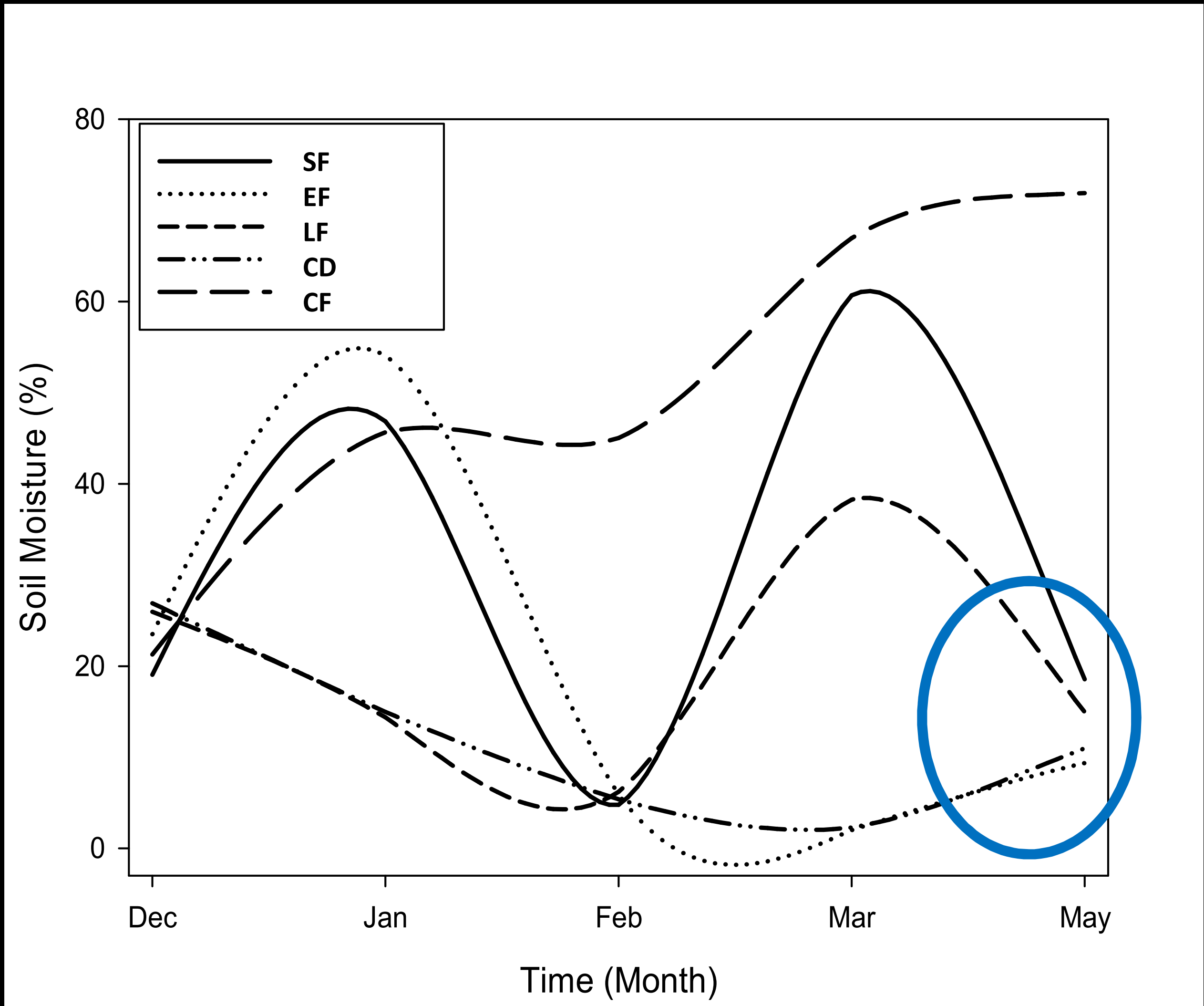
Soil Moisture

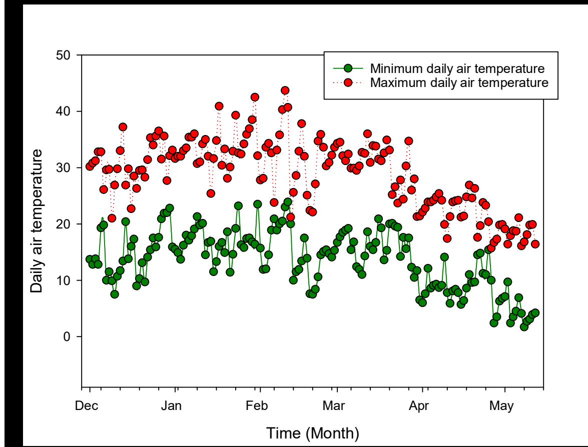


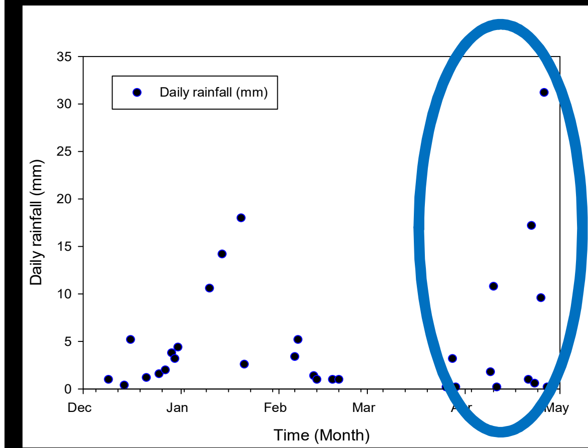




Soil Moisture



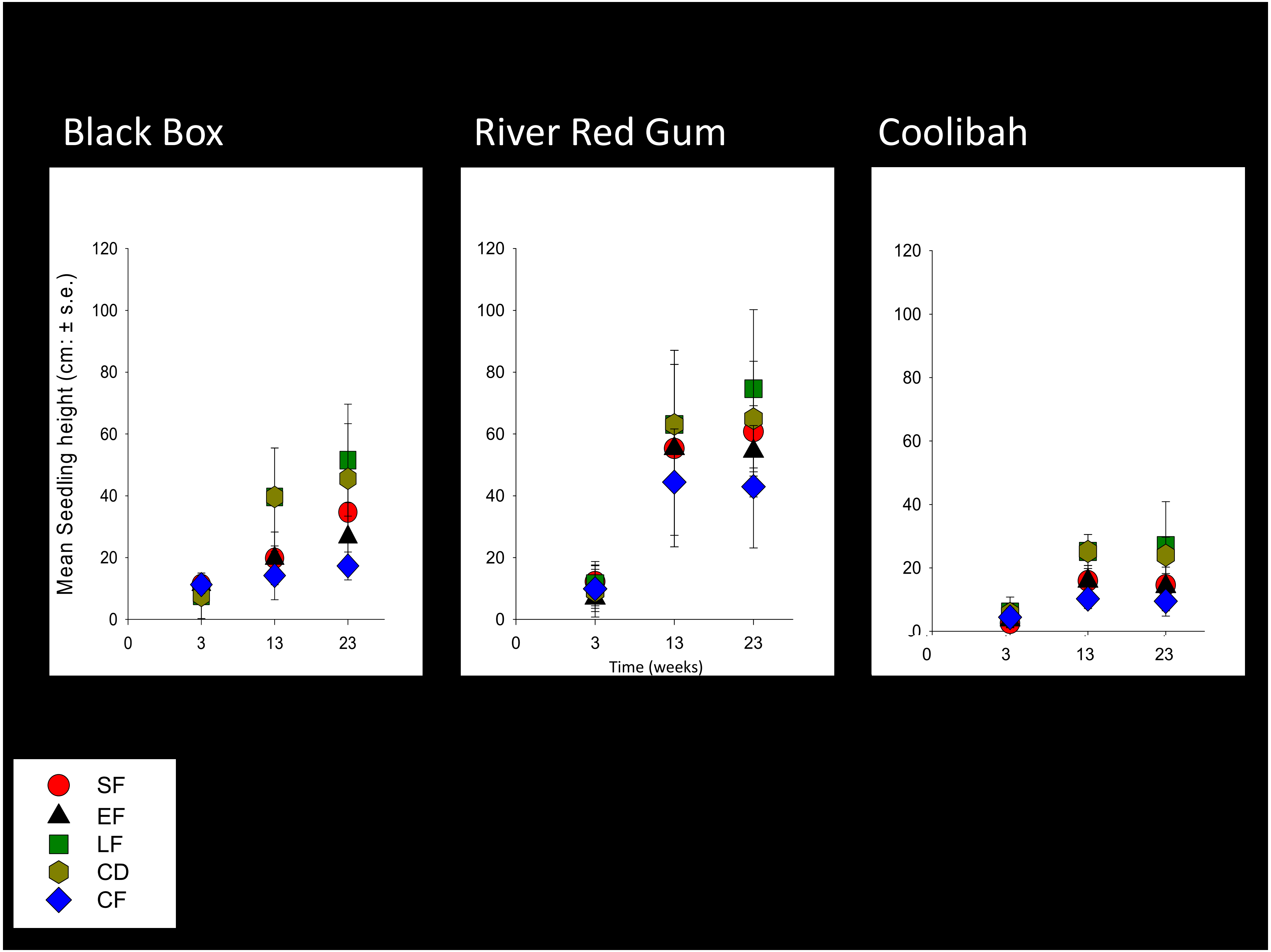




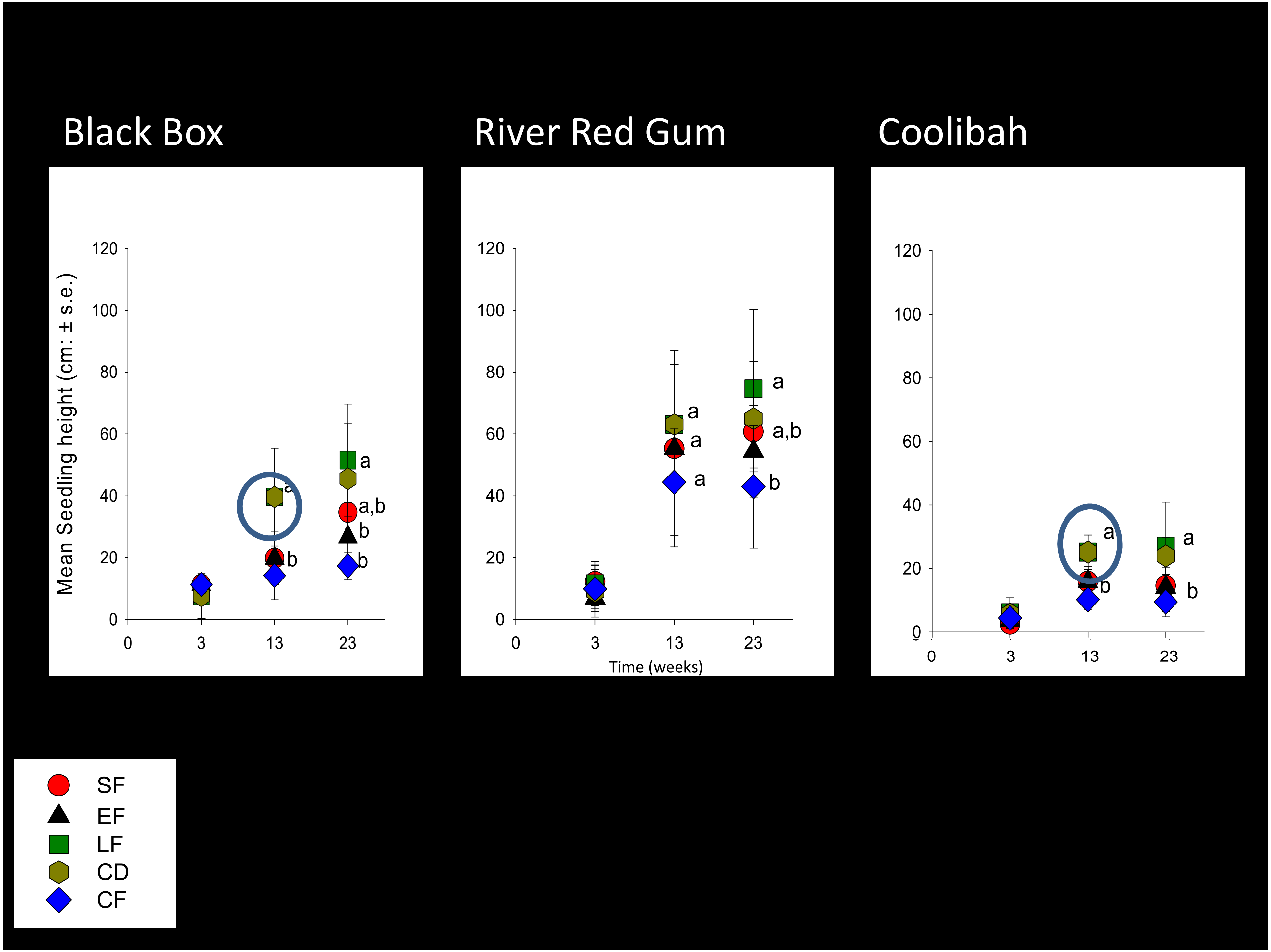
Mortality

- 13% Black box
- 22% Coolibah
- 16% River Red Gum

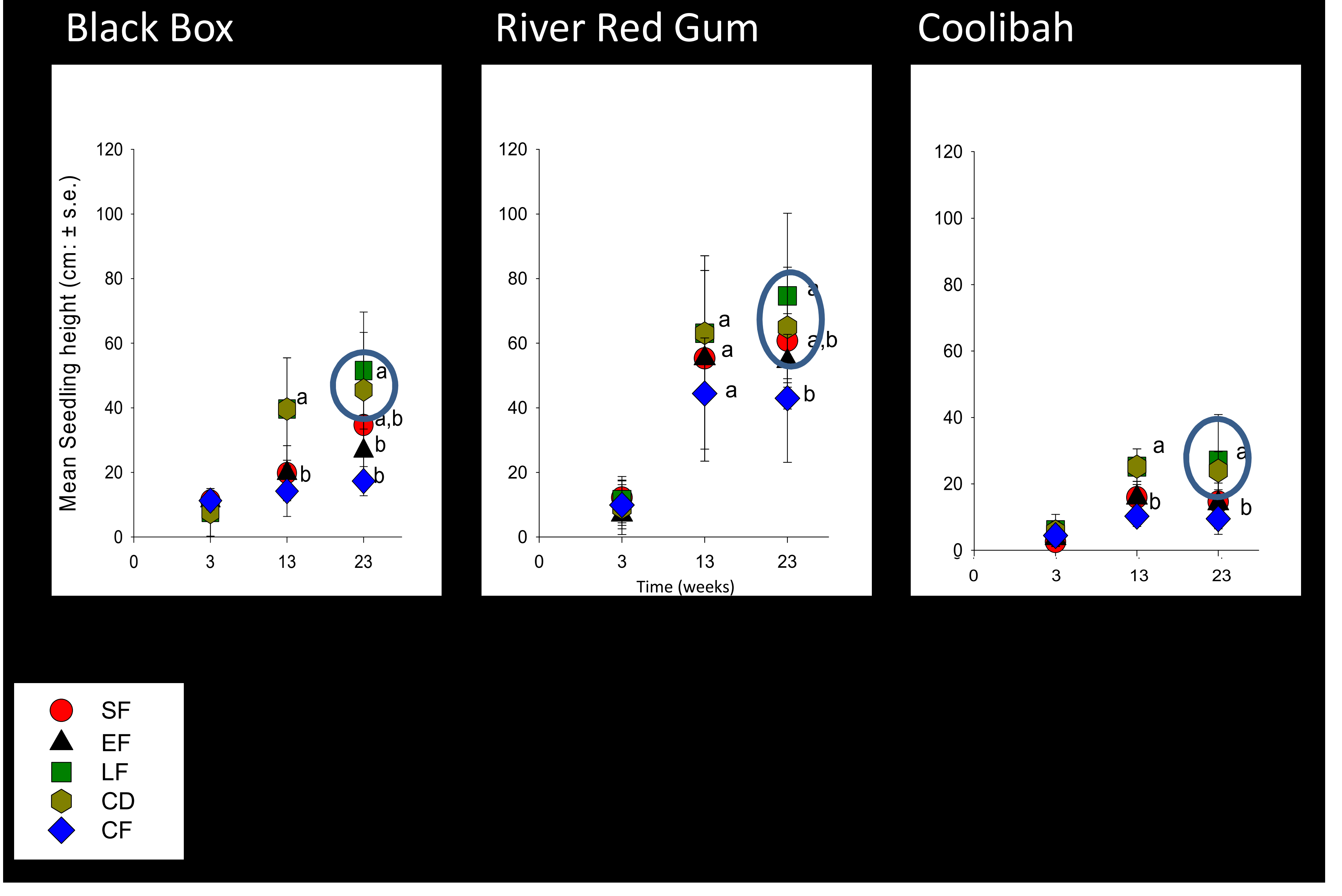
Above ground growth

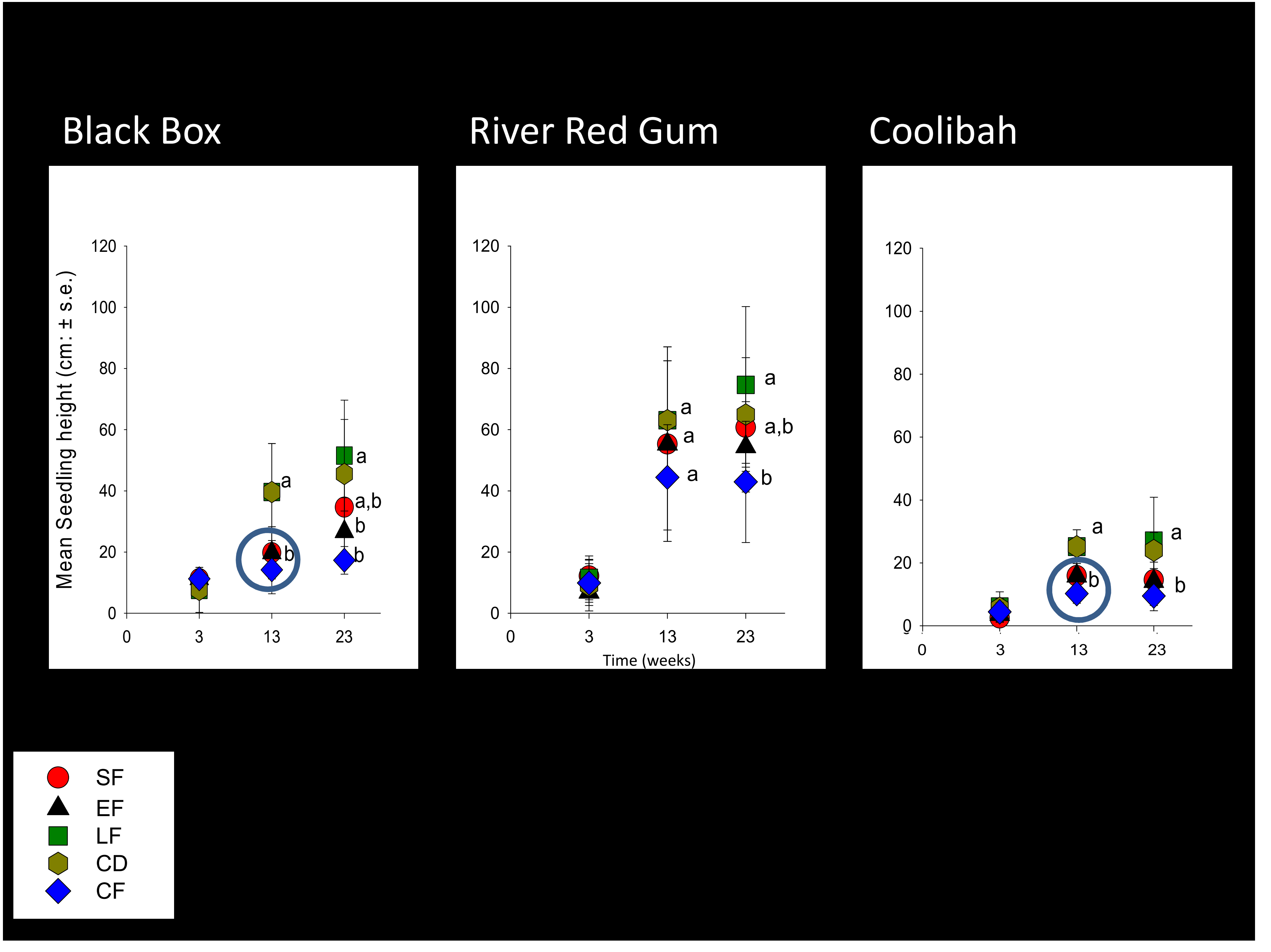


Black Box
River Red Gum
Coolibah
Mean Seedling height (cm: ± s.e.)
120
100
80
60
40
20
0
0
3
13
23
Time (weeks)
SF
EF
LF
CD
CF

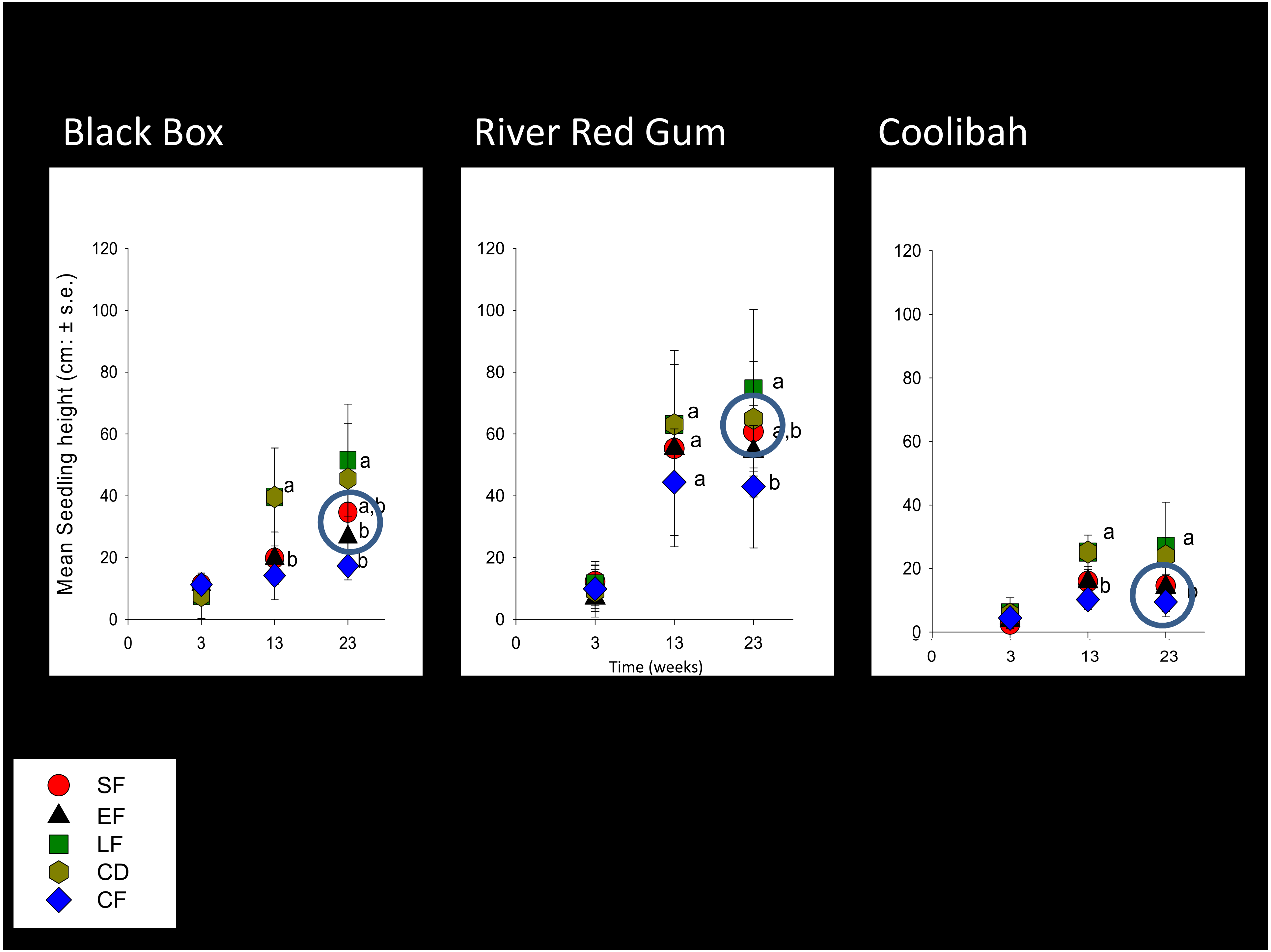


Black Box
River Red Gum
Coolibah
Mean Seedling height (cm: ± s.e.)
Time (weeks)
0
20
40
60
80
100
120
0
3
13
23
a
b
a,b
SF
EF
LF
CD
CF

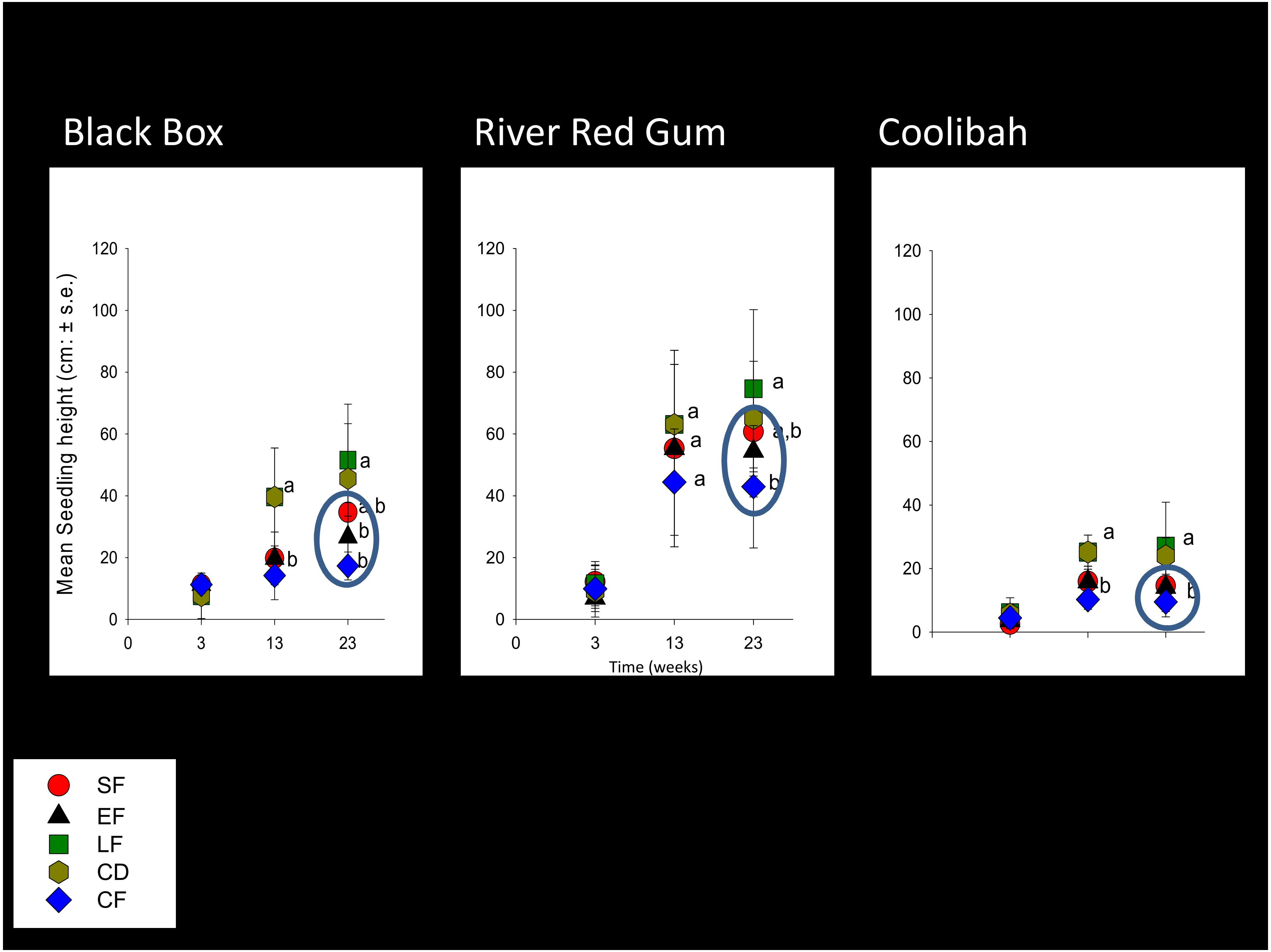

Black Box
River Red Gum
Coolibah
Mean Seedling height (cm: ± s.e.)
Time (weeks)
120
100
80
60
40
20
0
0
3
13
23
a
a,b
b
SF
EF
LF
CD
CF



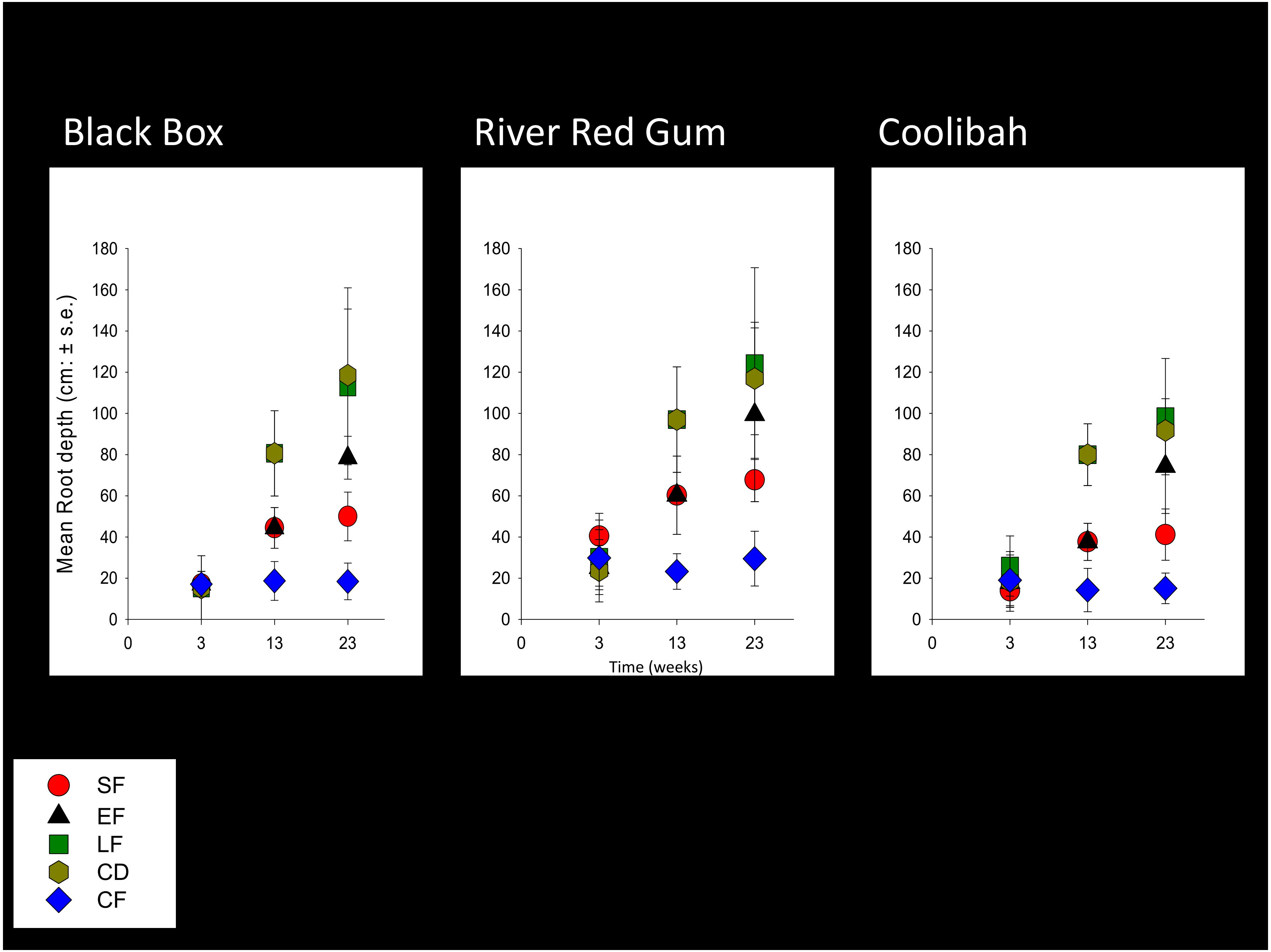
Black Box
River Red Gum
Coolibah
Mean Seedling height (cm: ± s.e.)
Time (weeks)
0
20
40
60
80
100
120
0
3
13
23
a
a,b
b
SF
EF
LF
CD
CF

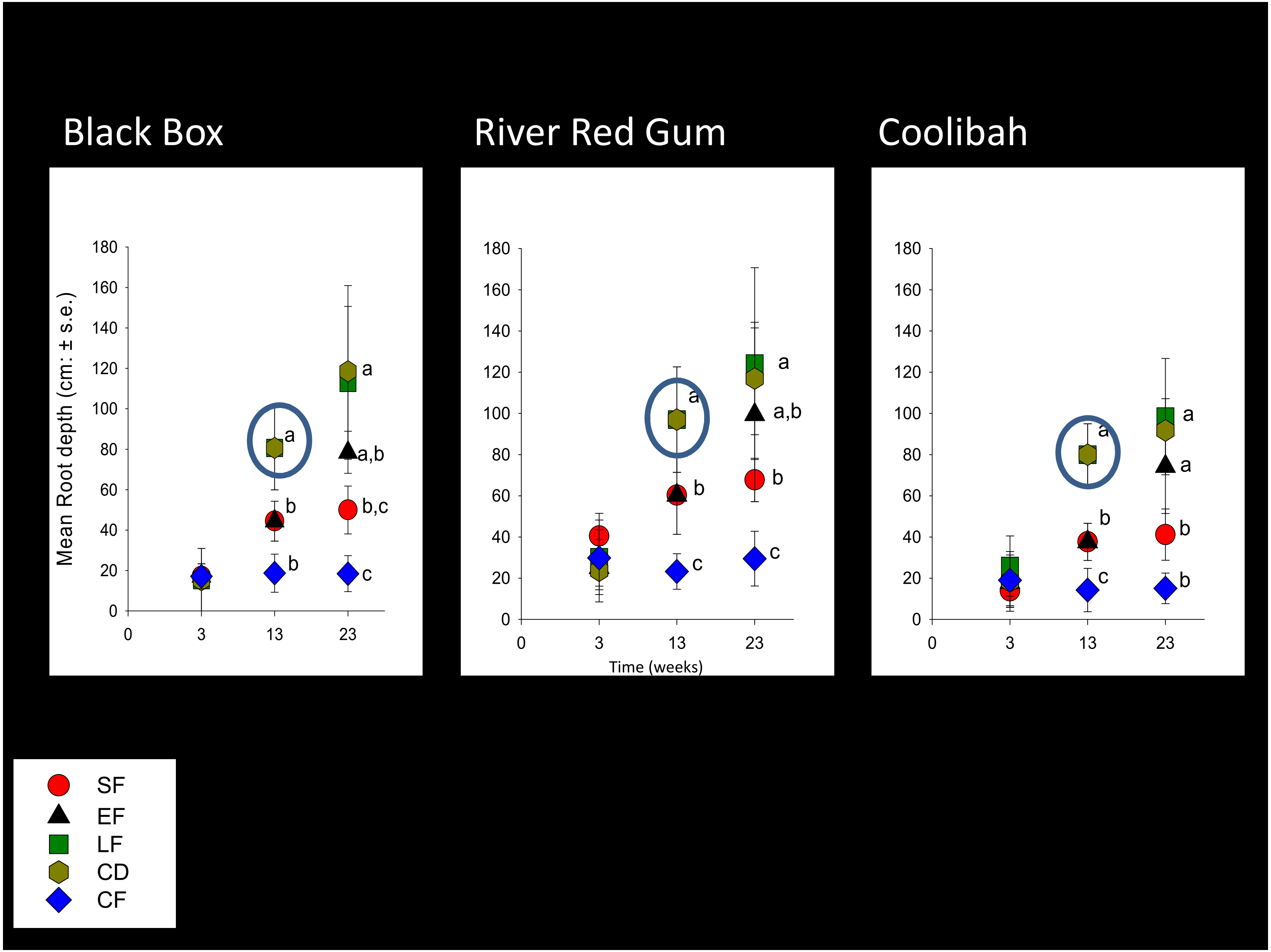


Black Box
River Red Gum
Coolibah
Mean Seedling height (cm: ± s.e.)
Time (weeks)
0
20
40
60
80
100
120
0
3
13
23
a
a,b
b
SF
EF
LF
CD
CF

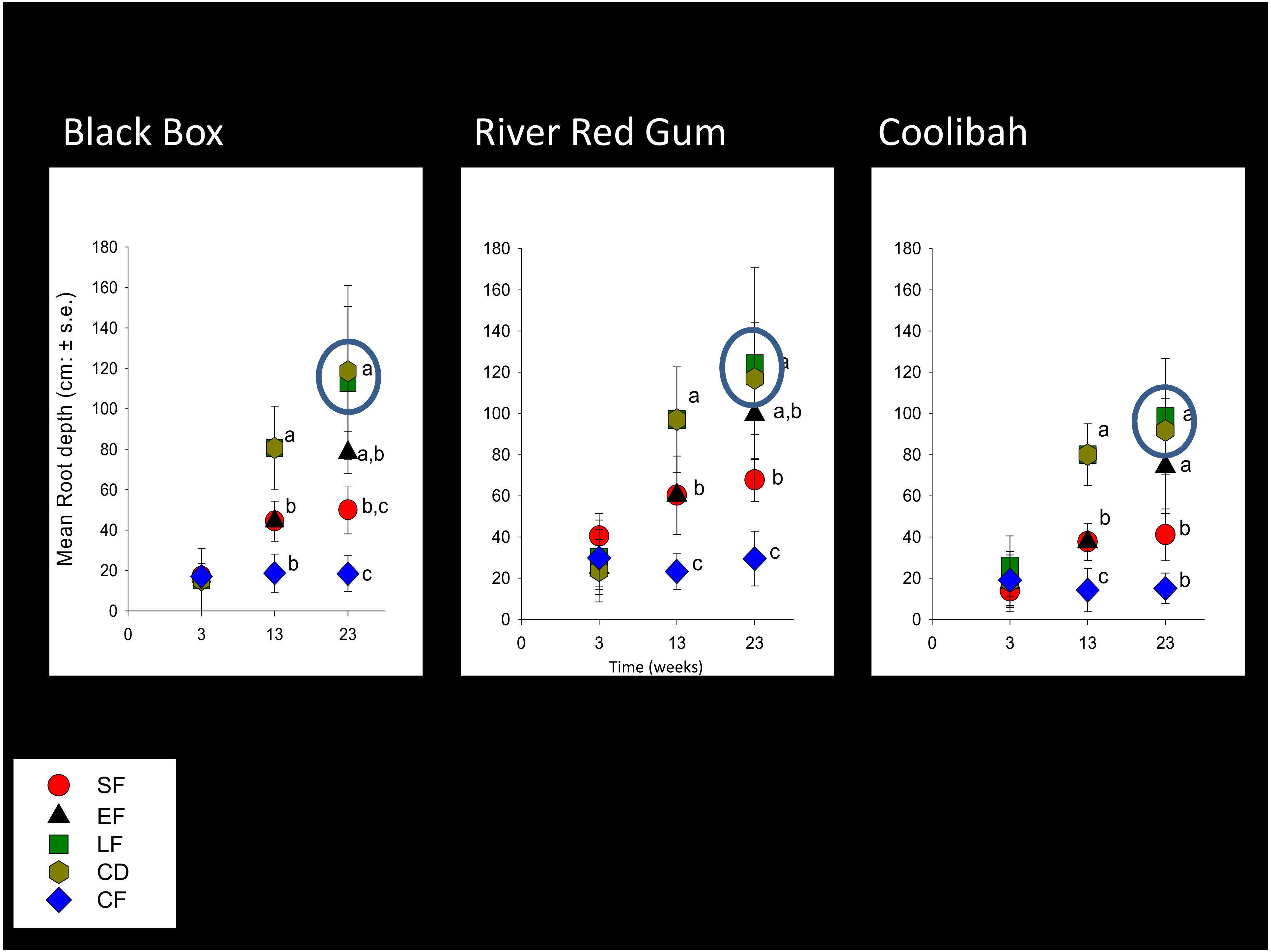

Black Box
River Red Gum
Coolibah
Mean Seedling height (cm: ± s.e.)
Time (weeks)
120
100
80
60
40
20
0
3
13
23
a
a,b
b
SF
EF
LF
CD
CF

Below ground growth

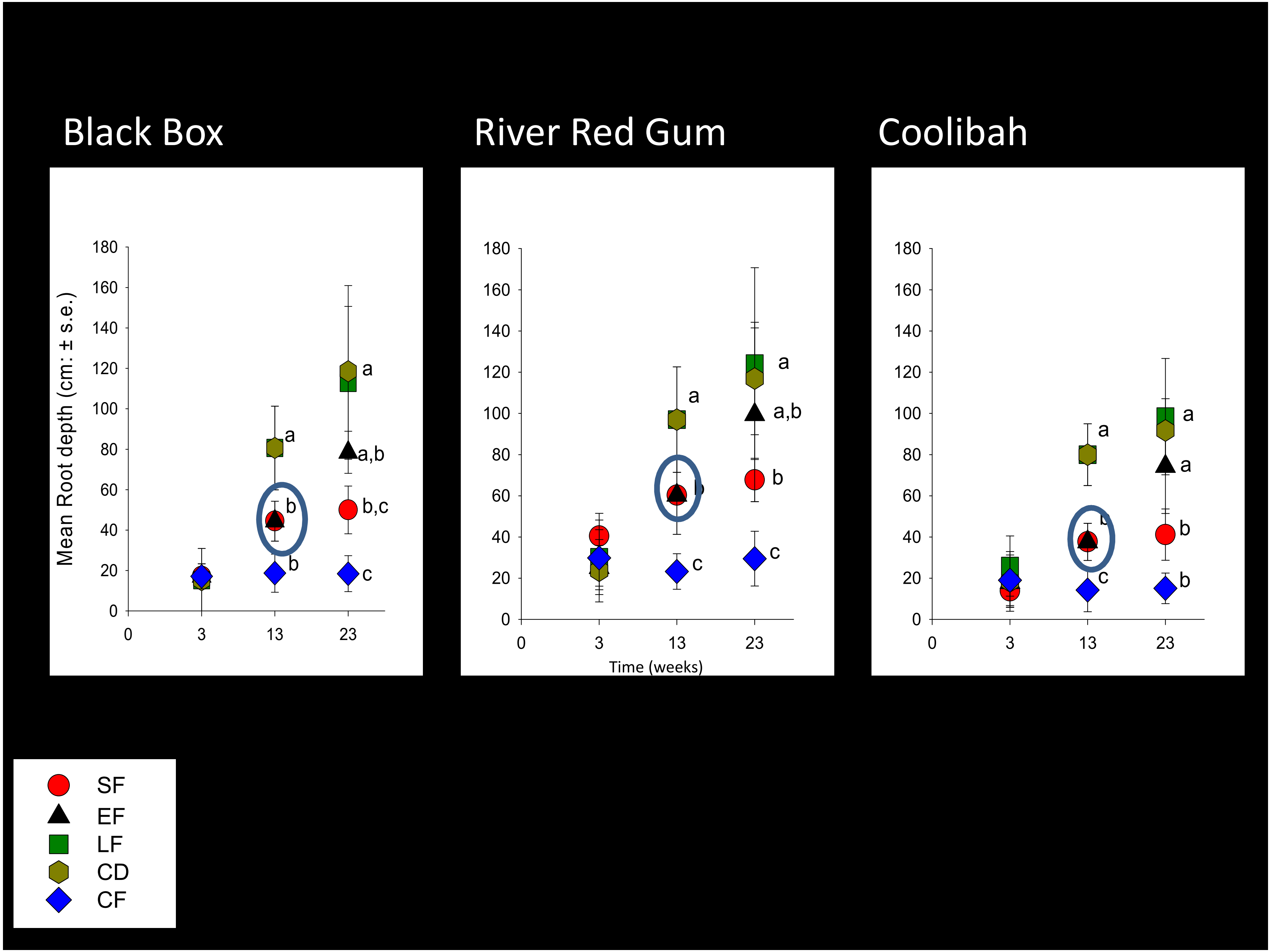

Black Box
River Red Gum
Coolibah
Mean Root depth (cm: ± s.e.)
0
20
40
60
80
100
120
140
160
180
0
3
13
23
Time (weeks)
SF
EF
LF
CD
CF



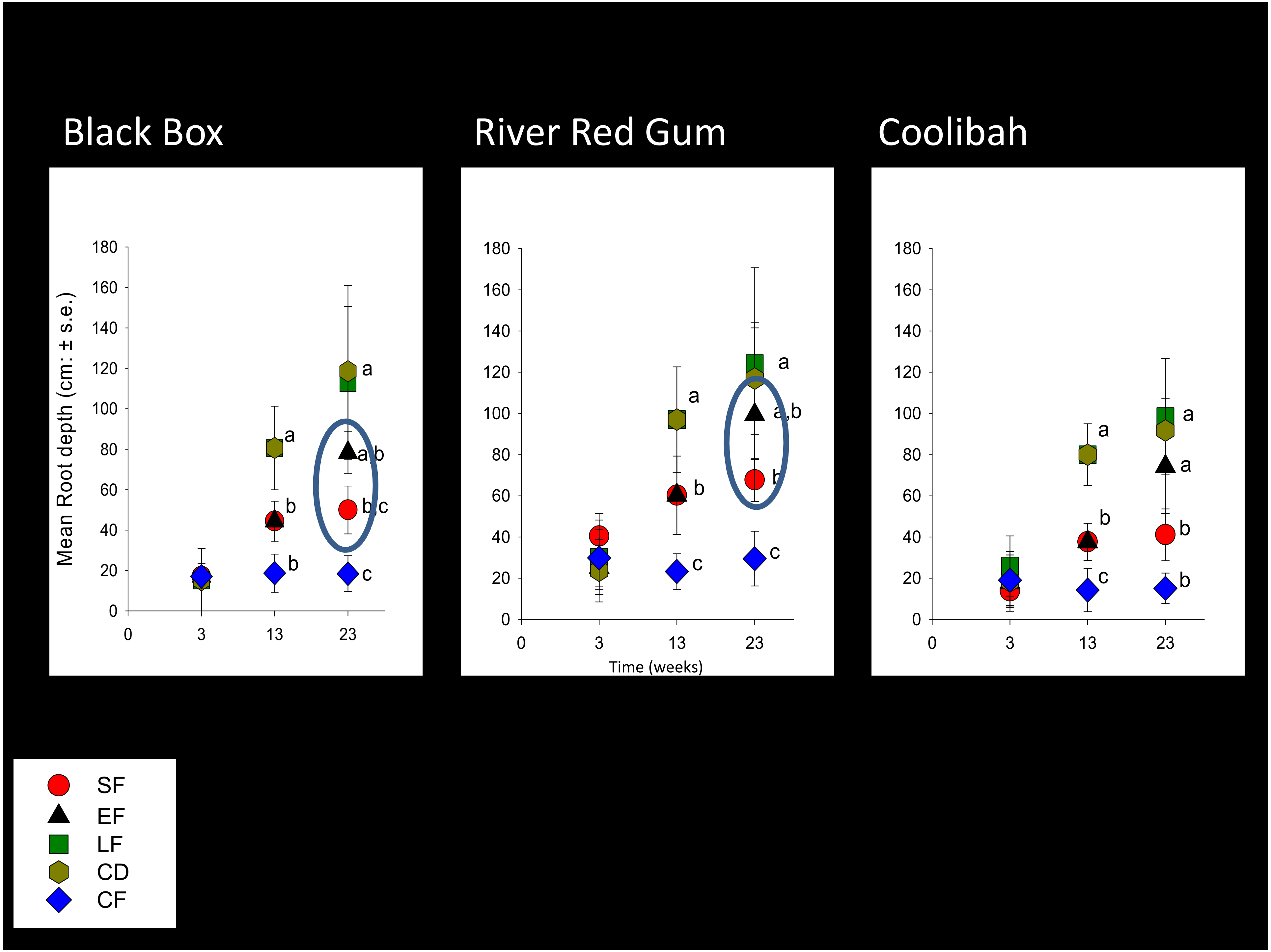
Black Box
River Red Gum
Coolibah
Mean Root depth (cm: ± s.e.)
Time (weeks)
SF
EF
LF
CD
CF



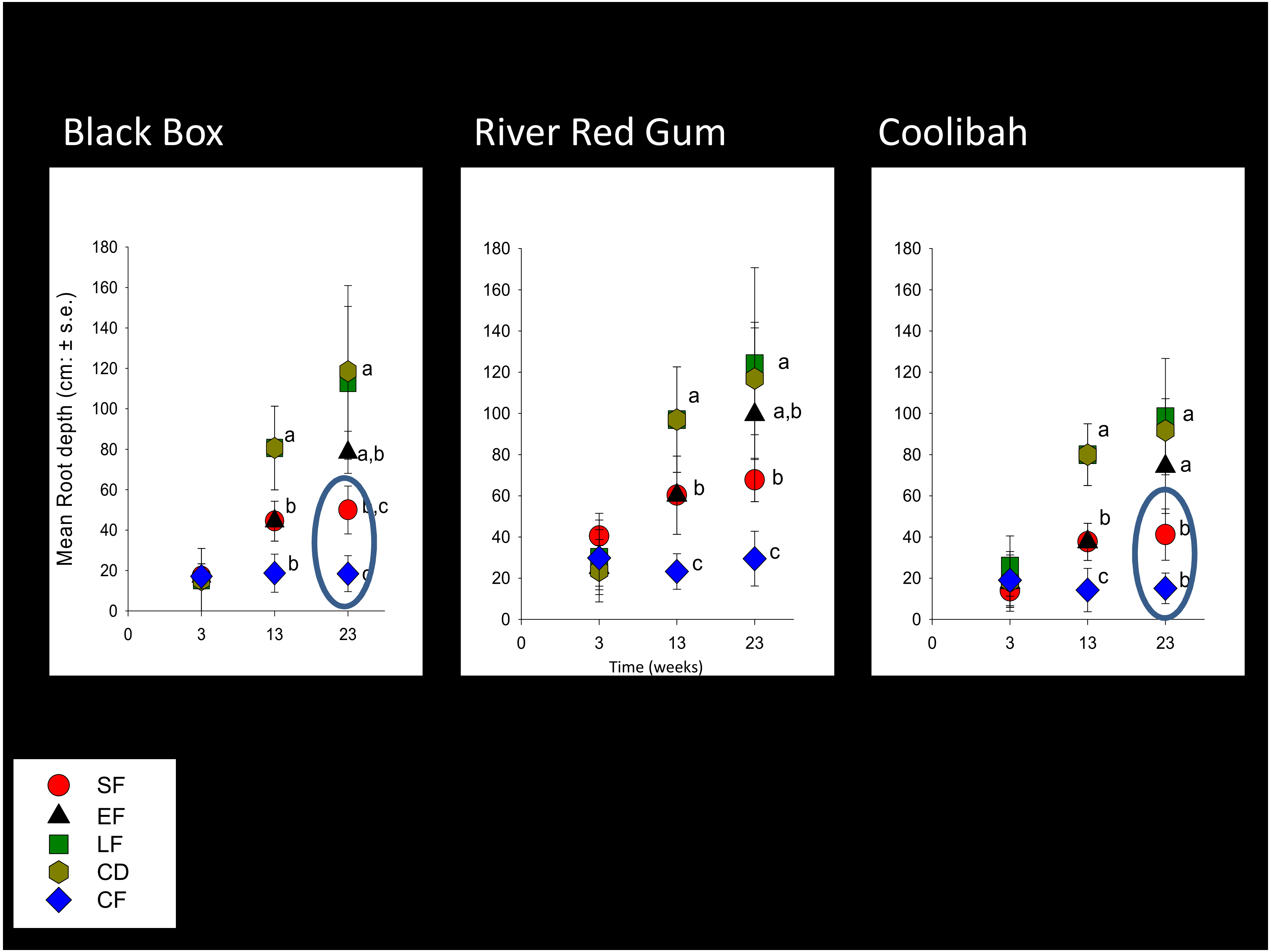
Black Box
River Red Gum
Coolibah
Mean Root depth (cm: ± s.e.)
Time (weeks)
SF
EF
LF
CD
CF



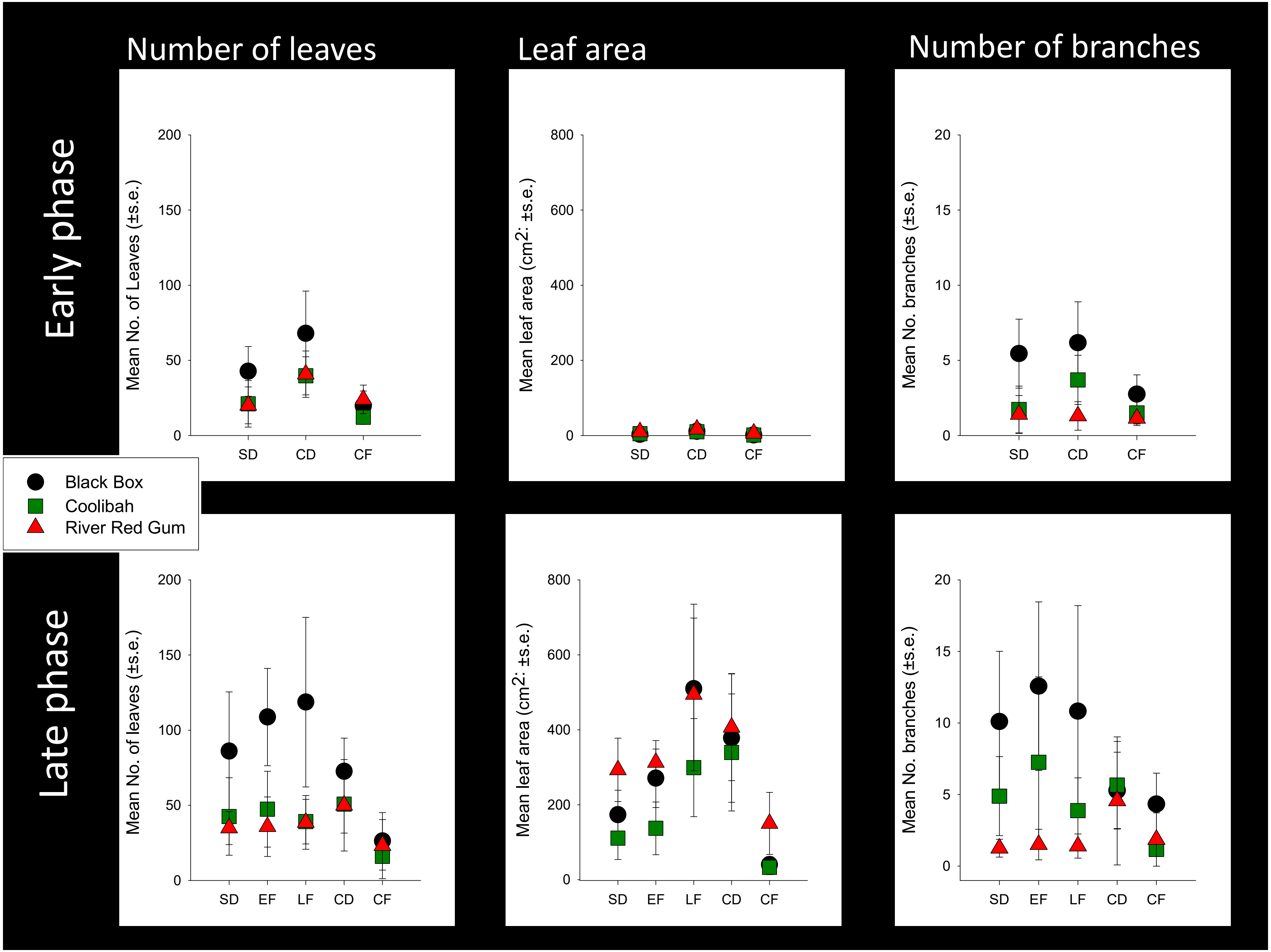
Black Box
River Red Gum
Coolibah
Mean Root depth (cm: ± s.e.)
Time (weeks)
180
160
140
120
100
80
60
40
20
0
0
3
13
23
a
a,b
b,c
b
c
a,b
SF
EF
LF
CD
CF



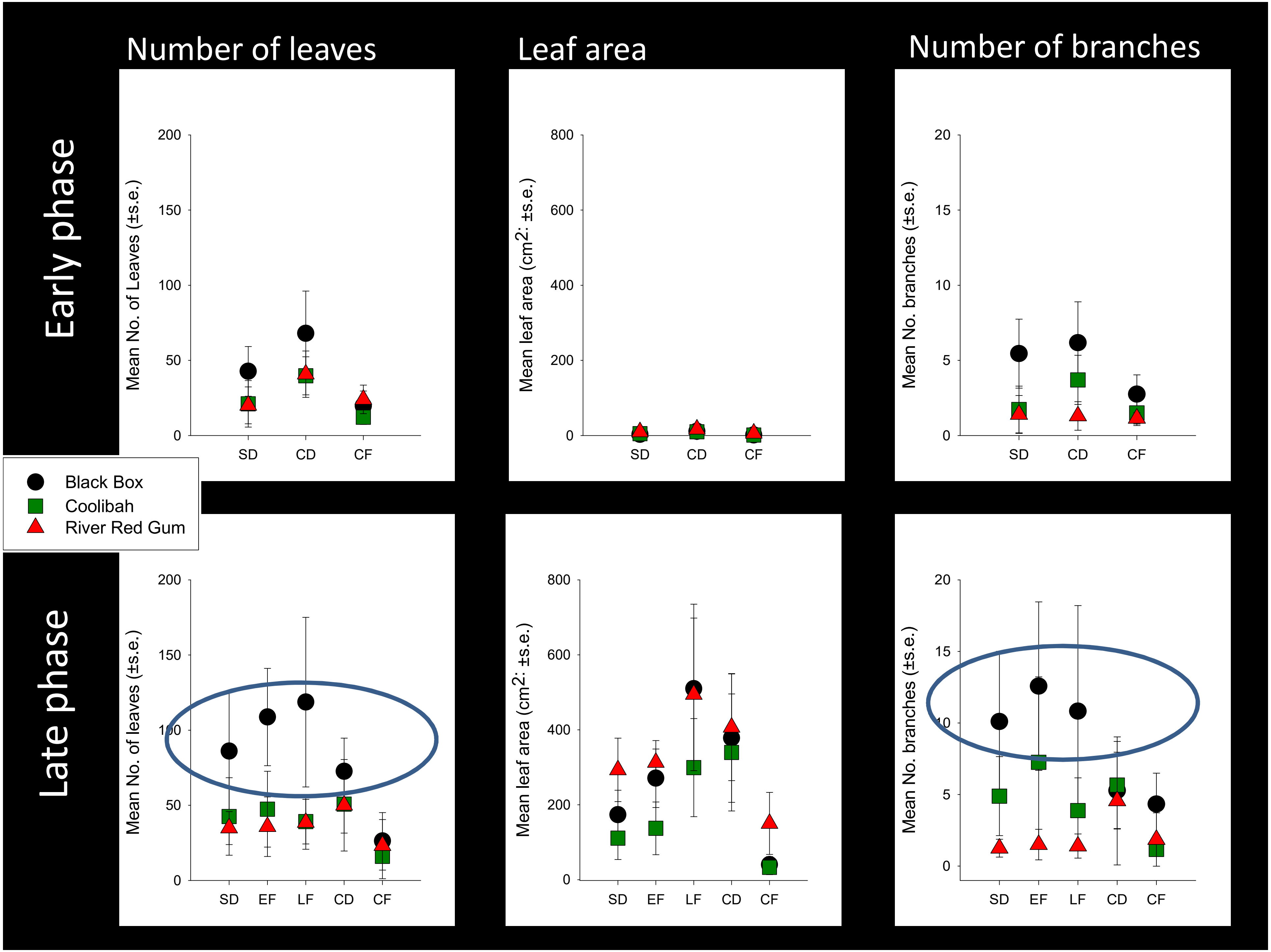
Black Box
River Red Gum
Coolibah
Mean Root depth (cm: ± s.e.)
Time (weeks)
0
20
40
60
80
100
120
140
160
180
3
13
23
a
b
c
a,b
b,c
SF
EF
LF
CD
CF

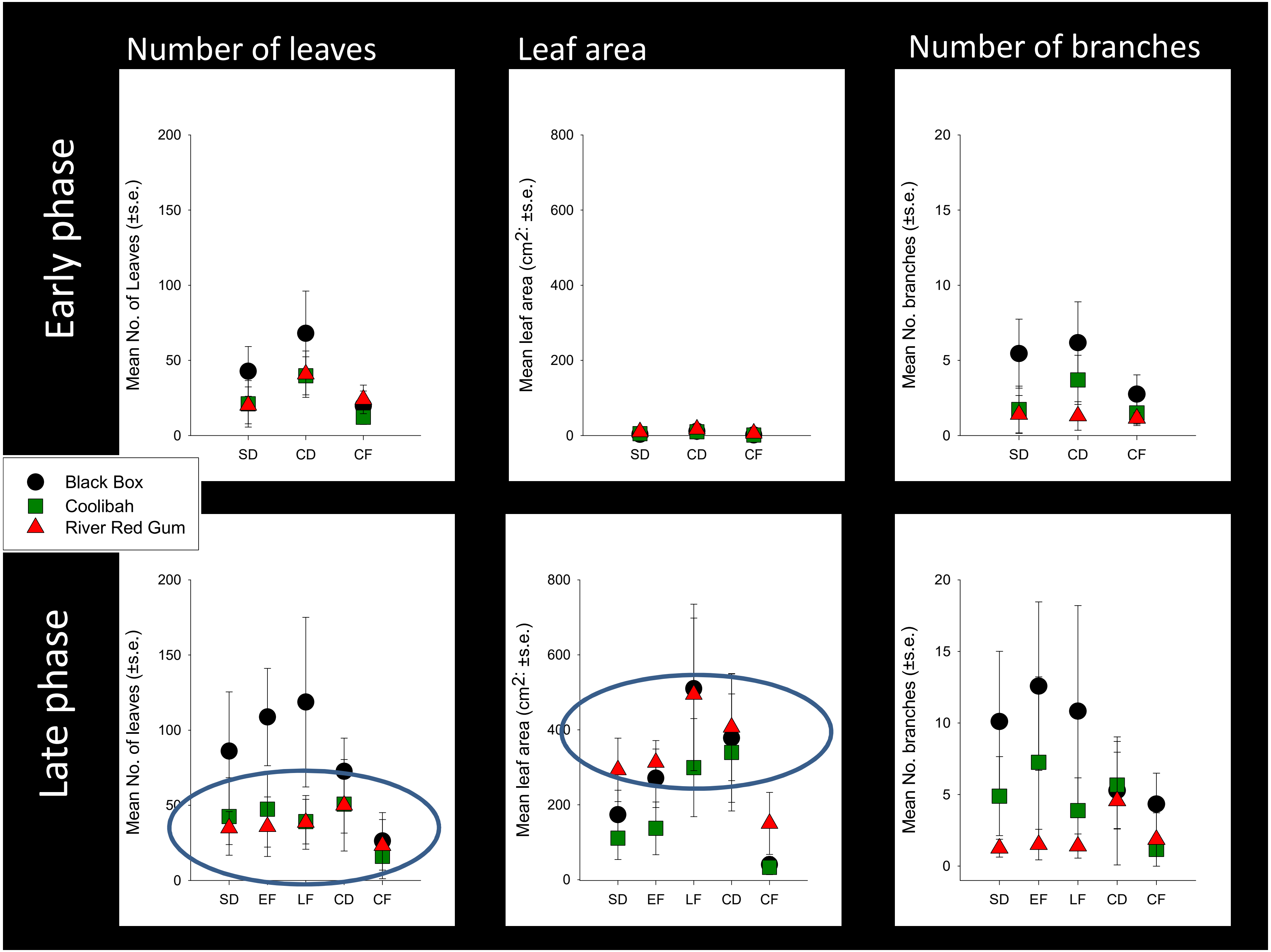

Black Box
River Red Gum
Coolibah
Mean Root depth (cm: ± s.e.)
Time (weeks)
0
3
13
23
0
20
40
60
80
100
120
140
160
180
a
a,b
b
b,c
c
a,b
SF
EF
LF
CD
CF

Leaves and branches



Number of leaves
Leaf area
Number of branches
Early phase
Late phase
Mean No. of Leaves (±s.e.)
Mean leaf area (cm2: ±s.e.)
Mean No. branches (±s.e.)
Mean No. of leaves (±s.e.)
SD
EF
LF
CD
CF
Black Box
Coolibah
River Red Gum


Number of leaves
Leaf area
Number of branches
Early phase
Late phase
Mean No. of Leaves (±s.e.)
Mean leaf area (cm2: ±s.e.)
Mean No. branches (±s.e.)
Mean No. of leaves (±s.e.)
SD
EF
LF
CD
CF
Black Box
Coolibah
River Red Gum


Number of leaves
Leaf area
Number of branches
Early phase
Late phase
Mean No. of Leaves (±s.e.)
Mean leaf area (cm2: ±s.e.)
Mean No. branches (±s.e.)
Mean No. of leaves (±s.e.)
200
150
100
50
0
800
600
400
200
0
20
15
10
5
0
SD
CD
CF
SD
EF
LF
CD
CF
Black Box
Coolibah
River Red Gum

Species specific interactions

Black Box

- Growth response most variable

River Red Gum

- Greater range of tolerance to hydrological regimes

Coolibah

- Climatic response?




Watering treatments

- Constant flood suppresses growth
- Seedlings performed well under a constant dry
- Seedlings benefited from an early dry period
- Initially flooding was not as beneficial as an early dry period
- Trends suggest increased rates of growth with a late flood




Conclusion

Sequential flooding

No difference

Sequential flooding

No difference

Same as constant & early flood

Importance of initial condition

Early Dry → Late flood

No difference

Early Dry → Late dry

Early Flood → Sequential flood

No difference

Early Flood → Late Dry

EWKR
Environmental Water Knowledge & Research

Thank you

For more information

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Website: http://ewkr.com.au/

Facebook: https://www.facebook.com/LaTrobeCFE/

Project Collaborators

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Commonwealth Environmental Water Office

LA TROBE UNIVERSITY

CSIRO

NSW GOVERNMENT Office of Environment & Heritage

UNSW SYDNEY

Arthur Rylah Institute for environmental research

JAMES COOK UNIVERSITY AUSTRALIA

TropWATER

Griffith UNIVERSITY

Government of South Australia

SARDI SOUTH AUSTRALIAN RESEARCH AND DEVELOPMENT INSTITUTE

Giving woody seedlings a fighting start

CHERIE CAMPBELL BEGINS WITH ROOTS AND ALL ADVICE ON GETTING THE BEST OUT OF THESE YOUNGSTERS.

Just like all of us, long-lived woody plant species need a healthy start to life to give them the best chance of growing big and strong. Providing woody seedlings with optimal conditions for growth and survival helps to ensure their success later in life.

The Vegetation Theme of the Murray–Darling Basin Environmental Water Knowledge and Research (MDB EWKR) project are seeking to determine which watering regimes give woody seedlings a fighting start. This research will focus on four long-lived woody plant species common to the Murray–Darling Basin: River Red Gum (*Eucalyptus camaldulensis*), Coolabah (*Eucalyptus coolabah*), Black Box (*Eucalyptus largiflorens*) and Tangled Lignum (*Duma florulenta*). All of these plant species are structurally dominant on various parts of the floodplain throughout the Basin. They play an important role in providing refuge, habitat and food sources for a wide range of species, and contribute to ecosystem services such as carbon and nutrient cycling. The importance of these species is recognised by their inclusion in the Murray–Darling Basin Authority's Basin-Wide Environmental Watering Strategy.

Seedlings represent the next generation for woody trees and shrubs, so their periodic germination and survival into adulthood is essential to ensuring the long-term survival of these populations and ecosystems. Seedling germination and survival has been observed to be highly variable across the Basin, with seedlings being scarce in some areas and abundant in others. Land and water managers are keen to foster the growth and survival of woody seedlings which will grow up to form the next generation of forests and woodlands.

A cross section of the PVC pipes showing seedling growth. Inset: Measuring and recording root and growth development. Both photos Ben Gawne.

Getting the best start

In order to give woody seedlings the best start in life we are investigating the watering regimes that provide the best conditions for growth of roots, stems and leaves. Roots provide woody seedlings with access to water and nutrients, as well as anchoring the plant to the soil. Leaves are the energy powerhouse, providing the plant with access to food. Opportunities that result in greater root growth—longer and bigger roots—are likely to provide the plant with greater access to water and nutrients, increasing their capacity to survive dry periods. Similarly, healthy seedlings are likely to be taller and bigger with lots of leaf area, giving them the opportunity to produce more energy.

In order to determine optimal watering regimes for seedlings, we set up experiments at Wonga Wetlands in Albury, New South Wales. Seedlings from River Red Gum, Black Box, Coolabah and Tangled Lignum were germinated in pots, then transferred to PVC tubes and placed in tanks with different watering treatments applied. The treatments included five contrasting flow regimes:

1. constant dry
2. constant flood
3. flood then dry
4. dry then flood
5. alternating flood and dry periods.

These flow regimes focus on the effect of permanent inundation or drying, inundation during both early and later seedling life stages, and multiple wetting and drying periods during seedling establishment.

Measuring and analysis

In total, approximately 350 seedlings were assessed as part of this experiment. Measurements of mortality, seedling height, number, and area of leaves and root length were collected from harvests undertaken at the start of the experiment, in the middle and at the end. We also calculated above and below ground biomass. Comparing the results between harvest times will enable growth rates and the effect of water regimes to be determined over time.

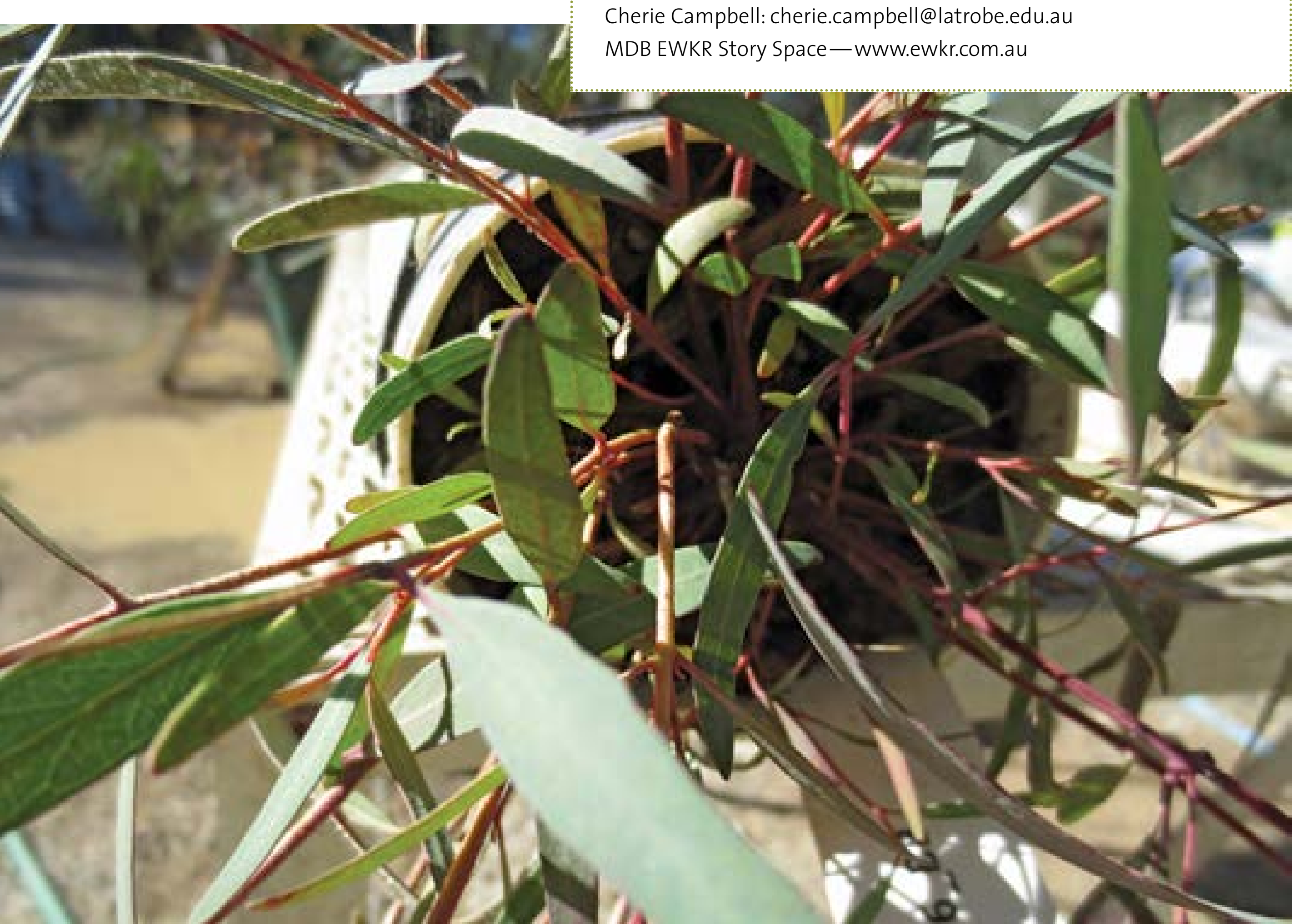
Analysis of data collected during this experiment will show the relationship between flow parameters such as duration, frequency and interflow dry period and woody seedling growth and establishment. We will then work with water managers to ensure the information on seedling water requirements will help them to make decisions that provide woody seedlings with the best possible start to life.

The MDB EWKR project is funded by the Australian Government's Commonwealth Environmental Water Office.

FOR FURTHER INFORMATION

Cherie Campbell: cherie.campbell@latrobe.edu.au
MDB EWKR Story Space—www.ewkr.com.au

Below: Extensive coppicing of a Black Box seedling. Photo Cherie Campbell.

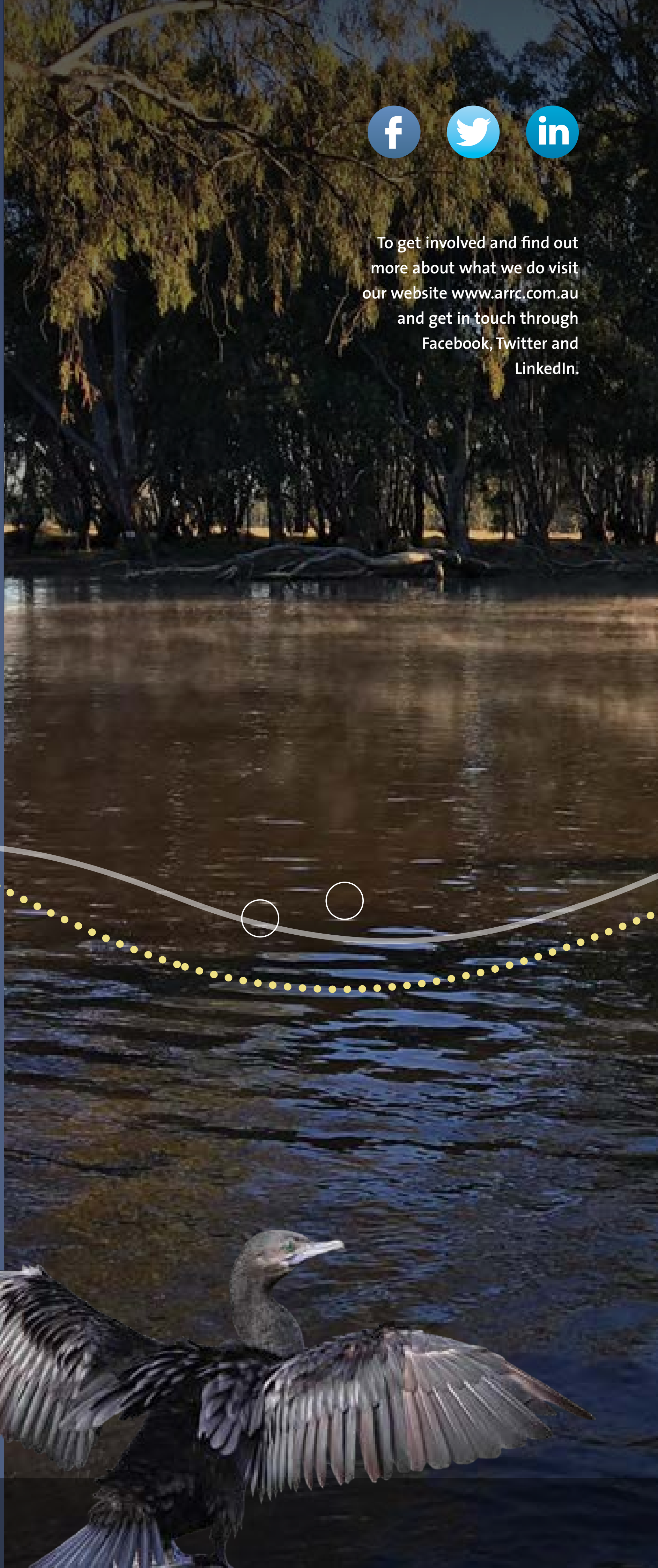


Australian River Restoration Centre

At the Australian River Restoration Centre we believe in sharing knowledge, restoring and protecting our rivers for all to enjoy and valuing people and the work they do. We do this by:

- Inspiring and supporting people passionate about rivers
- Creating and distributing *RipRap* magazine
- Sharing knowledge in multiple ways
- Collaborating and networking with a range of organisations
- Managing on-ground and science communication projects

www.arrc.com.au



To get involved and find out more about what we do visit our website www.arrc.com.au and get in touch through Facebook, Twitter and LinkedIn.

To find out when the next edition of *RipRap* is coming out, stay in touch through the ARRC blog, it is free to subscribe and you are also welcome to provide contributions to share with the wider ARRC community.

RipRap is ONLY available for purchase through the Australian River Restoration Centre.

RipRap

EDITION 40

Appendix V4.5: Seedling mesocosm paper

N.B. This is a full manuscript in preparation for submission to a scientific journal for publication. Inclusion as an output in this technical report doesn't preclude the ability to publish.

Contrasting establishment strategies amongst three dominant tree species of Australian desert floodplains

Campbell, C.J. [1✉], Capon, S.J.[2], James, C.S. [3], Durant, R.A.[4], Morris, K. [5], Thomas, R.F.[6,7], Nicol, J.M. [8], Nielsen, D. L. [4,9], Stoffels, R.[4,9,10] and Gehrig, S. L.[1].

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7. Centre for Ecosystem Science, School of Biological, Earth and Environmental Sciences, University of New South Wales, Sydney, NSW, Australia, 2052
8. South Australian Research and Development Institute (Aquatic Sciences), Henley Beach, South Australia, Australia, 5022
9. CSIRO Land and Water, Wodonga, Victoria, Australia, 3689
10. National Institute of Water & Atmospheric Research Ltd, Christchurch, NZ

1. Introduction

Response to disturbance is widely recognised as a major dimension underpinning variation in plant traits and strategies, especially those related to life history and reproduction (Wilson et al. 1999). In arid and semi-arid floodplains, plant habitats are characterised by erratic disturbance regimes driven by high levels of hydrologic variability (Brock et al. 2006). To inhabit these environments, plant species must therefore employ strategies that enable them to persist through both floods and drought, as well as the unpredictability with which these disturbances occur (Capon et al. 2016). Knowledge of these strategies is essential for predicting and managing plant responses to changing hydrological conditions such as those anticipated under climate change (Capon et al. 2013).

Many herbaceous plant species of desert floodplains exhibit ruderal traits, *sensu* Grime (1988), especially the maintenance of large, long-lived soil seed banks. These seed banks enable plant species to persist through the unsuitable conditions associated with both floods and drought (van der Valk 2018). These species only establish in the extant vegetation during favourable conditions for germination and growth, typically in the relatively brief periods following the recession of floodwaters (Brock et al. 2006). In contrast, mature woody plants in these environments demonstrate varying degrees of stress tolerance to both floods and drought, facilitated by a wide range of physiological and morphological traits (Capon et al. 2016). Seedlings of these woody species, however, are considerably more vulnerable to the stresses associated with hydrologic disturbances than their mature counterparts due to their smaller stature (Cooper et al. 1999; Gindaba et al. 2004). Indeed, seedling establishment, rather than seed supply or germination, is widely perceived to be the critical bottleneck shaping spatial and temporal population dynamics of floodplain tree species in arid and semi-arid regions (Streng et al. 1989; Hughes 1990; Cooper et al. 1999; Horton and Clark 2001; George et al. 2005; Maxwell et al. 2016). Variation in seedling establishment strategies in the face of hydrological variability, and the traits which underpin these, is therefore likely to be a significant determinant of the distribution and abundance of woody plant species in desert floodplains.

Germination of woody seedlings in arid and semi-arid floodplains is commonly associated with the favourable, moist conditions that occur following the recession of floodwaters (Capon et al. 2016). Seedlings often establish, for instance, along flood strand lines (Dexter 1970). Following early phases of establishment, however, seedlings face uncertain hydrological conditions and can experience either drought or inundation of varying depth and duration, sometimes in quick succession. Drought presents significant challenges for seedling survival and growth including reduced soil moisture and a lowering of the water table. Young seedlings may also be subject to greater grazing pressure during periods of drought if they offer a preferable food source over more xeric plants present during these times, many of which possess considerable deterrents to herbivores, e.g. spines. Flooding, on the other hand, may promote seedling growth through the provision of moisture and nutrients or, depending on the depth and duration of inundation, constitute multiple stressors to establishing seedlings such as reduced light and oxygen availability or mechanical damage (Blom et al. 1990; Maxwell et al. 2015).

A wide range of morphological and physiological traits facilitate flood and drought tolerance amongst tree and shrub seedlings (Capon et al. 2009; 2016). In particular, a capacity for rapid plastic responses to environmental fluctuations might be expected to be advantageous to seedlings establishing in hydrologically variable environments (Capon et al. 2009; Maxwell et al. 2016; Wang et al. 2017). Elongation of stems or roots, for example, is often associated with flood or drought tolerance respectively (Capon et al. 2016). In very unpredictable arid floodplains, however, seedlings may be more likely to display delayed development in response to flooding rather than adaptive morphological plasticity because of the high risk of being caught out of kilter with environmental conditions (Alpert & Simms 2002; Capon et al. 2009). Trade-offs may also exist amongst floodplain plants between flood tolerance and drought tolerance (Luo et al. 2008). On the other hand, some mechanisms of flood tolerance, such as the development of adventitious roots or a capacity for rapid stomatal closure, may also confer a degree of drought tolerance (Parolin 2001).

Seedling responses to floods and droughts are dependent on their size, age and/or condition (Capon et al. 2016). A taller seedling, for example, may be more likely to tolerate flooding if it extends above the water level than a shorter seedling which is completely submerged by the same inundation event. Furthermore, hydrologic conditions in a seedling's early life, and its responses to these, can in turn influence responses to later conditions (Wang et al. 2017). Extreme quiescence by plants during periods of flooding, for instance, might favour tolerance of rapid dehydration and water deficit during subsequent drying (Fukao et al. 2011). Similarly, investment in plasticity at later stages of development may be shaped by prior conditions, with early mesic conditions potentially constraining the capacity of some plants to cope with later extremes through plastic adjustments (Wang et al. 2017).

Establishment strategies of desert floodplain trees can therefore be conceived of as comprising: 1.) fixed traits, amongst which there may be either synergies or trade-offs between those which facilitate drought and flood tolerance; 2.) plastic responses to flooding and/or drought; and 3.) flexibility in plasticity (i.e. meta-plasticity) over time. Variation between species in these key dimensions of seedling responses to environmental heterogeneity can be expected to reflect current species' distributions as well as being important predictors of future range shifts (Wang et al. 2017). Seedlings of species which typically occur in wetter parts of floodplain gradients might therefore display more fixed and plastic traits associated with flood tolerance while exhibiting fewer plastic responses to drought than those from floodplain margins for which the reverse might occur. Additionally, it can be hypothesised that species exhibiting broader distributions across a floodplain gradient might exhibit greater levels of heterogeneity in trait responses to hydrological variation, reflecting both plasticity and meta-plasticity.

Here, we investigate responses to flooding and drought amongst seedlings of three common tree species of Australian desert floodplains: *Eucalyptus camaldulensis* Dehnh. (Myrtaceae), *Eucalyptus largiflorens* F.Muell. (Myrtaceae) and *Eucalyptus coolabah* Blakely & Jacobs (Myrtaceae). Amongst these species, *E. camaldulensis* commonly occurs in riparian and more frequently flooded floodplain habitats while *E. largiflorens* and *E. coolabah* tend to occupy higher parts of the floodplain or, in the case of the latter, more infrequently flooded riparian habitats where *E. camaldulensis* is either absent or very sparse. By conducting a large controlled experiment, we sought to identify the fixed traits and plastic responses favouring seedling establishment amongst these species in relation to flooding and drying and to investigate the effects of these disturbances during early life history stages on responses to subsequent conditions. Overall, we sought to relate the establishment strategies of these keystone species to current species' distribution patterns and to make predictions regarding their likely responses to projected climatic changes in order to inform current management practices, especially the delivery of environmental flows.

2. Methods

Study species

The three species we selected for study (*Eucalyptus camaldulensis* Dehnh. (Myrtaceae), *Eucalyptus largiflorens* F.Muell. (Myrtaceae) and *Eucalyptus coolabah* Blakely & Jacobs (Myrtaceae)) are all widespread and dominant floodplain trees of the Murray-Darling Basin which occupies a significant portion of south-eastern Australia. *E. camaldulensis* (river red gum) is a medium to tall (12-45 m) tree and the most widely distributed eucalypt in Australia, occurring in the riparian zones of most river catchments as well as on floodplains of many lowland rivers (Romanowski 2013; Colloff 2014). *E. largiflorens* (black box) is a small to medium tree (10–20 m tall) with a large spreading crown and drooping branches that forms open woodlands on floodplains and on the fringes of ephemeral lakes and water courses (Cunningham *et al.* 1992). Typically occurring at higher floodplain elevations than *E. camaldulensis*, *E. largiflorens* is widespread throughout western parts of the Murray-Darling Basin, particularly in the south, and displays a low tolerance to waterlogging (Roberts & Marston 2011). Finally, *E. coolabah* are among the most common trees in arid riverine environments in the north-west of the Murray-Darling Basin (Roberts & Marston 2011; Rogers & Ralph 2011) where they dominate infrequently inundated floodplains and riparian zones of ephemeral channels as well as often growing with *E. camaldulensis* in mixed floodplain woodlands (Roberts & Marston 2011; Rogers & Ralph 2011). *E. coolabah* is a medium sized tree (15–20 metres tall) varying in shape from erect to spreading (Roberts & Marston 2011).

Experimental methods

Seedlings of each species were obtained from a commercial nursery (Sandy Creek Trees, Allans Flat, Victoria). Once seedlings produced the first true leaves (after the initial cotyledons), they were transplanted to individual pots. These pots comprised PVC plumbing pipe, 90 mm in diameter and 70 cm in length, halved length-wise then sealed back together with duct tape and cable ties with the bottom of the pot enclosed by a PVC lid with drainage holes. Pots were filled with floodplain soil collected from the Murray River floodplain near Albury, New South Wales. Soil analysis, prior to commencing the experiment, indicated that the floodplain soil was a mixture of sand, silt and clay (41:41:18) with a water holding capacity of 21.21%, and a total carbon content of 3.09 % (following Nelson & Sommers 1982; Grimshaw 1989; Ilstedt *et al.* 2000).

The experiment was conducted outdoors at an experimental facility near Albury, NSW Australia (36°4'6.96"S, 146°51'15.48"E) from November 2016 (summer) to May 2017 (autumn). Seedlings in pots were randomly distributed across 15 fibreglass tanks (1 m diameter, 1 m deep). Each tank contained 15-21 seedlings with equal proportions of all three species, totalling 100 seedlings per species across the entire experiment. Initially, all tanks were filled to a set water level of ~15 cm below the soil surface using water from the Albury domestic water supply. Seedlings were then left in the tanks to acclimatise for two weeks, i.e. the establishment phase. Each fibreglass tank was then randomly assigned to one of five watering treatments, with three replicate tanks per treatment. At the commencement of these watering treatments, seedlings were 15 weeks old.

Watering treatments were applied over two consecutive 10-week phases, i.e. an early and a late phase, to examine effects of early conditions on responses to later conditions as follows:

1) Sequential flooding and drying (FDFD): in each phase, seedlings were flooded for four weeks then left to dry for six weeks;
2) Early flood then dry (FDDD): seedlings were flooded for four weeks and then dry for the remainder of the early phase and throughout the late phase;
3) Late flood (DDFD): seedlings were left to dry for the early phase (i.e. 10 weeks) then, in the late phase, flooded for four weeks followed by 6 weeks of subsequent drying;
4) Dry (DDDD): seedlings were left dry throughout both early and late phases; and
5) Flood (FFFF): seedlings were continuously flooded throughout both early and late phases of the experiment.

Flooding was achieved by filling tanks such that the soil surface was inundated to a depth of 2-3 cm. To dry tanks, a step-down approach was applied to mimic natural flood recession whereby the water level was lowered by 15 cm each week over a four-week period until the bottom of the pots retained 5 cm of water, mimicking access to sub-soil moisture. During the experiment, tanks were also exposed to ambient rainfall.

An initial harvest of 12 seedlings of each species was undertaken at the end of the establishment phase, immediately prior to the commencement of watering treatment, to obtain initial measurements of selected plant traits. During the experiment, seedlings were randomly selected from within the stratified sampling design and harvested at the end of the early phase (32 seedlings of each species) and the end of the late phase (remaining 56 seedlings of each species). A range of traits were measured for each harvested seedling (Table 1). We also assessed the mortality of all remaining seedlings at the end of each experimental phase. Additionally, we measured soil moisture on a weekly basis from two randomly selected pots per species from each tank (i.e. n= 6 seedlings per species per treatment per week). Soil moisture was also measured in all sampled pots at each harvest date. Soil moisture content (% volume) was measure with a Delta_T ML3 ThetaProbe Soil Moisture sensor Hand Held Reader (±1% accuracy) positioned in the top 5 cm of the soil.

Table 1. Seedling traits measured at the end of each experimental phase.

Parameter	Method
Seedling height	Measured from the base of the stem to the shoot tip (±1 mm).
Number of stems	Number of individual stems arising from the seedling base.
Leaf number	Number of leaves with a minimum length of 1 cm (*sensu* Mahoney & Rood 1991).
Leaf area	Leaves (>1 cm) were placed fresh onto a scanner and a photographic image captured. Total leaf area for each seedling was later determined using image analysis (ImageJ, National Institutes of Health, USA; Capon et al. 2009).
Root length	Distance from base of stem to end of longest root.
Above ground biomass	Stems and leaves separated from roots. Dried at 70°C for a maximum of 72 hours before weighing.
Below ground biomass	Roots separated from stem and washed to remove soil. Dried at 70°C for a maximum 72 hours before weighing.

Data analysis

Generalised linear mixed models (GLMM) were used to determine the effects on measured seedling traits (Table 1) at the end of both the early and late phases of the experiment. Additionally, we used GLMMs to investigate treatment effects on the probability of mortality and the ratio of above- to belowground biomass at the end of both early and late phases of the experiment. Models of all responses had a common base predictor function with fixed (species; watering treatment; species × watering treatment) and random (tank) terms (referred to as the 'full' model hereafter). 'Tank' was included as a random term to account for within-tank correlation among seedling responses. Response variables that were counts (non-negative integers) were modelled using Poisson LMM (variables 2 and 3), while probability of mortality was modelled using Binomial LMM. Gaussian LMMs were used to model all response variables defined by real, continuous numbers, assumed to follow a Normal distribution (response variables 4-9; Zuur et al. 2009). Probability of mortality was modelled using the entire data set, but seedlings that died during the experiment were not included when modelling any other response variable.

Model fits were assessed by examining diagnostic plots of standardised residuals against fitted and factor values. It was clear early in the modelling process that fits of the Poisson and Binomial LMMs resulted in minimal heterogeneity of errors among treatments. By contrast, there were clearly heterogeneous errors among treatments following Gaussian LMM. We therefore allowed further random terms in the Gaussian LMMs to account for heterogeneous variances (Pinheiro & Bates 2000). These additional random terms allowed for either species-specific variances, watering treatment-specific variances, or variances that vary across all species × watering treatment combinations. Consequently, the model selection strategy implemented for the Gaussian LMMs was slightly different from that of the Binomial and Poisson LMMs: Following Zuur et al. (2009) and Pinheiro and Bates (2000), we first determined the most parsimonious random structure for the Gaussian LMMs, retaining the full base predictor function (described earlier). After this first step, the second step of the selection process was the same across all GLMMs and involved determining which fixed terms may be dropped from the full model, reducing the full model until the most parsimonious model was selected. Akaike Information Criteria (AIC) and likelihood ratio tests (LRTs) were used to determine the most parsimonious models. P-values are unreliable for GLMM (Bates et al. 2015), so we only interpret biological 'significance' of results in light of graphical examination of effect sizes and confidence intervals (CIs).

All analyses were carried out in R (R Core Team 2017). Gaussian LMM was carried out using the nlme package (Pinheiro et al. 2017), while Poisson and Binomial LMM was carried out using the lme4 package (Bates et al. 2015). Temporal patterns in response variables for each species were displayed in SigmaPlot Version 14 (Systat Software, San Jose, CA).

To investigate seedling establishment strategies more holistically, we also conducted multivariate analyses to explore the variation in trait responses to watering treatments amongst the three species.

3. Results

3.1 Experimental conditions

During the study, air temperatures ranged from 2 °C – 44 °C and a total rainfall of 157 mm was recorded from the local gauge at Albury Airport (BOM 2017; Figure 1). Spikes in rainfall occurred around weeks 4 and 5 and between weeks 16 and 20. Soil moisture strongly reflected imposed watering treatments but did not always follow a smooth trajectory, probably due to the exposure to ambient weather conditions (Figure 1).

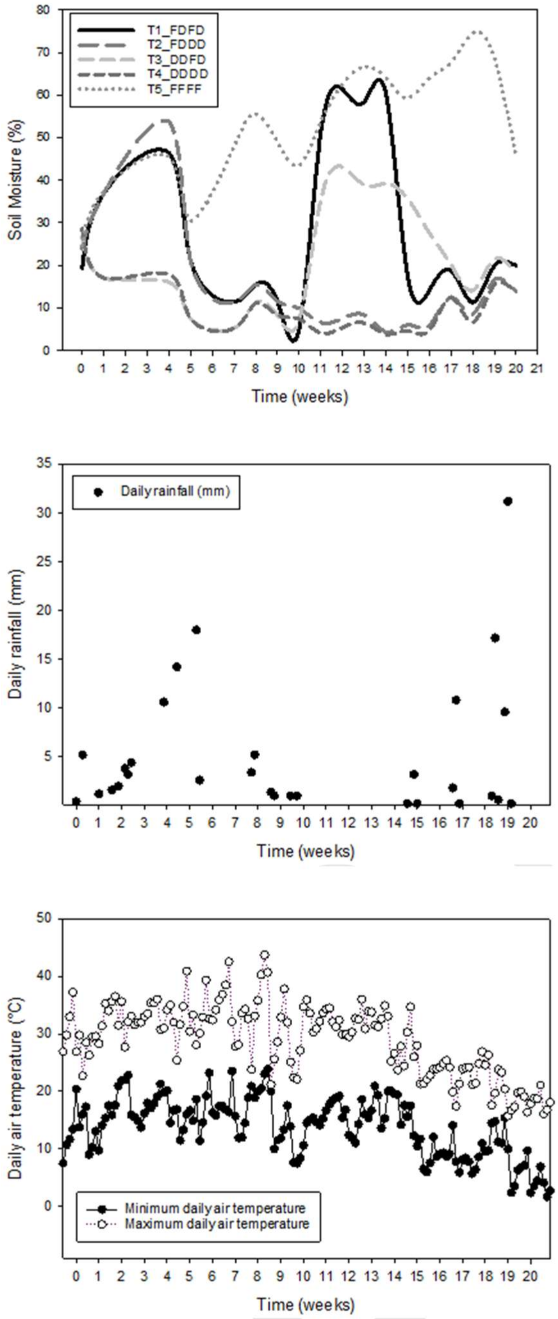


Figure 1. Rainfall and mean soil moisture recorded under each watering treatment during the experiment.

3.2 Seedling mortality

From a total of 300 seedlings, 51 seedlings died during the experiment. Fifteen of these seedlings died in a single tank, data from which was subsequently excluded from further analyses. In the remaining 14 tanks, mortality ranged from 0 % to 28.6 %. Probability of seedling mortality did not differ significantly according to species or watering treatment. Binomial LMMs with the lowest AIC and highest log-likelihood for seedling mortality at the end of both the early and late phases were those that contained only the intercept and random term ‘Tank’. LRTs also indicated that the full model was not significantly more likely than the model containing only the intercept and ‘Tank’ (early phase: $\chi 2$ = 16.08, P = 0.31; late phase: $\chi 2$ = 11.83, P = 0.62).

3.3 Trait responses to watering treatments

Seedling height

Seedlings of all three species responded to watering treatments with a relatively rapid initial increase in height that generally plateaued during the late phase of the experiment (Fig. 2). Gaussian LMMs for both experimental phases indicated an effect on seedling height of both species and watering treatment but no significant interaction. Including the interaction term in the model did not result in a significant increase in log-likelihood (early phase: L = 4.94, P = 0.29; late phase: L = 11.86, P = 0.16). *Eucalyptus camaldulensis* seedlings were taller than *E. coolabah* and *E. largiflorens* seedlings, regardless of water treatment or phase (Fig. 3a and 4a). *Eucalyptus largiflorens* seedlings were generally taller than *E. coolabah* seedlings, especially by the end of the late phase, except under constant flooding (FFFF) where they exhibited comparable height (Fig. 4a).

Seedling biomass

By the end of both early and late phases, *E. camaldulensis* seedlings had greater above- and below-ground biomass, but lower ratios of above- to belowground biomass, compared with either *E. largiflorens* or *E. coolabah* seedlings (Fig. 3b-d, Fig. 4b-d). Overall, above- and belowground biomass tended to be highest in all species under the constant dry (DDDD) and late flood (DDFD) treatments and lowest under the constant flood treatment (FFFF; Fig. 2). Conversely, above- to belowground biomass ratios were greatest under the constant flood treatment (FFFF) with root growth of all three species inhibited under these conditions (Fig. 2).

The optimal model for all biomass response variables was the Gaussian LMM without an interaction term, with models only differing in the random terms that accounted for heterogeneity in variances. Including the interaction term in the models did not result in a significant increase in log-likelihood for above-ground mass (early phase: $L = 4.52$, P = 0.34; late phase: $L = 7.76$, P = 0.46), below-ground biomass (early phase: $L = 3.01$, P = 0.56; late phase: $L = 14.10$, P = 0.08) or above- to belowground biomass ratio (early phase: $L = 5.13$, P = 0.27; late phase: $L = 10.19$, P = 0.25). While this suggests an absence of significant species-specific responses, subtle inter-specific differences were apparent. Although seedling heights generally plateaued by the end of the early phase, seedlings of *E. camaldulensis* and, to a lesser extent, *E. largiflorens* continued to accumulate total biomass during the late phase under most watering treatments except constant flooding (FFFF). *E. coolabah,* however, only gained total biomass during the late phase under the drier treatments (DDDD and DDFD). Under sequential flooding and drying (FDFD), only *E. camaldulensis* gained significant total biomass during the late phase.

By the final harvest, there was subtle variation in biomass allocation amongst *E. camaldulensis* seedlings under the early (FDDD), flood (FFFF) and constant dry (DDDD) treatments, but no difference in total biomass (Fig. 4b-d). In contrast, *E. coolabah* and *E. largiflorens* gained most biomass during the experiment under the late flood (DDFD) and constant dry (DDDD) treatments but not the early flood treatment (FDDD), indicating that these species were sensitive to the timing of the flood relative to seedling age (Fig. 4b-d).

Leaf number and area

Accumulation of leaves and leaf area varied considerably in relation to watering treatment between species (Fig. 2, 3e and 4e). With respect to leaf count, the full Poisson LMM was the most likely model at the end of both experimental phases. LRT showed that dropping the interaction term significantly reduced log-likelihood (early phase: $\chi^2 = 50.23$, P < 0.01; late phase: $\chi^2 = 180.92$, P < 0.01). By the end of the early phase, the interactive effect of watering treatment and species on leaf count was apparent in the tendency of *E. largiflorens* seedlings to grow more leaves in response to flooding variability (FD) and continuously dry conditions (DD) than either *E. camaldulensis* or *E. coolabah* (Fig. 3e). By the end of the late phase, *E. largiflorens* seedlings still had greater numbers of leaves than the other species, especially under the early flood (FDDD) and late flood treatments (DDFD; Fig. 4e). In contrast, leaf counts for *E. camaldulensis* and *E. coolabah* seedlings exhibited relatively little variation across watering treatments, with only a slight reduction in leaf numbers evident under the constant flood treatment.

Patterns in total leaf areas and mean leaf area per seedling differed across the water treatments and species (Fig. 3f and 4f). Total leaf areas for all three species tended to be highest under the late flood (DDFD) or constant dry (DDDD) treatments with lowest total leaf areas for all three species occurring under constant flooding. At the end of the early phase, *E. camaldulensis* had the highest total leaf area per seedling across all watering treatments (Fig. 3f). The full LMM including the interaction term was selected as the optimal model, with LRT showing that dropping just the interaction term alone significantly reduced log-likelihood ($L = 32.73$, $P < 0.001$). Interactions between watering treatment and species for total leaf area were manifested in a greater reduction in total leaf areas for *E. largiflorens* and *E. coolabah* under constant flooding relative to *E. camaldulensis* (Fig. 3f). By the end of the late phase, however, total leaf areas of *E. largiflorens* seedlings were comparable to those of *E. camaldulensis* under some treatments (FDDD, DDFD and DDDD) while totals for *E. coolabah* were generally lower regardless of treatment (Fig. 4f). The optimal model after the late phase was one without the interaction term with LRT showing that dropping the interaction term did not significantly reduced log-likelihood ($L = 0.12$, $P = 0.94$).

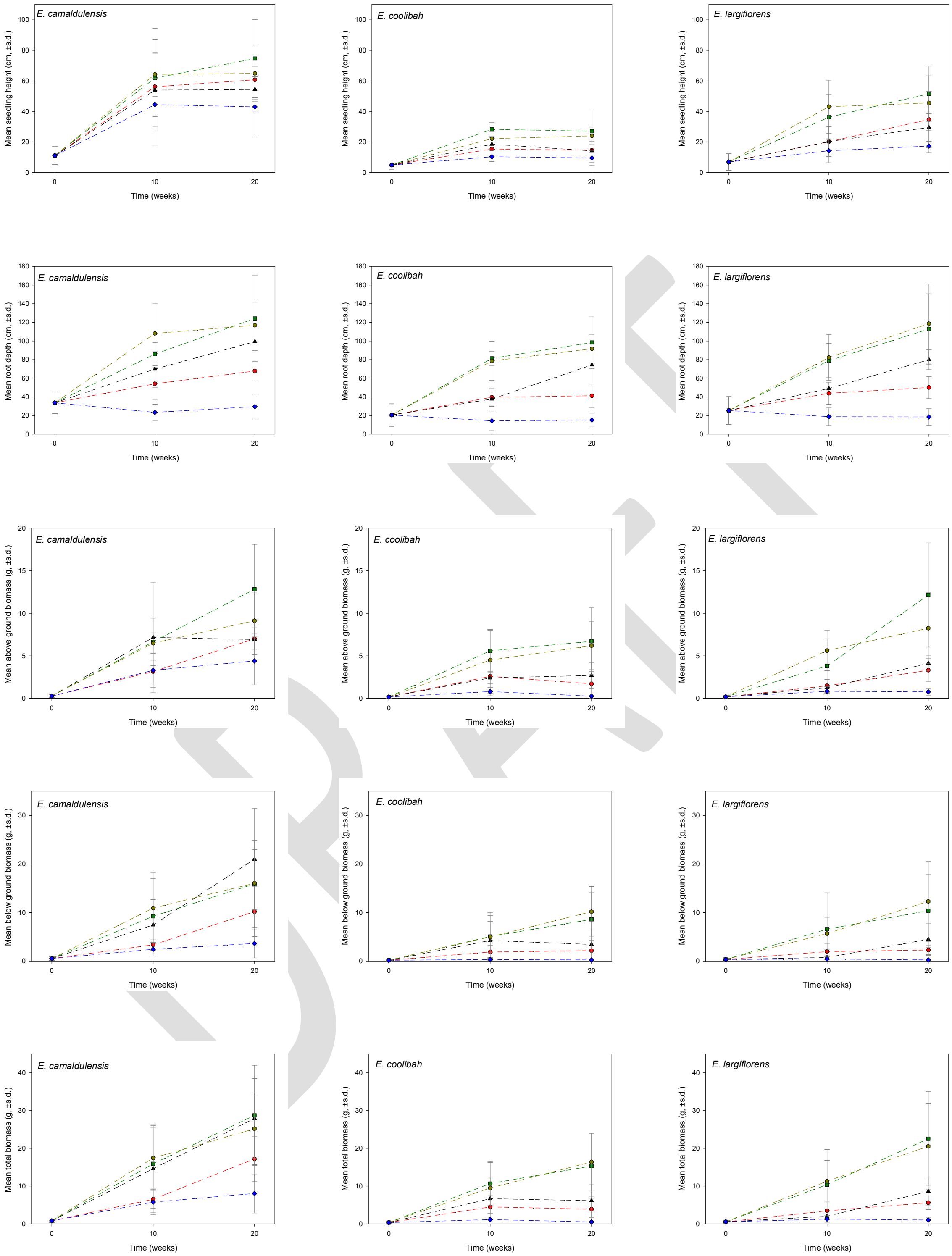
No significant differences in leaf area between species were apparent by the end of the early phase of the experiment, with all three species tending to have lower mean leaf areas under constant flooding conditions (Fig. 2 and 3g). The optimal model was one without the interaction term with LRT showing that dropping the interaction term did not significantly reduced log-likelihood ($L = 7.07$, $P = 0.13$). By the end of the late phase, however, LRT showed that dropping the interaction term alone significantly reduced log-likelihood ($L = 18.32$, $P = 0.02$), indicating a species-specific mean leaf area response to watering treatments by this stage (Fig. 4g). *Eucalyptus camaldulensis* had higher mean leaf areas than the other species, with differences most apparent under the early flood (FDDD) and sequential flooding and drying (FDFD) treatments. *Eucalyptus coolabah* and *E. largiflorens* responded similarly with small leaves (low mean leaf area) under the constant flood (FFFF) and early and sequential flooding and drying treatments (FDDD, FDFD) but E. *coolabah* showed greater mean leaf area under the later flood treatment relative to *E. largiflorens*. The greatest mean leaf area in all three species occurred under either the late flood treatment (DDFD: *E. camaldulensis* and *E. coolabah*) or constant drying (DDDD: *E. largiflorens*).

Root length

Root development was significantly affected by watering treatment in all three species, with greater root lengths reached under the constant dry (DDDD) and later flood treatments (DDFD), especially compared with the constant flood and sequential flooding and drying treatments (Fig. 2, 3h and 4h). This response did not vary between species. The LMMs without the interaction terms were selected as the optimal model for root length at the end of both experimental phases as including the interaction term did not result in a significant increase in log-likelihood (early phase: L = 5.18, P =0.27: late phase: L = 5.38, P = 0.72). Average root depth at the end of the early phase was greater than the length of the pots (>70 cm) for all three species under treatments which were dry during this period and close to the length of pots in several other species-treatment combinations. Of the 81 live seedlings harvested at the end of the early phase, 33 had root lengths greater than the length of the pots, including nine *E. largiflorens* and nine *E. coolabah* seedlings, all from treatments which had not yet received any inundation. The remaining 15 seedlings were *E. camaldulensis,* 10 of which were recorded from dry early phase treatments while the remaining five were from the early or consecutive flood treatments (FDDD, FDFD).

Number of stems

The optimal model for the number of stems at the end of the early phase was the model without the interaction term between species and watering treatment. Dropping the interaction did not significantly reduced model likelihood (χ^2 = 6.47, P = 0.17). *Eucalyptus largiflorens* produced more stems than either *E. camaldulensis* or *E. coolabah* but responses to watering treatments at the end of the early phase were similar between species overall, with fewer stems occurring under the constant flood treatment (Fig. 3i). By the end of the late phase, some species-specific responses to the treatments were apparent (Fig. 4i). The full Poisson LMM was the most parsimonious in the candidate model set for stem number. Dropping the interaction between species and watering treatment significantly reduced model likelihood (χ^2 = 67.65, P < 0.001). *Eucalyptus camaldulensis* rarely produced more than one stem except under constant drying (DDDD; Fig. 2). Conversely, *E. largiflorens* produced greater numbers of stems under the variable flooding treatments (FDFD, FDDD and DDFD) compared with either of constant drying (DDDD) or flooding (FFFF; Fig. 2).


E. camaldulensis
E. coolibah
E. largiflorens
Mean seedling height (cm, ±s.d.)
Mean root depth (cm, ±s.d.)
Mean above ground biomass (g, ±s.d.)
Mean below ground biomass (g, ±s.d.)
Mean total biomass (g, ±s.d.)
Time (weeks)

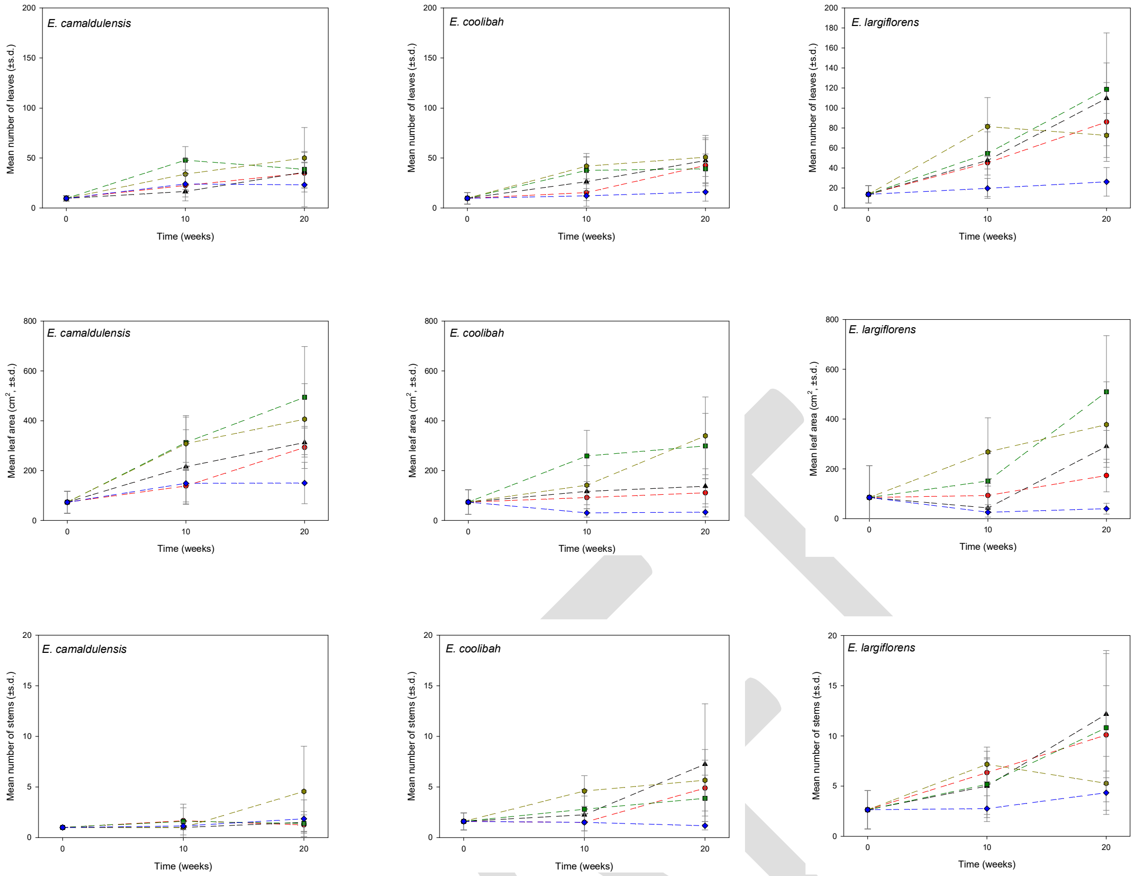


Figure 2. Mean responses (± 95% CI) of eight seedling traits to five watering treatments over the duration of the experiment, i.e. at the end of the establishment phase, end of the early phase and end of the late phase, for three floodplain tree species: *E. camaldulensis*, *E. coolabah* and *E. largiflorens*. Symbols denote watering treatments: red circles = FDFD; black triangles = FDDD; green squares = DDFD; olive hexagons = DDDD; and blue diamonds = FFFF.

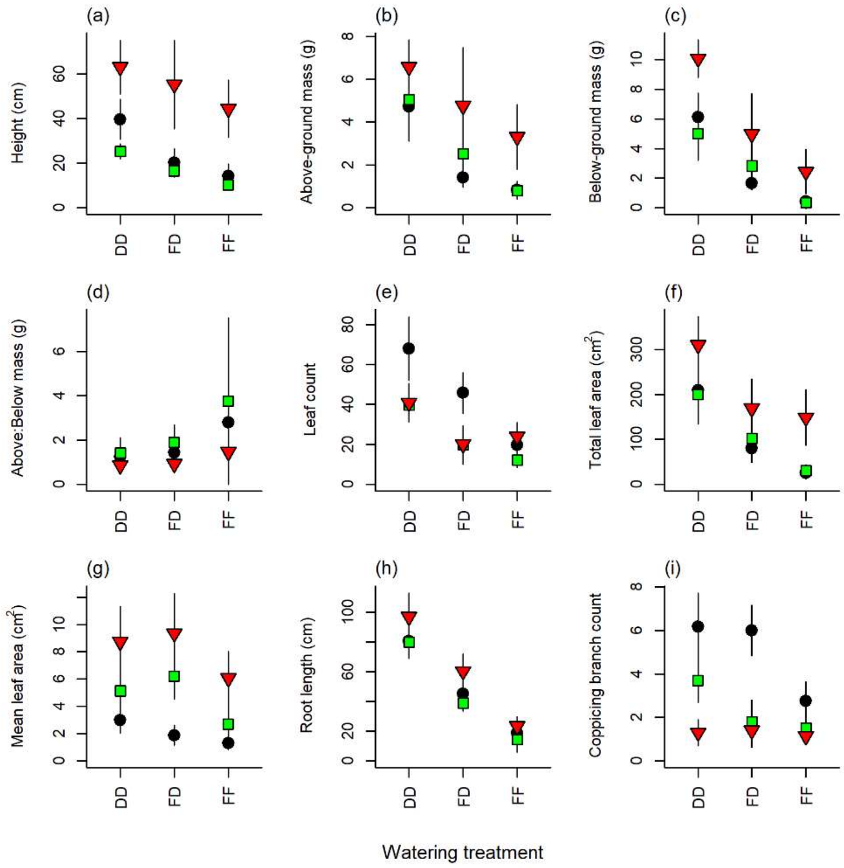


Figure 3. Mean (+/- 95% CI) response of selected traits at the end of the early phase for *E. largiflorens* (black circles), *E. coolabah* (green squares), and *E. camaldulensis* (red triangles) to watering treatments: constant drying (DD); early flood treatment (FD) and constant flooding (FF). N.B. Results are combined here for seedlings under watering treatments which only differed during the late phase of the experiment.

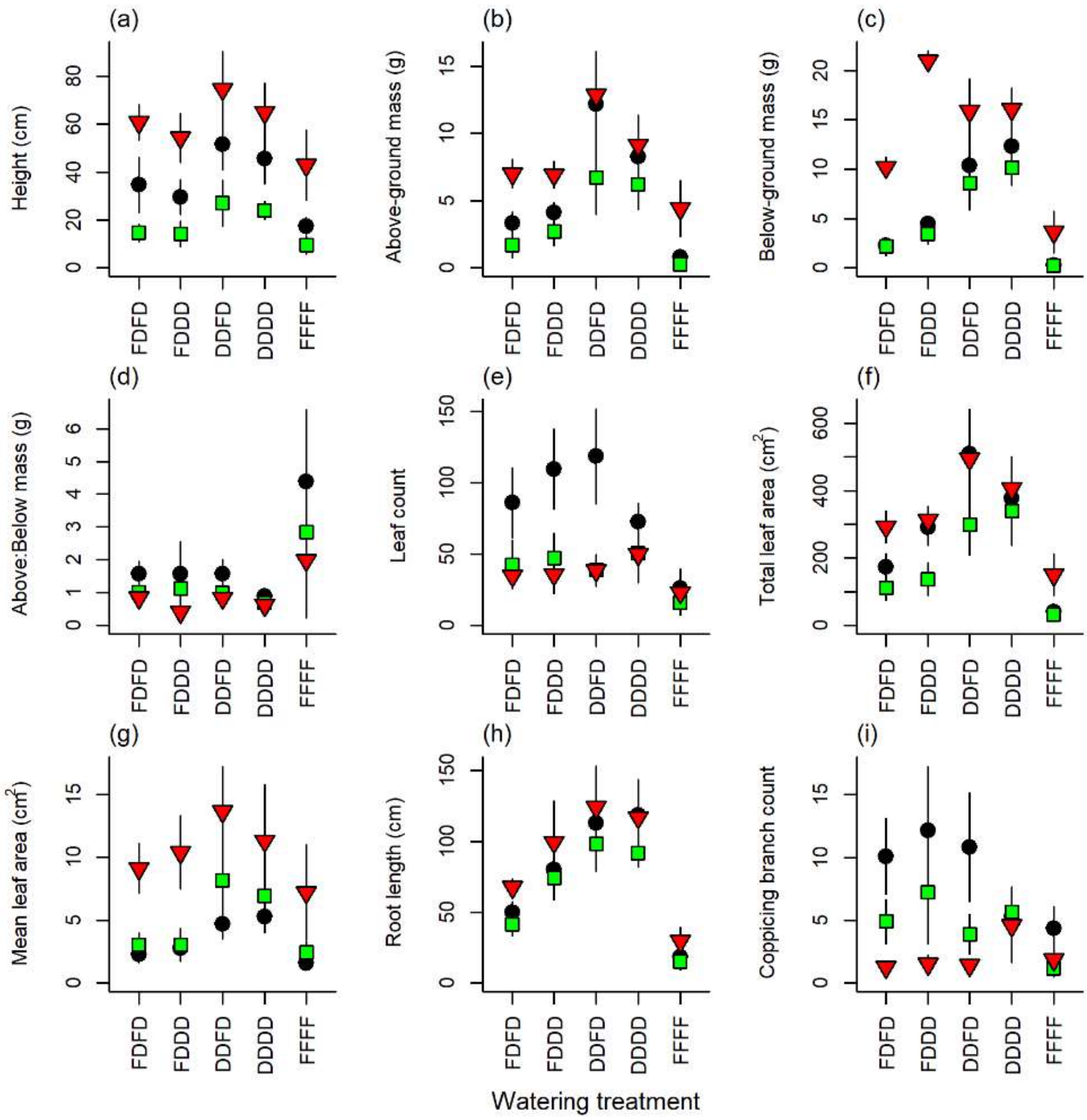


Figure 4. Mean (+/- 95% CI) response, at the end of the late phase for *E. largiflorens* (black circles), *E. coolabah* (green squares), and *E. camaldulensis* (red triangles) to five watering treatments: sequential flooding and drying (FDFD); early flood (FDDD); later flood (DDFD); constant dry (DDDD); and constant flood (FFFF).

3.4 Heterogeneity of seedling responses to watering treatments

Significant differences in the heterogeneity of seedling responses to watering treatments were apparent between species (Fig. 5). At the end of the early phase, a relatively clear gradient was apparent amongst species with *E. coolabah* occupying an intermediate space relative to *E. camaldulensis* and *E. largiflorens* which respectively separated in the ordination in relation to seedling height (*E. camaldulensis*) and number of leaves and stems (*E. largiflorens*). By the end of the late phase of the experiment, *E. camaldulensis* remained distinct from the other two species, particularly in relation to seedling height and total biomass. The variation in seedlings of *E. coolabah* and *E. largiflorens,* however, were much more similar by this time.

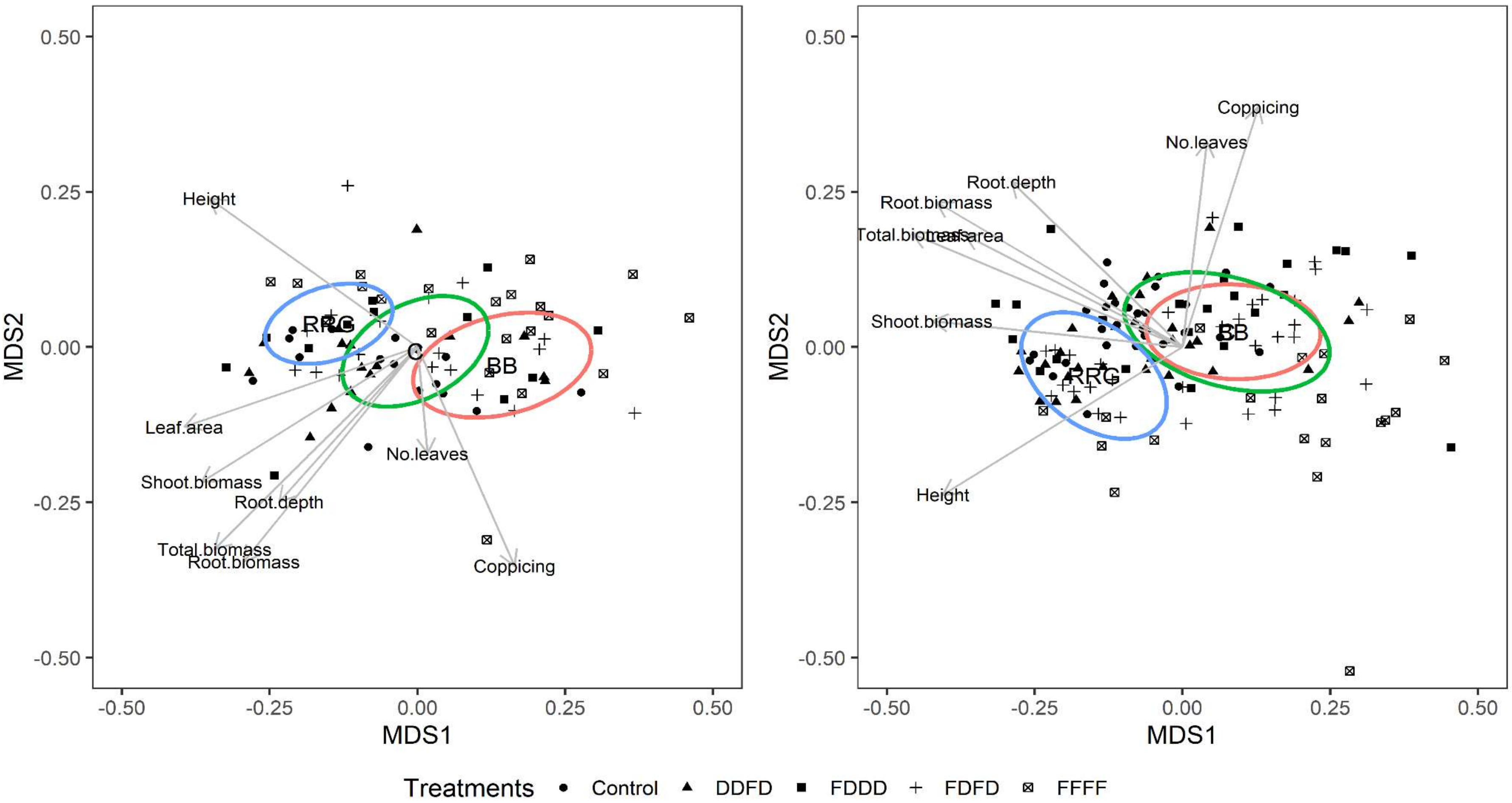


Figure 5. nMDS ordinations of seedlings of *E. largiflorens* (red circles), *E. coolabah* (green circles), and *E. camaldulensis* (blue circles) based on responses of selected traits (Table 1) to five experimental watering treatments: sequential flooding and drying (FDFD); early flood (FDDD); later flood (DDFD); constant dry (DDDD); and constant flood (FFFF). Results at the end of the early experimental phases are shown in the left panel while the right panel shows results from the end of the late experimental phase. Vectors show intrinsic variables (i.e. seedling traits) significantly correlated with the ordinations.

4. Discussion

4.1 Seedling responses to flooding and drying

Desert floodplains are characterised by both hydrologic variability and unpredictability; however, these vary across the floodplain gradient. For tree seedlings establishing in more frequently flooded parts of the floodplain, flooding may be a more likely occurrence than for those seedlings occurring in drier, marginal floodplain areas and vice versa. In this experiment, constant flooding supressed growth in almost all measured traits in all three species, but especially with respect to belowground growth and particularly amongst *E. largiflorens* and *E. coolabah*. Nevertheless, there was a high level of tolerance to flooding amongst all species with no significantly greater mortality of seedlings occurring under continuous flooding over 20 weeks. Extreme quiescence during periods of shallow inundation therefore appears to be the dominant mechanism of tolerating flooding in all three species considered here. This capacity to delay development and limit biomass accumulation and the growth of shoots or roots or the accumulation of leaves appears to enable a high degree of flood tolerance in these species. Leaf shedding was also observed amongst all three species in response to flooding during the later phase which can also be perceived as an adaptive response to flooding (Capon et al. 2009). Overall, this strategy probably facilitates flood tolerance by minimising resource requirements and exposure to stressors (Capon et al. 2009).

An exception to this 'freeze' response to flooding was evident amongst *E. camaldulensis* seedlings subject to early flooding which demonstrated initial aboveground growth, especially in terms of seedling height. Such a fixed pattern for early stem elongation is likely to be advantageous for a species such as *E. camaldulensis,* which inhabits immediate riparian and more frequently inundated habitats. Seedlings are more likely to face further inundation than those species inhabiting drier parts of the floodplain. Gaining height quickly is therefore an important strategy for seedlings to adopt since they are more likely to be able to survive and continue growth if they extend above floodwaters.

While flooding clearly presents a significant stress to establishing seedlings, especially in early life history stages, most seedlings across the floodplain gradient are likely to experience drought of varying intensities well before they reach maturity. Rapid development of a deep root system may be one approach via which seedlings can prepare for the eventuality of drought by maximising their capacity to access subsurface soil moisture. In our experiment, all species exhibited rapid root extension in response to drying. Drying also appeared to induce the growth of more numerous but smaller leaves in all species, but particularly in *E. largiflorens*. Other adaptive responses to drying could not be observed in our experiment, however, because the rapid root elongation enabled all plants to reach moisture and avoid drought during the early phase of the experiment.

4.2 Effects of early conditions on responses to subsequent conditions

Flooding during the early phase of the experiment affected aboveground growth responses to subsequent drying in all three species. In *E. camaldulensis* and *E. coolabah*, early flooding tended to continue to limit growth during following dry conditions, especially for seedlings of the latter. In contrast, aboveground growth in *E. largiflorens* recommenced in response to drying in seedlings that were flooded during the early phase. In all three species, however, seedlings that were flooded during the early phase of the experiment, all responded to drying by rapid extension of root length. In the case of *E. camaldulensis,* this belowground development also involved significant allocation of biomass to roots. Such development of lateral roots may also represent an adaptive trait with respect to flooding since this may assist in anchoring seedlings during future inundation.

Early flooding was associated with the subsequent development of more stems in response to later drying in *E. coolabah* and *E. largiflorens* as well as increased leaf acquisition in the late phase. *Eucalyptus camaldulensis* and *E. largiflorens* also exhibited shoot elongation in response to later flooding where seedlings had been dried in the early phase. Variable, repeated flooding and drying tended to stifle growth in the late experimental phase compared to drier treatments, especially with respect to root depth and biomass.

4.3 Variation in species establishment strategies

Although there were many similarities in trait responses to watering treatments amongst the three species, overall variation was apparent between the three species considered representing subtle differences in strategy. *Eucalyptus camaldulensis* seedlings, for instance, can be seen as having a ‘go hard and fast’ establishment strategy with rapid initial growth, probably as a strategy to put on height before subsequent flood events (which are likely to be sooner for this species given its distribution lower on the floodplain and in more frequently flooded areas). This approach may also enable *E. camaldulensis* seedlings to outcompete other species, e.g. dense herbs which can establish following floodwater recession. *Eucalyptus camaldulensis* seedlings also tended to develop a single main stem with fewer but larger leaves than the other species. Where multiple stems did develop, this was mainly under the continuous dry treatment. Finally, more adventitious roots developed in *E. camaldulensis* during our experiment than in the other species.

Eucalyptus coolabah seedlings, in comparison to *E. camaldulensis,* did not gain much height during the experiment but rather put a lot more relative effort into developing root length. *Eucalyptus coolabah* occurs in hydrologically unpredictable, flashy systems where floods are likely to be of relatively short duration and time between floods highly variable. Putting a lot of effort into developing deep roots may therefore represent a strategy that enables seedlings to access groundwater as quickly as possible to ensure access to alternate water sources given the high likelihood of drought.

Finally, *E. largiflorens* seedlings typically displayed a multi-stemmed growth form and produced lots of branches with many small leaves. While the majority of leaves produced were small, the number of leaves was such that the average leaf area was comparable to *E. camaldulensis*. *Eucalyptus largiflorens* occur on higher parts of the floodplain compared with *E. camaldulensis* and, as such, usually experience less frequent floods of shorter duration and depth. Hence, the need to establish a single, tall dominant stem quickly is likely to be less important for *E. largiflorens* than for *E. camaldulensis*. The benefit of the multi-stemmed, lots of small leaves growth form observed in our experiment could potentially have developed as a response to grazing, or other benefits related to increasing wood density (Chave et al. 2009).

4.4 Management implications

A key finding of this experiment with respect to environmental water management includes the high importance of inter-flood dry periods for promoting seedling establishment in these species. Following successful germination, an initial dry period is likely to be very beneficial for promoting root length and biomass accumulation and to enable seedlings to develop height and, therefore, there capacity to survive drought or further flooding. Managed flows, if required, might therefore be best applied approximately 6 months after germination.

Our results also suggest that where management seeks to control undesirable seedling establishment, prolonged flooding or flooding applied shortly after germination is likely to be necessary. After this time, however, seedlings are likely to be very tolerant of flooding. The timing of managed floods for the purpose of controlling unwanted seedlings therefore need to occur as soon after germination as practicable, and ideally within the first three months, to have a stunting effect on seedling growth. Ideally, floods delivered for this purpose need to be of sufficient depth to over-top targeted seedlings.

Eucalyptus coolabah and *E. largiflorens* are likely to be more sensitive to the timing of floods relative to their age.

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Appendix V4.6: Seedling Mesocosm Research Activity Report

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Document purpose: Summary document to capture the outputs and outcomes from the Seedling Mesocosm component of the MDB EWKR Vegetation theme. This document complements and refers to other outputs rather than duplicates information.

- ## Research Question

What drives vegetation responses to watering actions?

- With a focus on the response of woody floodplain seedlings

Specifically, this research sought to improve the understanding of flow requirements for seedling establishment of woody floodplain trees and shrubs. We were interested in the relationships between flow parameters such as duration, frequency, interflood-dry period, sequential, cumulative events and seedling establishment. How do sequential flooding and drying events affect seedling growth? How important are patterns of root development to overall growth and survival? How does the initial condition of seedlings affect their response to a flooding/drying treatment?

- ## Methods

To help determine the current knowledge status regarding woody floodplain seedling recruitment a literature review was undertaken at the start of the component (Durant et al. 2016a; Appendix V4.1). For additional information about the flow requirements of river red gum, black box, coolibah and lignum seedlings refer to Durant et al. 2016a.

Using information from the literature review, mesocosm experiments focusing on seedling establishment were developed. For detailed experiment methods refer to Durant et al. 2016b. *Recruitment of long-lived floodplain vegetation: mesocosm study experimental design* (Appendix V4.2) as well as methods described in Campbell et al. (draft), *Establishment strategies of dominant trees of highly variable floodplains* (Appendix V4.5). This section provides a summary of the seedling experimental methods and highlights any deviations from the above methods document.

Species

Initially four key woody floodplain species were identified as target species, consisting of three eucalypt tree species, river red gum (*Eucalyptus camaldulensis* Dehnh.), black box (*Eucalyptus largiflorens* F.Muell.) and coolibah (*Eucalyptus coolabah* Blakely and Jacobs), and one native floodplain shrub species, tangled lignum (*Duma florulenta* Meissner). For short species descriptions refer to Campbell et al (draft), *Establishment strategies of dominant trees of highly variable floodplains* (Appendix V4.5).

Seedling propagation

The germination of the four species was sub-contracted to a commercial nursery (Sandy Creek Trees, Allans Flat, Victoria). For the eucalypt species seed was sourced from the following provenances: river red gum – Kiewa Valley, VIC; black box – Ararat, VIC; coolibah – Alice Springs, NT. The nursery was unable to source seed for tangled lignum so this was collected by MDFRC staff from the Lower Murray region (Bottle Bend NSW and Colignan, VIC). Despite having collected sufficient lignum seed, there was poor germination success and poor seedling survival through the establishment phase. Only 36 lignum seedlings were available at the start of the experiment. As a result, lignum has been excluded from the research outputs. A brief outcome from the reduced number of lignum seedlings has been provided in the results below, however these should be interpreted with caution.

Flow treatments

Water treatments were applied over two, 10-week phases termed early and late (Table 1). Treatments were comprised of various combinations of flooding and drying (Table 1), reflecting different intra-annual flow regimes.

1) Sequential flooding and drying (FDFD). Seedlings flooded and dried in a sequence of flooding for four weeks then dry for six weeks and repeated
2) Early flood then dry (FDDD). Seedlings flooded for four weeks and then dry for remainder of experiment.
3) Late flood (DDFD.). Seedlings dry for ten weeks then flooded for four weeks. Dry for remainder of experiment.
4) Dry (DDDD). Seedlings remain dry for duration of experiment.
5) Flood (FFFF). Seedlings flooded continuously for duration of experiment.

For the flood phase, tanks were filled to flood pots to a depth of 2-3 cm (Figure 1). For the dry phase a step-down approach was applied to mimic natural flood recession. The water level was lowered by 15 cm each week over a four-week period until the bottom of the pots retained 5 cm of water, mimicking access to sub-soil moisture.

Seedling growth measurements, observational monitoring and sacrificial harvests

To obtain a baseline of parameters an initial harvest of 12 seedlings of each eucalypt species was undertaken at the end of the establishment phase, immediately prior to the application of the five treatments. During the experiment eucalypt seedlings were randomly selected within the stratified sampling design and sacrificially harvested, at the end of Phase 1 (32 seedlings of each species) and at the end of the experiment (remaining 56 seedlings of each species) (Table 2). At each sacrificial harvest seedlings were measured for: mortality, height, number of stems (coppicing), development of aerial roots, number of leaves and leaf area, root length, above-ground biomass and below-ground biomass (Table 3).

Additional observational monitoring occurred during Phase 1 and Phase 2 (Table 1) where seedlings were measured for mortality, height, number of stems (coppicing), development of aerial roots, and number of leaves (Table 3). Coppicing (number of branches) and aerial/surface root development were added during the end of Phase 1 sacrificial harvest when it became apparent these were being developed. Results for the sacrificial harvest at the end of the establishment phase were estimated from photos taken of individual seedling pots at the time of harvest.

Figures 1 to 3 show the tank and pot set-up and demonstrate various aspects of the harvesting.

Alterations for lignum

As we only had 36 lignum seedlings, tangled lignum has a reduced sampling regime. Seedlings were still distributed across all five watering treatments, however there were only two sacrificial harvests, one at the end of the establishment phase and one at the end of the experiment. There were five observational monitoring times (Table 2).

Soil type

Standard floodplain soil was collected from the Murray River floodplain near Albury. Soil analysis, prior to commencing the experiment, indicated that the floodplain soil was a mixture of sand, silt and clay (41:41:18) with a water holding capacity of 21.21, and a total carbon content of 3.09% (following Grimshaw 1989, Ilstedt *et al.* 2000, Nelson & Sommers 1982).

Data preparation and statistical analyses

For details about data preparation and analysis refer to Campbell et al (draft), *Establishment strategies of dominant trees of highly variable floodplains* (Appendix V4.5).

Table 1. Sequences of flooding/drying identified for mesocosm study. Green indicates Drying treatment (where pots will be watered with 5cm of water in the bottom of tanks), blue indicates shallow flooding (2-3cm) above the top of the pot. Red lines indicate sacrificial & observational harvesting and the thick Black line observational measurements.

	Time (Weeks)																						
Treat-ments	Establi-shment		Early Phase										Late Phase										
Week no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Date	28 Nov	5 Dec	12 Dec	19 Dec	26 Dec	2 Jan	9 Jan	16 Jan	23 Jan	30 Jan	6 Feb	13 Feb	20 Feb	27 Feb	6 Mar	13 Mar	20 Mar	27 Mar	3 Apr	10 Apr	17 Apr	24 Apr	1 May
1																							
2																							
3																							
4																							
5																							

Table 2: Number of pots for each sampling period, for each species and watering treatment

Phase	Treatment no.	River Red Gum	Black Box	Coolibah	Lignum	No. pots to sample
Establishment sacrificial harvest begins week 3	1,2,3,4,5	12	12	12	6	42
End of watering treatment and observational harvest for early phase occurs week 7	1, 2, 5	12	12	12	6	84
	3, 4	12	12	12	6	
End of early phase sacrificial harvest begins week 13	1, 2	12	12	12	6	108 + 18 observations (Lignum)
	3, 4	12	12	12	6	
	5	12	12	12	6	
End of watering treatment and observational harvest for late phase occurs week 17	1	12	12	12	6	186
	2	12	12	12	6	
	3	12	12	12	6	
	4	12	12	12	6	
	5	4	4	4	6	
End of late phase (end of experiment) sacrificial harvest begins week 23	1	12	12	12	6	186
	2	12	12	12	6	
	3	12	12	12	6	
	4	12	12	12	6	
	5	4	4	4	6	

Table 3: Variables measured at observational monitoring times and sacrificial harvesting

Variables	Observational monitoring	Sacrificial harvesting
Soil moisture (%) - Surface soil moisture content as % volume determined via use of a soil moisture probe (Lynch 2006)	√	√
Mortality - Live (l) or dead (d) - number of seedlings that die recorded by date/time	√	√
Seedling height (cm) - measured to the shoot tip (±1 mm)	√	√
Coppicing - number of stems at the base of the plant - *(NB. added at the end of Phase 1 sacrificial harvest)*	√	√
Aerial / surface roots - have above-ground roots developed (y/n) - *(NB. added at the end of Phase 1 sacrificial harvest)*	√	√
Leaf numbers - leaf number calculated on leaves with minimum 1 cm length (Mahoney & Rood 1991)	√	√
Leaf Area (cm^2) - place leaves on scanner and determine area via image analysis (e.g. Bioscan Image analyser)		√
Root depth/ length (cm) - on day of harvest soil root column is exposed and length of root system measured to its lowest point in the soil (Neave & Florence 1994)		√
Biomass of shoot, root and leaf components (g) - separation of roots from stem/leafs at the root-shoot junction and weighed separately after being dried in oven at 70°C temperature for 48 hours (Horton & Clark 2001)		√

Figure 1: Example of experimental tanks with seedlings pots under dry (left) and wet (right) treatments

Figure 2: Cross-section of a seedling pot showing early (above) and later (below) root development

Figure 3: Coppicing branches (left), measuring seedling height (centre), and scanning leaf area (right)

• Results

Communication of the seedling mesocosm results can be found in the following appendices within MDB EWKR Vegetation Theme summary report (the document to which this is an appendix).

- Presentation: Durant et al 2018. *Early, late or constant – what are long-lived woody floodplain seedlings looking for?* 58th Australian Freshwater Sciences Society Congress, Adelaide, 23-28 September 2018 (Appendix V4.3)
- Article: *Giving woody seedlings a fighting start*, RipRap V40, 2017, pp 19-20, Australian River Restoration Centre, Canberra (Appendix V4.4)
- Paper: Campbell et al. (draft), *Establishment strategies of dominant trees of highly variable floodplains (*target journal Journal of Experimental Botany*)* (Appendix V4.5)

A video is also available online: StorySpace video, http://ewkr.com.au/valiant-vegetation/

Results are only presented here where they are additional to results presented in Campbell et al. (draft), *Establishment strategies of dominant trees of highly variable floodplains* (Appendix V4.5).

The above paper presents the results for the three eucalypt species: river red gum, black box and coolibah. Additional results relating to lignum are presented below.

Lignum

Of the 36 seedlings available at the start of the experiment, 32 survived with only four seedlings dying during the experiment. These four seedlings were from four different treatments and four different tanks.

Based on the limited number of lignum seedlings available, the results indicate that the constant flood treatment has a suppressive effect on all seven variables measured for lignum (Figure 4).

Results for the other treatments differ across the measured variables (Figure 4). Height was greatest under the later flood treatment (DDFD), while root length was greatest under the dry (DDDD) and early flood treatments (FDDD). Above-ground, below-ground and total biomass were all greatest under the later flood treatment (DDFD), however below-ground biomass under the dry treatment (DDDD) was very similar. Mean number of leaves were greatest under the early flood (FDDD) and later flood (DDFD) treatments. Typically only a limited number of main stems were produced (between 1 and 5 coppicing branches). The number of coppicing branches produced was similar under the dry (DDDD), later flood (DDFD) and sequential flood (FDFD) treatments.

The variability in responses across all the measured variables means these results are unlikely to be significant.

Figure 4: Response of seven growth parameters to five flood treatments, measured at two points in time (est – end of establishment phase and P2 – end of Phase 2) for lignum (*Duma florulenta*).

• Discussion / applications

The outcomes from this component directly relate to the management of water for seedling establishment of woody floodplain trees.

The key findings can be summarised as:

- The three eucalypt tree species displayed different growth strategies
    - River red gum displayed an opportunistic strategy, capturing resources quickly. River red gum seedlings put on height quickly and produced a single dominant stem with few, larger leaves. River red gum seedlings have a greater likelihood of being flooded again soon; putting on height quickly is likely to be advantageous in terms of outcompeting other species, such as grasses, and surviving subsequent flooding.
    - Black box displayed a drought stress strategy. These seedlings produced a multi-stemmed branching structure with lots of small leaves (with a total leaf area similar to river red gum). Black box seedlings are most likely to experience drying stress. The multi-stemmed branching structure and small leaves may be adaptations to drought and or grazing pressure.
    - Coolibah displayed a conservative strategy, with seedlings putting comparatively more effort into root length as opposed to height. This is likely to reflect the unpredictability of the floodplain environments coolibah typically occurs in and their reliance on groundwater as adult trees, a finding of the Queensland floodplain vegetation component of EWKR (DSITI, DNRM 2017).
- Common responses
    - Constant inundation suppresses growth
    - Inter-flood dry periods are important for growth, particularly the development of roots.
    - Coolibah and black box are more sensitive to the timing of floods, with both species performing better under a later flood as opposed to an earlier flood.

Understanding the flow requirements for woody seedling establishment of different species, including different growth strategies, enables watering events to be targeted to specific traits associated with seedling establishment for particular species, such as root length or height.

While constant flooding suppresses growth, seedlings were observed to be very flood tolerant. Inundation will not always lead to mortality, particularly if the inundation depth isn't sufficient to overtop seedlings. If the control of seedlings is a desirable management outcome, flooding needs to occur very early in their life to improve the likelihood of mortality, especially if these seedlings have established in habitats where drying stress is likely to be lesser, e.g. lake beds, creeks etc.

Inter-flood dry periods were determined to be important for growth, particularly the development of roots. Root length and biomass were significantly suppressed under constant flooding. While it is unclear what the long-term impacts may be on the development of tap roots, we hypothesise that prolonged waterlogged conditions during early seedling establishment may lead to suppression of long tap roots and greater development of surface roots. Well-developed tap roots are vital for access to groundwater as well as anchorage and stability as an adult tree. Consequently, seedlings establishing under prolonged waterlogged conditions may be less tolerant of subsequent drying.

Both black box and coolibah seedlings performed better under a later flood as opposed to an earlier flood. The implications for management are that if you're designing watering events for coolibah or black box establishment then allow a dry period (of ~ 6 months) post germination before providing top up flows. This comes with the caveat that individual site conditions, such as soil type, soil moisture, temperature and rainfall will influence the need for top up inundation.

• Conclusions / further work

A number of opportunities or considerations for further work have been identified:

- Influence of interacting non-flow drivers:

This experiment looked at the effects of five different watering treatments reflecting different intra-annual watering regimes. However, there is emerging evidence that seedling occurrence in the landscape is not always well explained by flow parameters alone. It would be valuable to investigate the interacting influence of non-flow drivers. The experiment (or aspects of the experiment) could be repeated incorporating the influence of one or more non-flow drivers, such as:

  - Salinity (soil and groundwater)
  - Soil type / compaction
  - Grazing

- Modify the 'constant dry' regime

The constant dry regime included five cm of water at the base of the tank to mimic access to sub-surface moisture. However, a large number of the seedlings developed roots so quickly they were able to access this water early in the experiment. Repeat the experiment with a truly 'constant dry' regime to determine the effect of no access to groundwater and no access to soil moisture.

- Effect of seedling provenance

Seedlings of the same species all had the same provenance (seed source) and these may not have been typical for the Murray-Darling Basin (coolibah – Alice Springs; black box – Ararat; river red gum – Kiewa Valley). Repeating the experiment, with seed collected from different locations within the MDB, would test the effect of seed provenance on seedling establishment and provide details of the variability of responses within species from different locations.

- Lignum

Lignum was originally included in the experiment, however poor germination success of lignum seedlings led to the data being excluded. It would be good to further investigate the potential reasons for the lignum failure and repeat the experiment with lignum seed collected from a number of provenances.

- Restrictions to root growth

Typically root development was rapid and by half-way through the experiment (end of Phase 1) roots of many seedlings had reached the base of the pots. If the experiment was repeated it would be good to consider the potential to use longer pots. However, there are health and safety considerations with this and consideration should be given to alternate ways to remove and insert pots into treatment tanks. The existing pots (~75cm length) are extremely heavy and hard to handle when full of waterlogged soil.

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Output type	Output title / reference	Brief description	Work Component	Activity	Availability
Scientific publication	Campbell et al. (submitted). *Blue, green and in-between; setting objectives for and evaluating wetland vegetation responses to environmental flows.* Submitted to Ecological Indicators	Paper proposing four principles to guide the development of robust objectives and evaluation approaches for the adaptive management of environmental flows with respect to vegetation outcomes.	V1. Conceptualisation	V1.3 Reporting	Appendix V1.1 When published it will be available via the journal
Technical report	Conceptualisation Research Activity Report	Plain english description of work undertaken in the Conceptualisation component	V1. Conceptualisation	V1.3 Reporting	Appendix V1.2
Presentation	Campbell et al. (2016). *Vegetation outcomes: what are we seeking and why?* Australian Society of Limnology Conference, Ballarat, 27th September 2016	Pdf copy of slides presented at the conference	V1. Conceptualisation	V1.3 Reporting	Appendix V1.3
Article	*Grow with the flow* , RipRap V40, 2017, pp 16-18, Australian River Restoration Centre, Canberra	Article for the RipRap magazine	V1. Conceptualisation	V1.3 Reporting	Appendix V1.4 http://ewkr.com.au/grow-with-the-flow/ Copies of the RipRap magazine can be obtained from the Australian River Restoration Centre
External workshop notes	Nov2015_WorkshopInfo_circulated	Folder containing: 0. Email/cover letter 1. Workshop Agenda Nov 2015 2. Workshop summary 3. Workshop notes 3a. Guiding principles 3b. Additional recruitment notes 4. Workshop participation list 5. Metadata spreadsheet P1 - P9. Pdf copies of 9 presentations given at the workshop by different participants	V2. Data integration and synthesis	V2.1 Planning and data workshop	Centre for Freshwater Ecosystems, La Trobe University, online server G:\SHE - Life Sciences\MDFRC\Projects\DSEWPC\465 MDB EWKR\Themes\Vegetation\4. Coordination_Reporting\End_Project_Reports\Theme_summary\V2 DISC\Nov2015_WorkshopInfo_circulated
Dataset	DISC Dataset 1: Combined TLM understorey data	Collated, formatted and quality checked dataset combining understorey data from The Living Murray program from Hattah Lakes, Lindsay-Mulcra-Wallpolla Islands, Chowilla Floodplain, Gunbower Forest, Koondrook-Perricoota Forest	V2. Data integration and synthesis	V2.2 Data collation	Centre for Freshwater Ecosystems, La Trobe University, online server G:\SHE - Life Sciences\MDFRC\Projects\DSEWPC\465 MDB EWKR\Themes\Vegetation\4. Coordination_Reporting\End_Project_Reports\Theme_summary\V2 DISC\DISC_Dataset1_Combined_Final_May2019 See also Appendix V5.1, Theme data inventory
Dataset	DISC Dataset 2: Hattah Lakes TLM understorey wetland data	Formatted and quality checked dataset for Hattah Lakes TLM understorey wetland data	V2. Data integration and synthesis	V2.3 Data analysis	Centre for Freshwater Ecosystems, La Trobe University, online server G:\SHE - Life Sciences\MDFRC\Projects\DSEWPC\465 MDB EWKR\Themes\Vegetation\4. Coordination_Reporting\End_Project_Reports\Theme_summary\V2 DISC\DISC_Dataset2_HattahWL_Final_May2019 See also Appendix V5.1, Theme data inventory
Scientific publication	James et al. (draft). *Disentangling flow-vegetation relationships and legacy effects to inform environmental flows.* Target journal Ecological Applications	Paper investigating the relative importance of hydrological and climate variables, both separately and in combination with each other, on the response of wetland and dryland plant species.	V2. Data integration and synthesis	V2.4 Reporting	Appendix V2.1 When published it will be available via the journal
Model	DISC Vegetation response model	Code developed to model the response of vegetation to a range of hydrological and climate variables. Model can be applied to other datasets, given data can be formatted in a particular way and required hydrological and climate variables are available	V2. Data integration and synthesis	V2.3 Data analysis	Centre for Freshwater Ecosystems, La Trobe University, online server All EWKR R scripts are available at: https://github.com/CassieJames See also Appendix V5.1, Theme data inventory
Technical report	Data integration and synthesis Research Activity Report	Plain english description of work undertaken in the DISC component	V2. Data integration and synthesis	V2.4 Reporting	Appendix V2.2
Technical report	Field Assessment Experimental Design report	Internal methods document to ensure consistency	V3. Field site assessments and germination trials	V3.1 Field work planning	Appendix V3.1
Dataset	EWKR Field Dataset 1: Extant field site assessment	Collated field data collected from 180 sites, across four locations: Lower Murray, Mid Murray, Macquarie Marshes and Narran Lakes	V3. Field site assessments and germination trials	V3.2 Field surveys	Centre for Freshwater Ecosystems, La Trobe University, online server G:\SHE - Life Sciences\MDFRC\Projects\DSEWPC\465 MDB EWKR\Themes\Vegetation\4. Coordination_Reporting\End_Project_Reports\Theme_summary\V3 Field and germination\EWKR_Field_Dataset1_Extant_Final_April2019 See also Appendix V5.1, Theme data inventory
Dataset	EWKR Field Dataset 2: Seed bank germination trials	Collated germination trial data from 180 sites, across four locations: Lower Murray, Mid Murray, Macquarie Marshes and Narran Lakes	V3. Field site assessments and germination trials	V3.2 Field surveys	Centre for Freshwater Ecosystems, La Trobe University, online server G:\SHE - Life Sciences\MDFRC\Projects\DSEWPC\465 MDB EWKR\Themes\Vegetation\4. Coordination_Reporting\End_Project_Reports\Theme_summary\V3 Field and germination\EWKR_Field_Dataset2_Seedbank_Final_30April2019 See also Appendix V5.1, Theme data inventory
Presentation	Campbell et al 2018. *From the four corners of the Basin: assessing vegetation responses to flow regimes* . Ecological Society of Australia conference, Brisbane, 25-29 November 2018.	Pdf copy of slides presented at the conference	V3. Field site assessments and germination trials	V3.3 Reporting	Appendix V3.2
Scientific publication	Campbell et al (draft). *Vulnerability of resilient systems to the Anthropocene.* Target journal Global Change Biology	Paper describing the influence of location, flood return frequency and vegetation class on the composition and abundance of seed bank vegetation.	V3. Field site assessments and germination trials	V3.3 Reporting	Appendix V3.3

Output type	Output title / reference	Brief description	Work Component	Activity	Availability
Technical report	Field site assessment and germination trials Research Activity Report	Plain english description of work undertaken in the field and germination component	V3. Field site assessments and germination trials	V3.3 Reporting	Appendix V3.4
Technical report	Recruitment of long-lived floodplain vegetation: literature report	Initial literature review to help inform design of the seedling mesocosm experiments	V4. Seedling mesocosm studies	V4.1 Mesocosm planning	Appendix V4.1
Technical report	Recruitment of long-lived floodplain vegetation: mesocosm study experimental design	Internal methods document	V4. Seedling mesocosm studies	V4.1 Mesocosm planning	Appendix V4.2
Dataset	Mesocosm Dataset 1: Seedling mesocosm experiments	Data from three floodplain eucalypt species, five watering treatments and nine monitored variables	V4. Seedling mesocosm studies	V4.2 Seedling experiments	Centre for Freshwater Ecosystems, La Trobe University, online server G:\SHE - Life Sciences\MDFRC\Projects\DSEWPC\465 MDB EWKR\Themes\Vegetation\4. Coordination_Reporting\End_Project_Reports\Theme_summary\V4 Seedling mesocosm\Mesocosm_Dataset1_Final_April2019 See also Appendix V5.1, Theme data inventory
Video	StorySpace video	Video depicting the mesocosm experiments	V4. Seedling mesocosm studies	V4.2 Seedling experiments	Video is avaiable on the EWKR StorySpace website http://ewkr.com.au/valiant-vegetation/
Presentation	Durant et al 2018. *Early, late or constant – what are long-lived woody floodplain seedlings looking for?* 58th Australian Freshwater Sciences Society Congress, Adelaide, 23-28 September 2018	Pdf copy of slides presented at the conference	V4. Seedling mesocosm studies	V4.3 Data analysis and reporting	Appendix V4.3
Article	*Giving woody seedlings a fighting start* , RipRap V40, 2017, pp 19-20, Australian River Restoration Centre, Canberra	Article for the RipRap magazine	V4. Seedling mesocosm studies	V4.3 Data analysis and reporting	Appendix V4.4 http://ewkr.com.au/giving-woody-seedlings-a-fighting-start/ Copies of the RipRap magazine can be obtained from the Australian River Restoration Centre
Scientific publication	Campbell et al (draft). *Establishment strategies of dominant trees of highly variable floodplains. T* arget journal, Journal of Experimental Botany	Paper describing the establishment strategies of three dominant floodplain eucalypt species to different watering treatments and the implications for management of recruitment for these species	V4. Seedling mesocosm studies	V4.3 Data analysis and reporting	Appendix V4.5
Technical report	Seedling Mesocosm Research Activity Report	Plain english description of work undertaken in the seedling mesocosm component	V4. Seedling mesocosm studies	V4.3 Data analysis and reporting	Appendix V4.6
Dataset	Vegetation Theme Coordination Dataset 1: Vegetation Theme Data and Model Inventory	Excel spreadsheet describing the datasets and models produced as part of the EWKR Vegetation Theme. Includes a description of the dataset and information about data custodians and availability of the dataset	V5. Theme coordination, leadership and reporting	V5.10 End of project reporting	Appendix V5.1
Dataset	Vegetation Theme Coordination Dataset 2: Vegetation Theme Outputs	Excel spreadsheet describing the outputs produced as part of the EWKR Vegetation Theme. Includes output title, brief description, relevant research component and availability	V5. Theme coordination, leadership and reporting	V5.10 End of project reporting	Appendix V5.2 (this document)
Dataset	Vegetation Theme Coordination Dataset 3: Vegetation Theme Engagement and Communication activities	Excel spreadsheet listing engagement and communication activities undertaken around EWKR Vegetation Theme. Includes formal activities (workshops) as well as more ad hoc activities and capatalising on opportunistic ways to share information and foster cross-project awareness and potential collaboration.	V5. Theme coordination, leadership and reporting	V5.10 End of project reporting	Appendix V5.3
Presentation	Campbell et al 2019. *Vegetation theme: predicting outcomes in response to flow and other drivers.* MDB EWKR end of project Forum, Canberra, 21 March 2019	Pdf copy of slides presented at the final Forum	V5. Theme coordination, leadership and reporting	V5.10 End of project reporting	Appendix V5.4

The Murray-Darling Basin
Environmental Water Knowledge
Research project

Vegetation theme: predicting outcomes in response to flow and other drivers

Cherie Campbell, Sam Capon, Cassie James, Kay Morris, Jason Nicol, Daryl Nielsen, Rachael Thomas, Susan Gehrig

EWKR
Environmental Water Knowledge & Research

LA TROBE UNIVERSITY

CSIRO

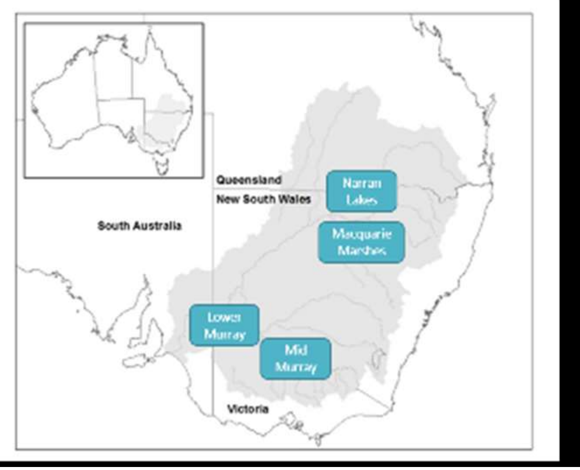
Australian Government
Commonwealth Environmental Water Office

Leadership group

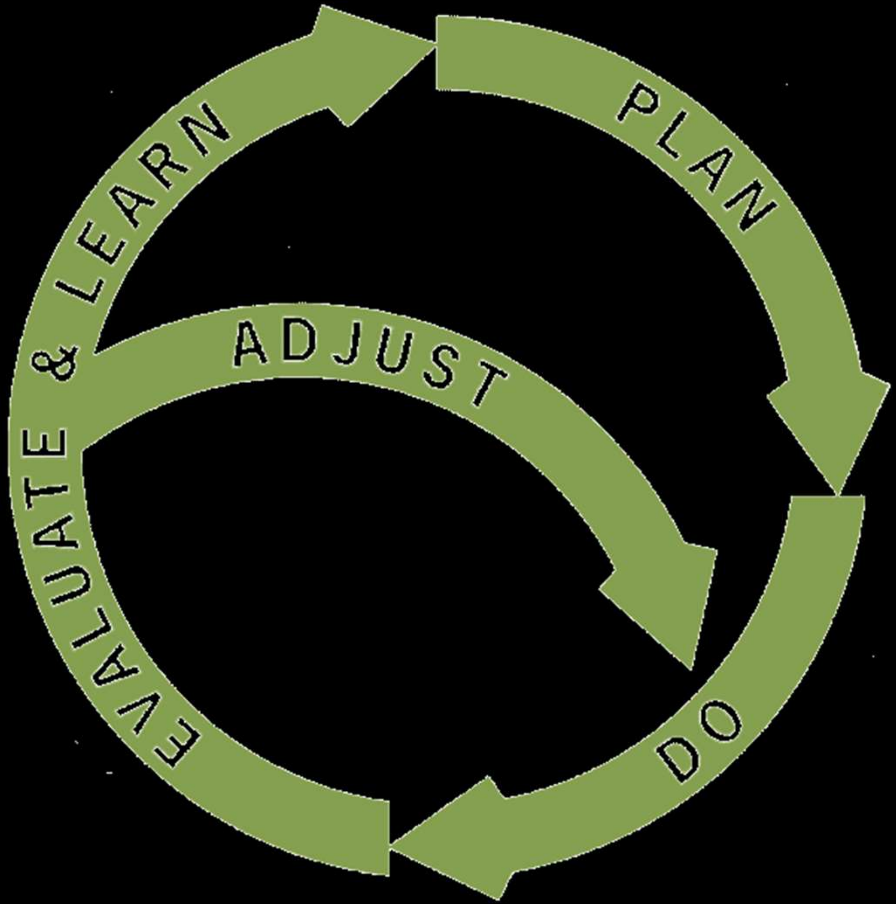
- ARI (DELWP)
- ARI (GU)
- CSIRO
- MDFRC / LTU
- NSW OEH/ UNSW
- SARDI
- TropWater (JCU)

Overarching question

- *What are the drivers of sustainable populations and diverse communities of water-dependent vegetation?*
- Scope
  - Non-woody vegetation
  - Woody recruitment
  - 4 EWKR sites

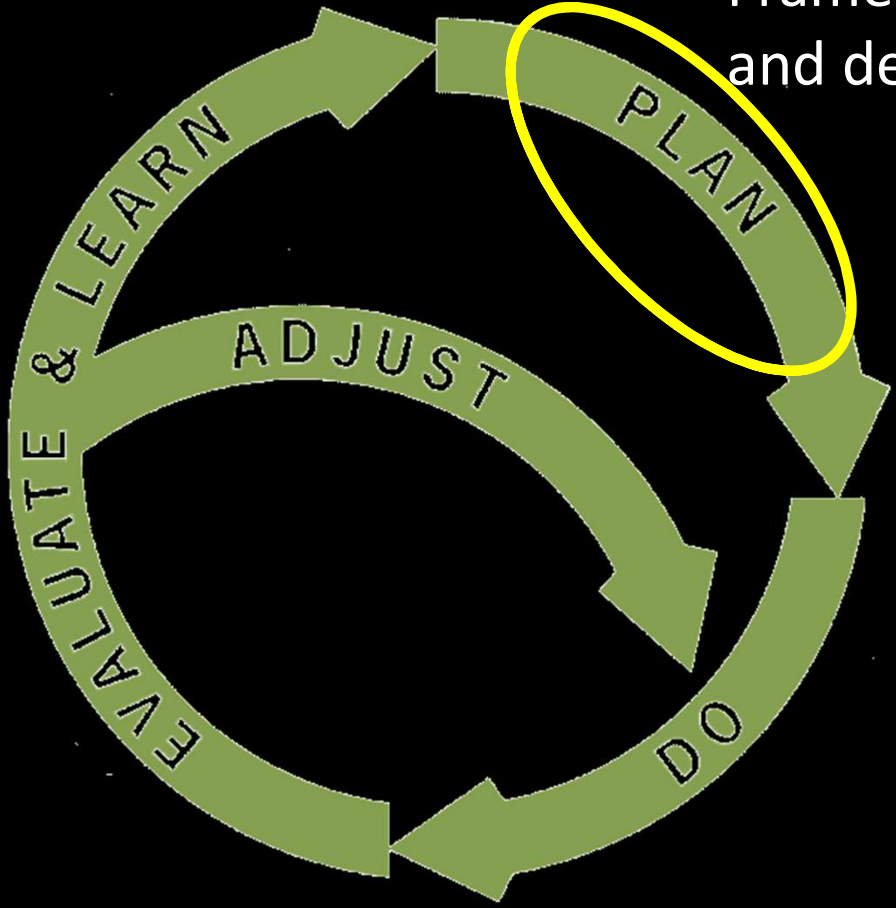


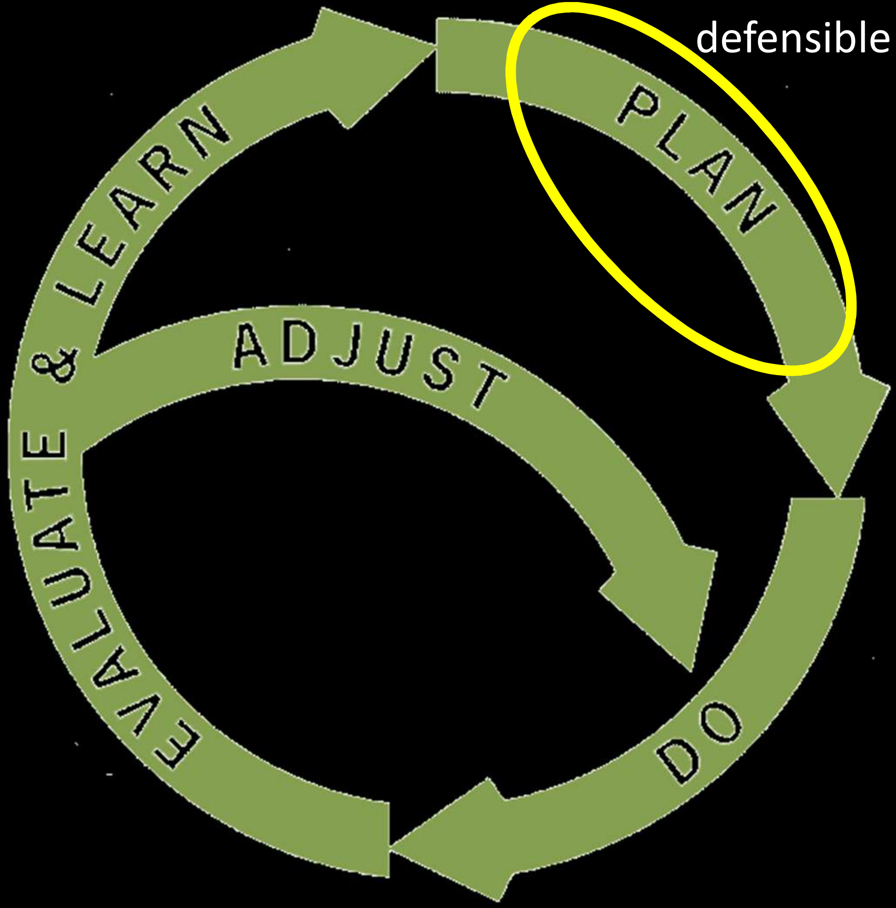
Adaptive environmental water management



How do our EWKR research questions relate to the adaptive management cycle?

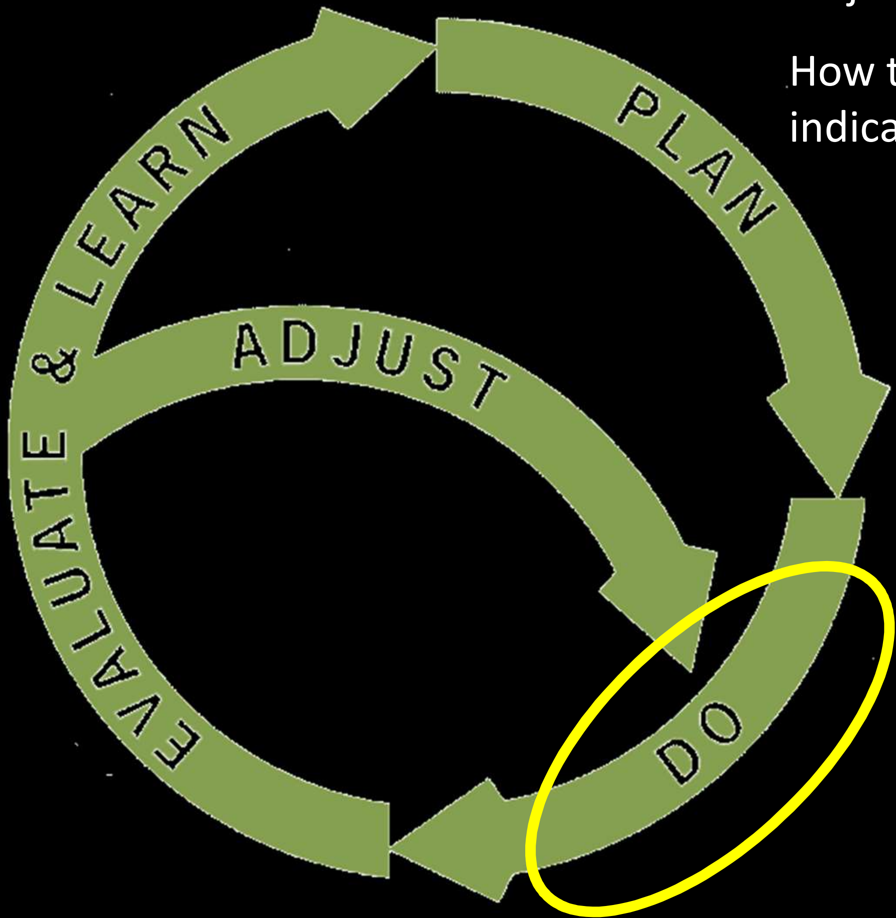
Framework to develop robust and defensible objectives





Framework to develop robust and defensible objectives

How to identify SMART indicators



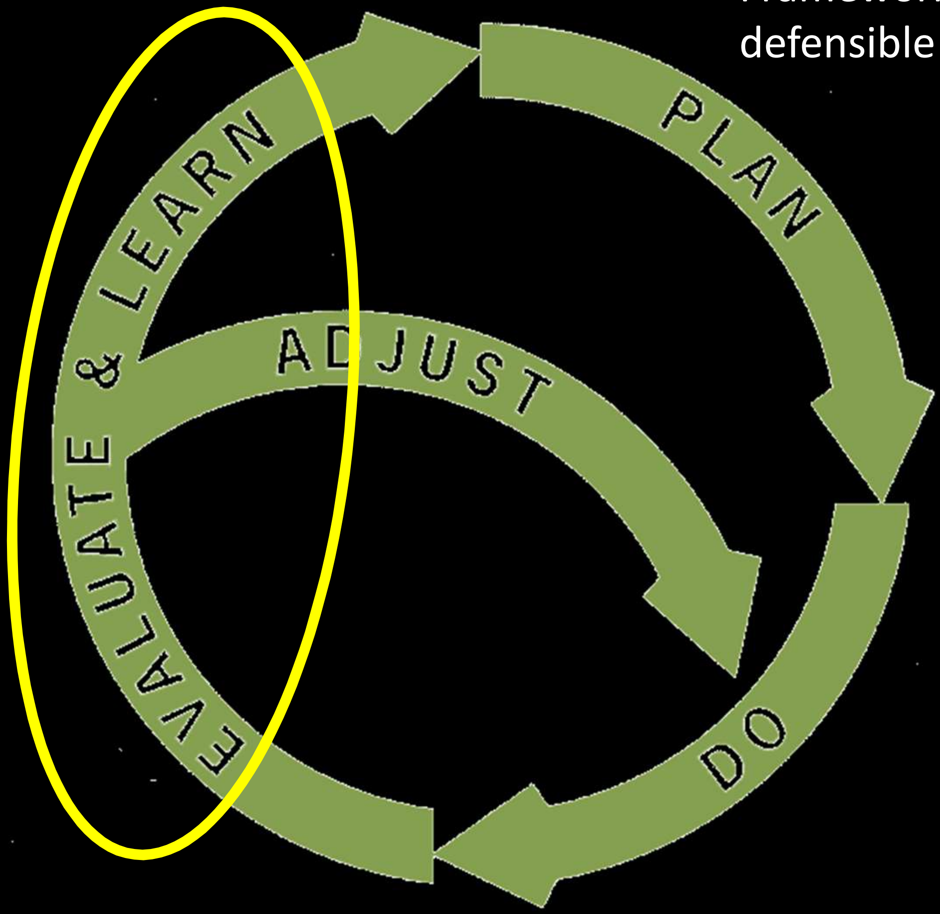
Framework to develop robust and defensible objectives

How to identify SMART indicators

What drives vegetation responses to watering actions?

- Flow regime (pulse, short to long-term)
- Climate
- Vegetation structure
- Soil seed banks

How can we learn more from existing data?



Framework to develop robust and defensible objectives

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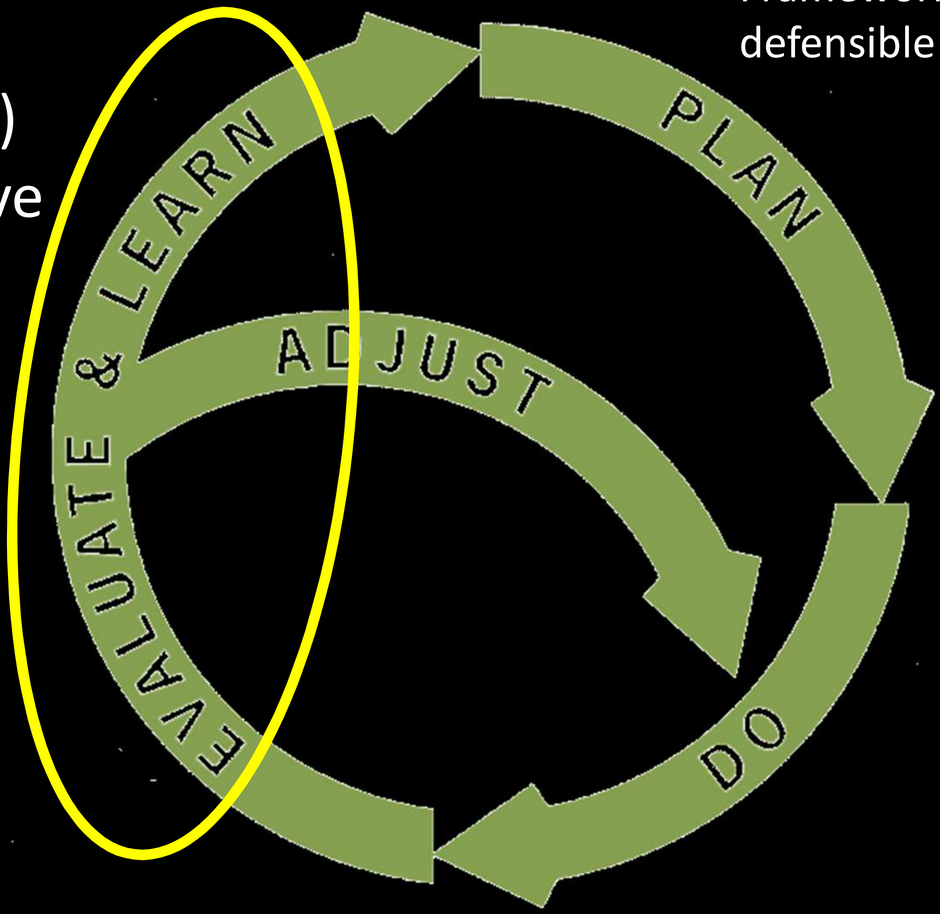

Framework to develop robust and defensible objectives

How to identify SMART indicators

What drives vegetation responses to watering actions?

- Flow regime (pulse, short to long-term)
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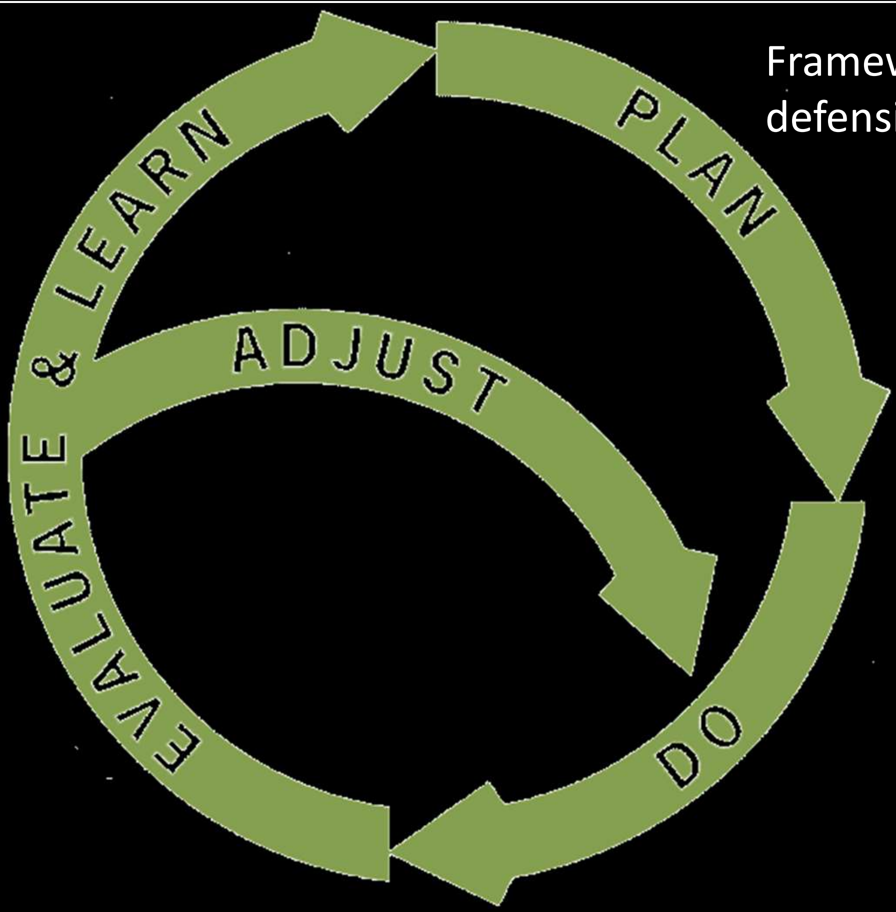
- Soil seed banks

EWKR vegetation theme research questions

How can we best monitor and evaluate (collect and analyse data) to inform adaptive management?

How can we learn more from existing data?



Framework to develop robust and defensible objectives

How to identify SMART indicators

What drives vegetation responses to watering actions?

- Flow regime (pulse, short to long-term)
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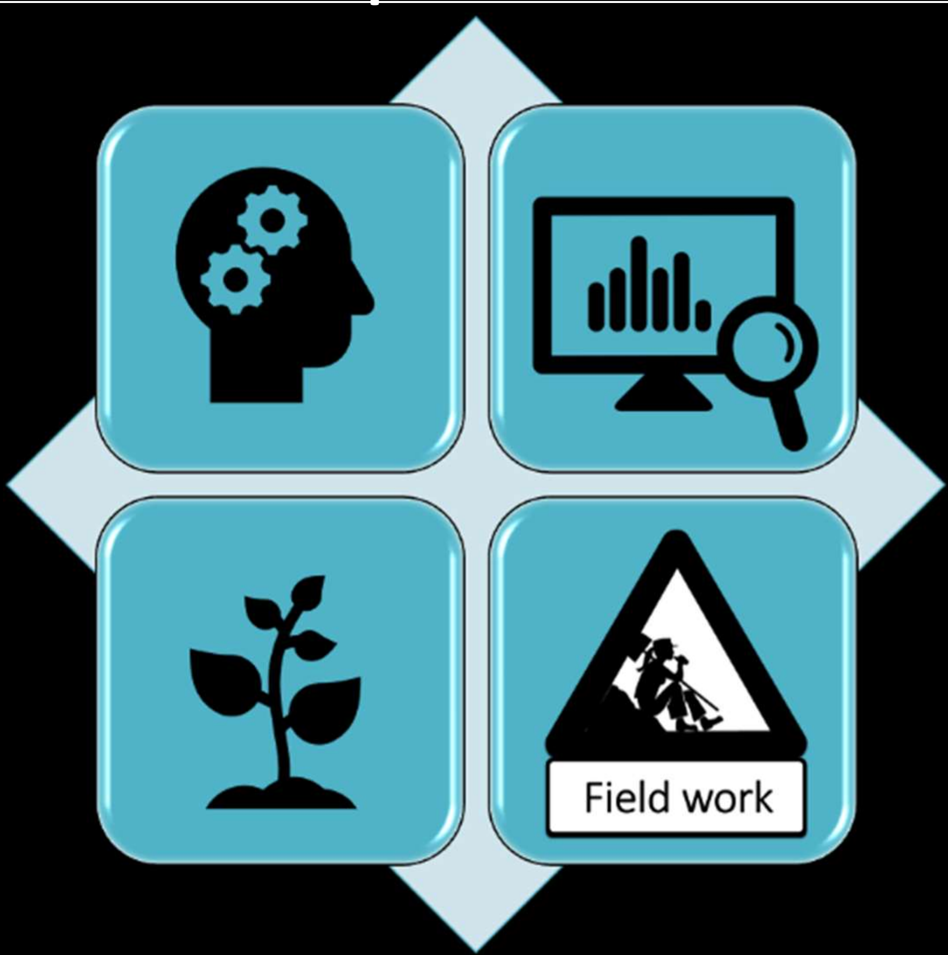
Research components



Water dependent vegetation?



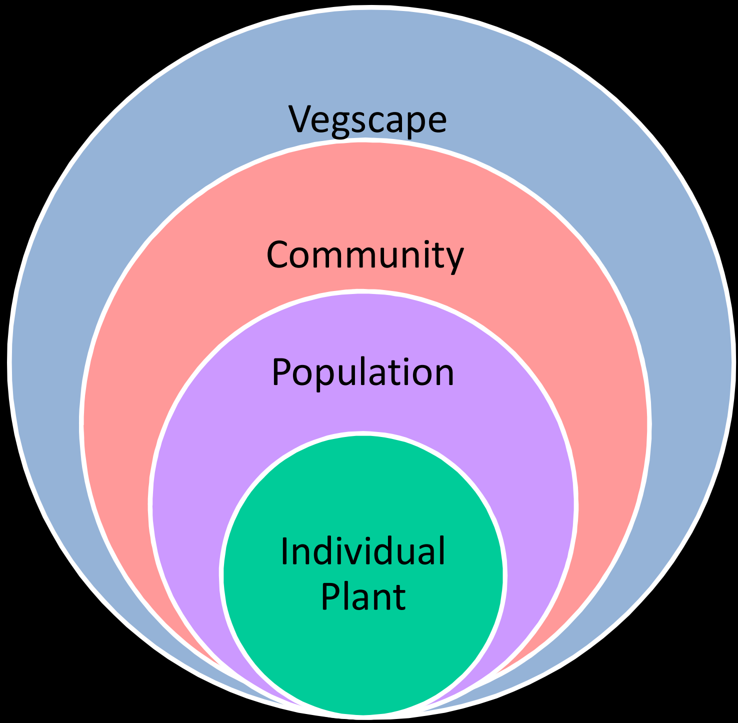



Plan: what are we watering for and why?

- The 'what and the why'
- What does vegetation response mean?

Plan

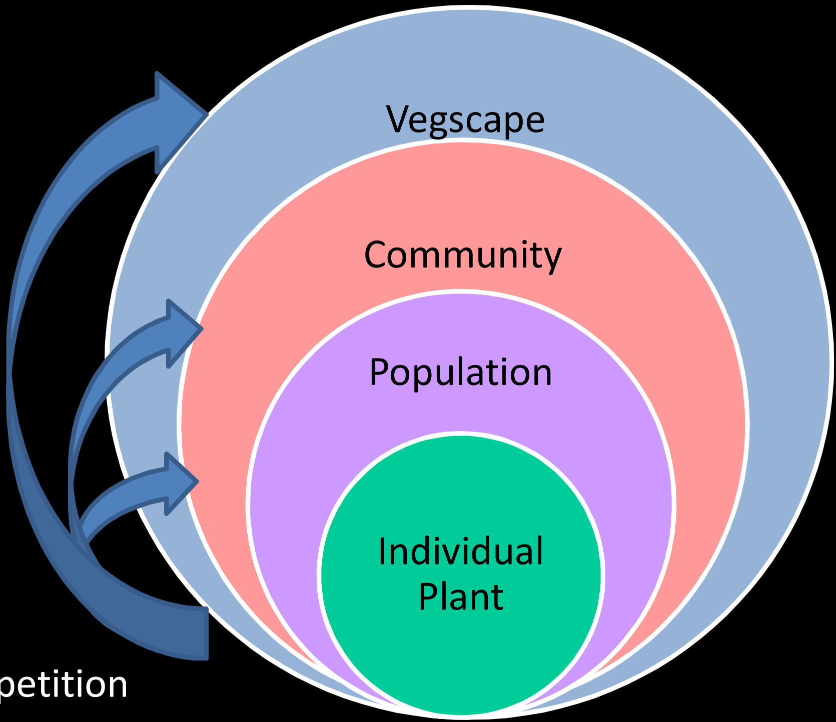
- Function and structure
- Lignum
  - Extent, presence/absence vs structure



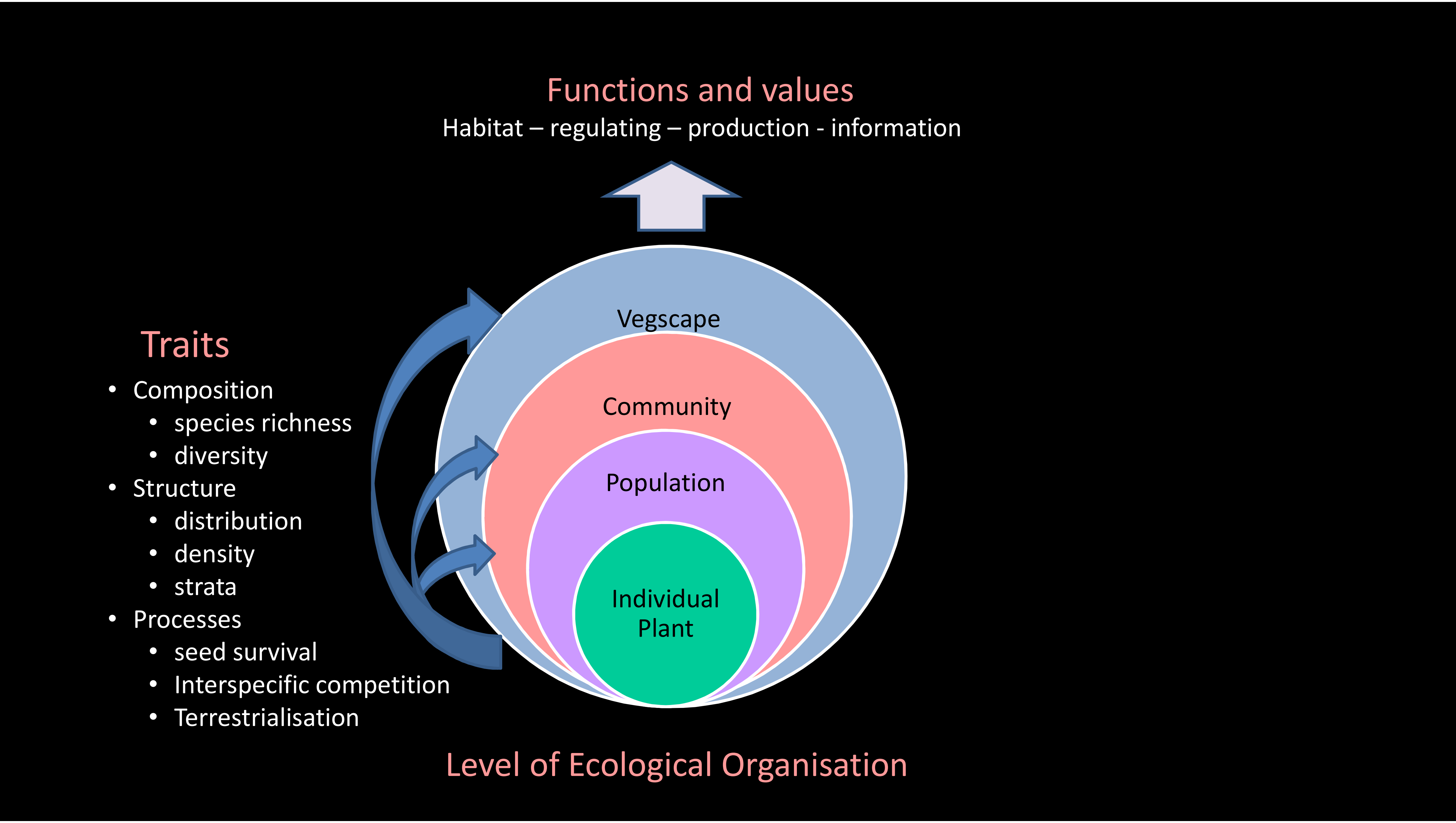
Level of Ecological Organisation

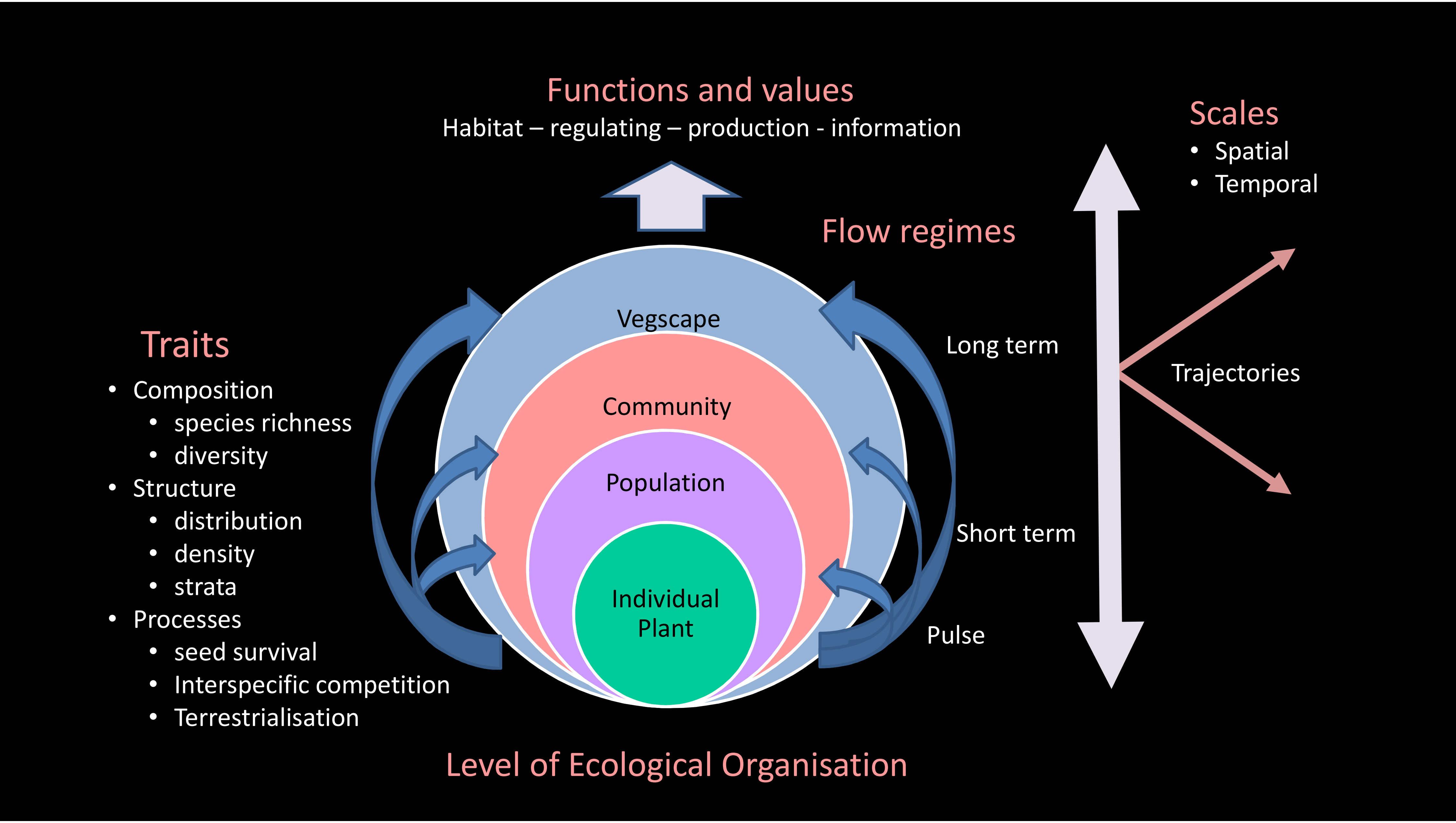
Traits

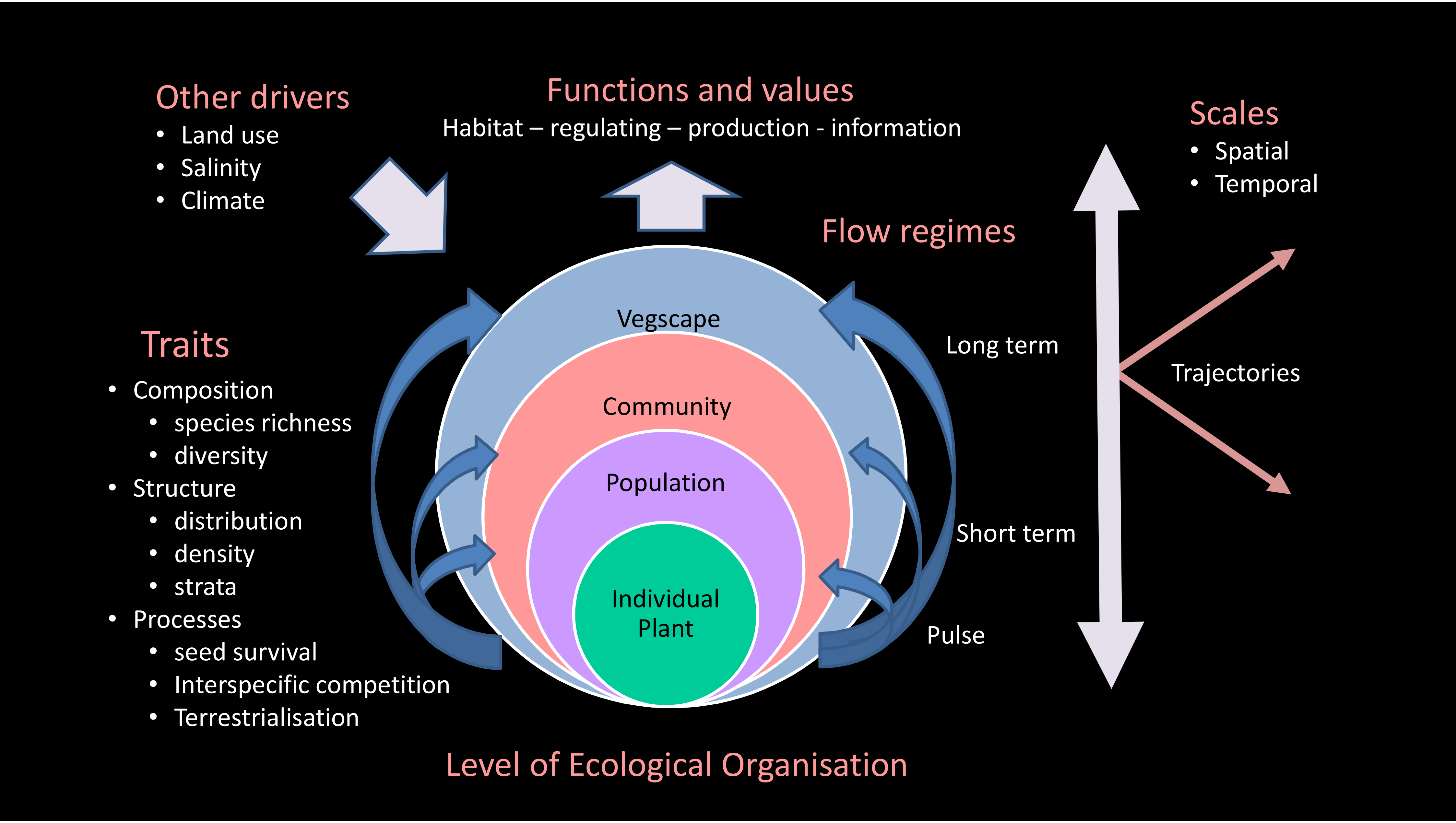
- Composition
    - species richness
    - diversity
- Structure
    - distribution
    - density
    - strata
- Processes
    - seed survival
    - Interspecific competition
    - Terrestrialisation



Level of Ecological Organisation


Functions and values
Habitat – regulating – production - information
Traits
• Composition
• species richness
• diversity
• Structure
• distribution
• density
• strata
• Processes
• seed survival
• Interspecific competition
• Terrestrialisation
Vegscape
Community
Population
Individual Plant
Level of Ecological Organisation


Functions and values
Habitat – regulating – production - information
Scales
• Spatial
• Temporal
Flow regimes
Traits
• Composition
• species richness
• diversity
• Structure
• distribution
• density
• strata
• Processes
• seed survival
• Interspecific competition
• Terrestrialisation
Vegscape
Community
Population
Individual Plant
Long term
Short term
Pulse
Trajectories
Level of Ecological Organisation


Other drivers
Land use
Salinity
Climate
Functions and values
Habitat – regulating – production - information
Scales
Spatial
Temporal
Flow regimes
Traits
Composition
species richness
diversity
Structure
distribution
density
strata
Processes
seed survival
Interspecific competition
Terrestrialisation
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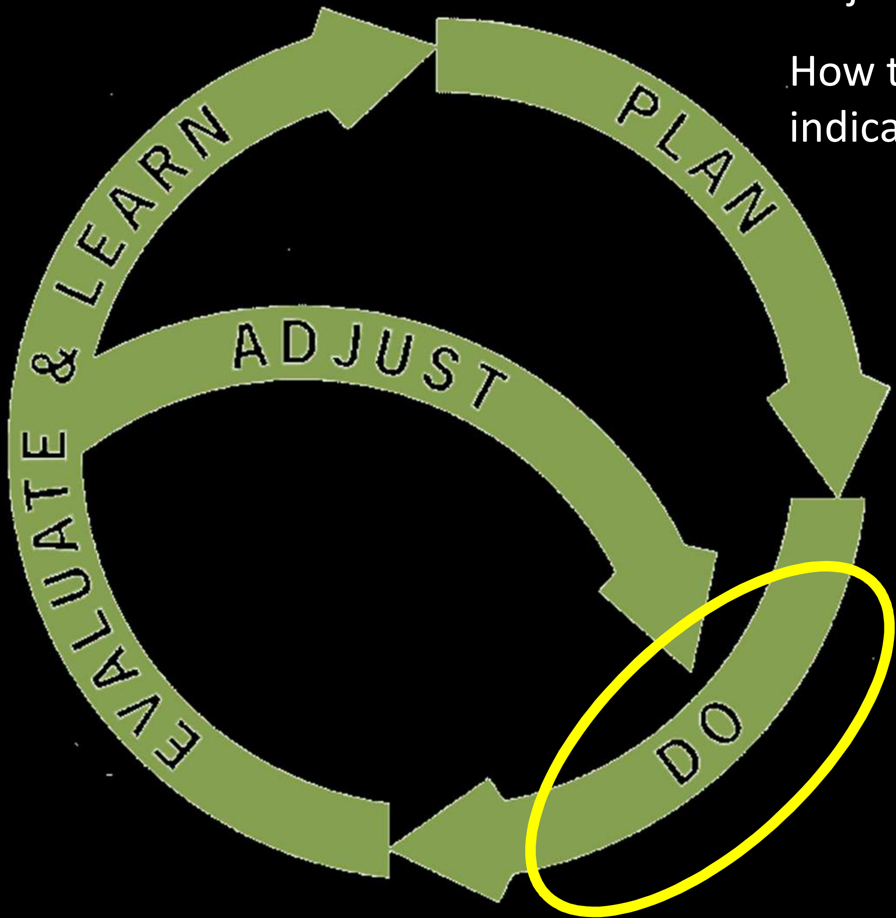
Level of Ecological Organisation

Framework to develop robust and defensible objectives

How to identify SMART indicators

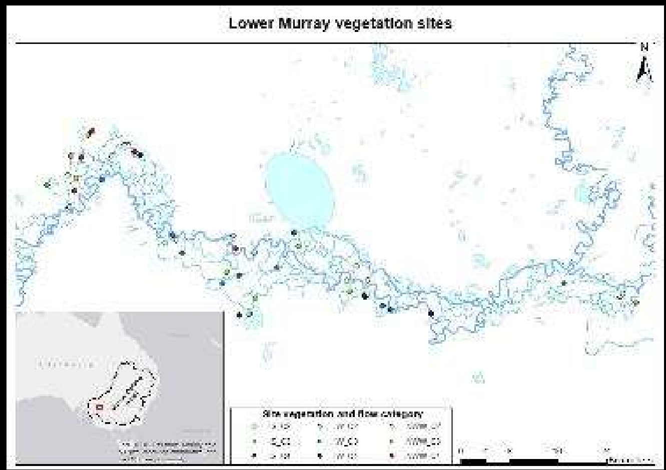


What drives vegetation responses to watering actions?

- Flow regime (pulse, short to long-term)
- Climate
- Vegetation structure
- Soil seed banks

Do

- What drives vegetation responses to watering actions?
  - Flow regime (pulse, short to long-term,)
  - Climate
  - Vegetation structure
  - Soil seed banks



Do: woody seedling responses

- Vulnerable stage, key to distribution and population sustainability
- Woody seedlings are sparse, patchy and variable in space and time
  - Not a clear relationship with flood history
  - Existing canopy may limit recruitment
  - No resident soil seed bank
- Influence of flood pulse

Do: woody seedling responses

- Seedling establishment in response to 5 watering treatments (dry, wet, alternating)
- Three species
  - River Red Gum
  - Black Box
  - Coolibah

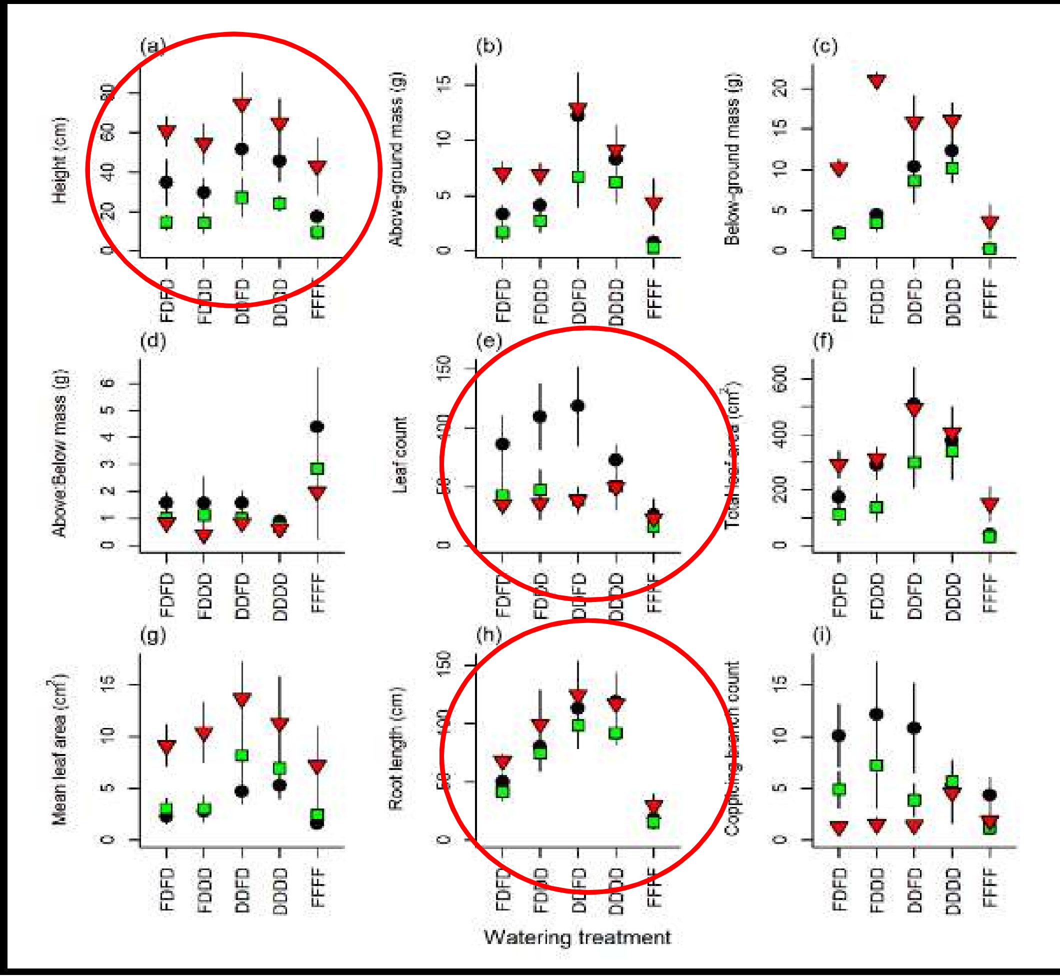
Do: Seedling mesocosm results

- Measured individual traits
  - Height, root length, biomass

River Red Gum

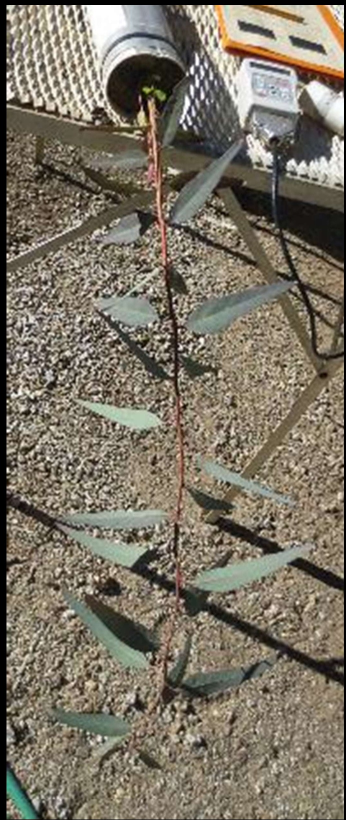
Black Box

Coolibah



Do: Seedling mesocosm results

- Strategies reflect distribution and likely inundation regime
- Different strategies for the 3 species



Do: Seedling mesocosm results

- Constant flooding suppresses growth
  - But very flood tolerant
  - For control need to flood very early in their life (~3 months)

Do: Seedling mesocosm results

- Importance of inter-flood dry period
  - Provide a dry period following germination to enable root development and growth

Do: Seedling mesocosm results

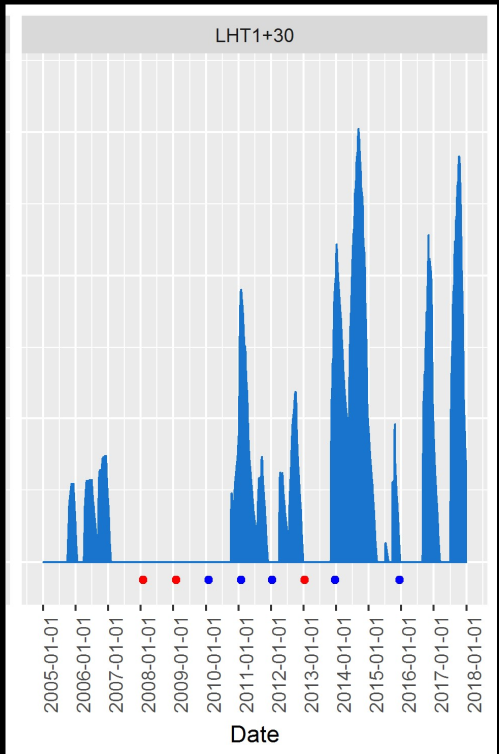
- Coolibah and Black Box more sensitive to the timing of floods
  - Did better under a later flood
  - Allow a dry period of 6 months or so before top up flooding

Do: non-woody vegetation response

- What drives non-woody vegetation responses to watering actions?
  - Watering history (flow pulse, short to long-term,)
  - Climate
  - Vegetation structure
  - Soil seed banks
- We did this through multi-lines of evidence
  - Big Data synthesis and analysis
  - Field surveys and germination trials

Do: non-woody vegetation response

- Can we use existing long-term data to determine the influence of flow and climate history on vegetation responses?



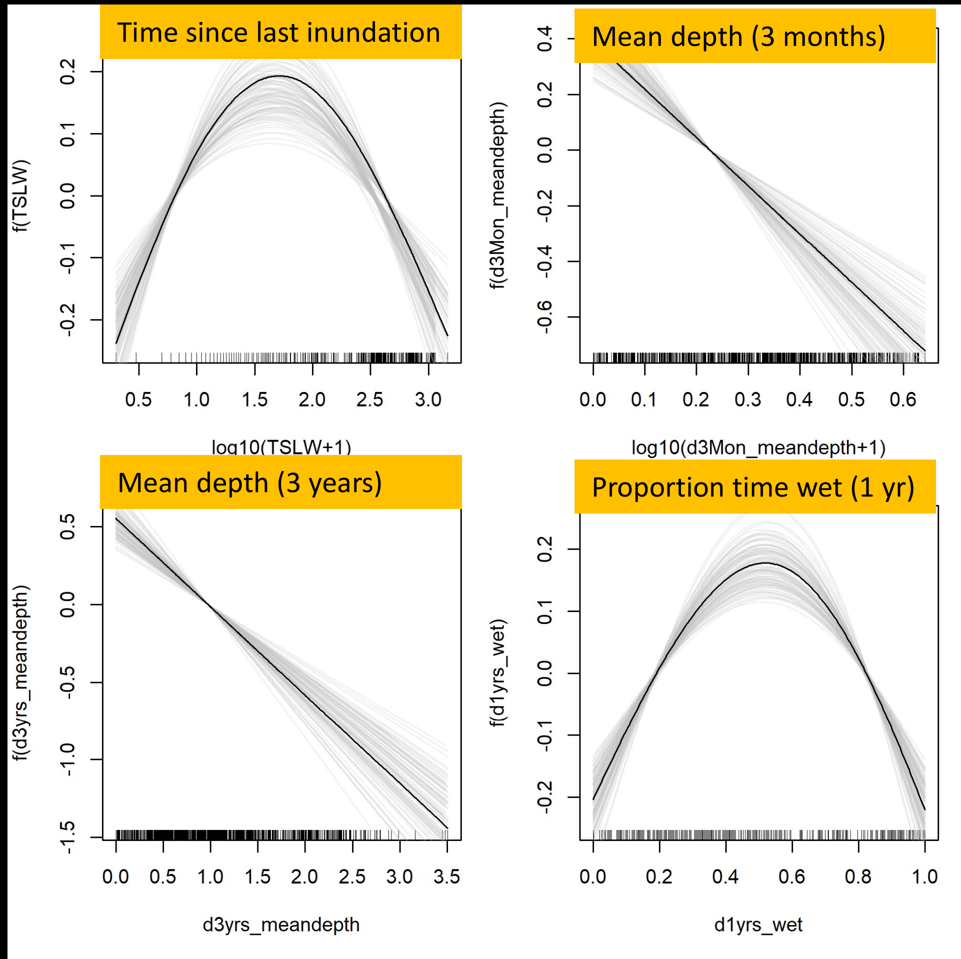
Hattah Lakes TLM data 2008-16

Do: non-woody vegetation response

Wetland plants

- Water depth
- Time-since-last inundation
- Proportion time wet

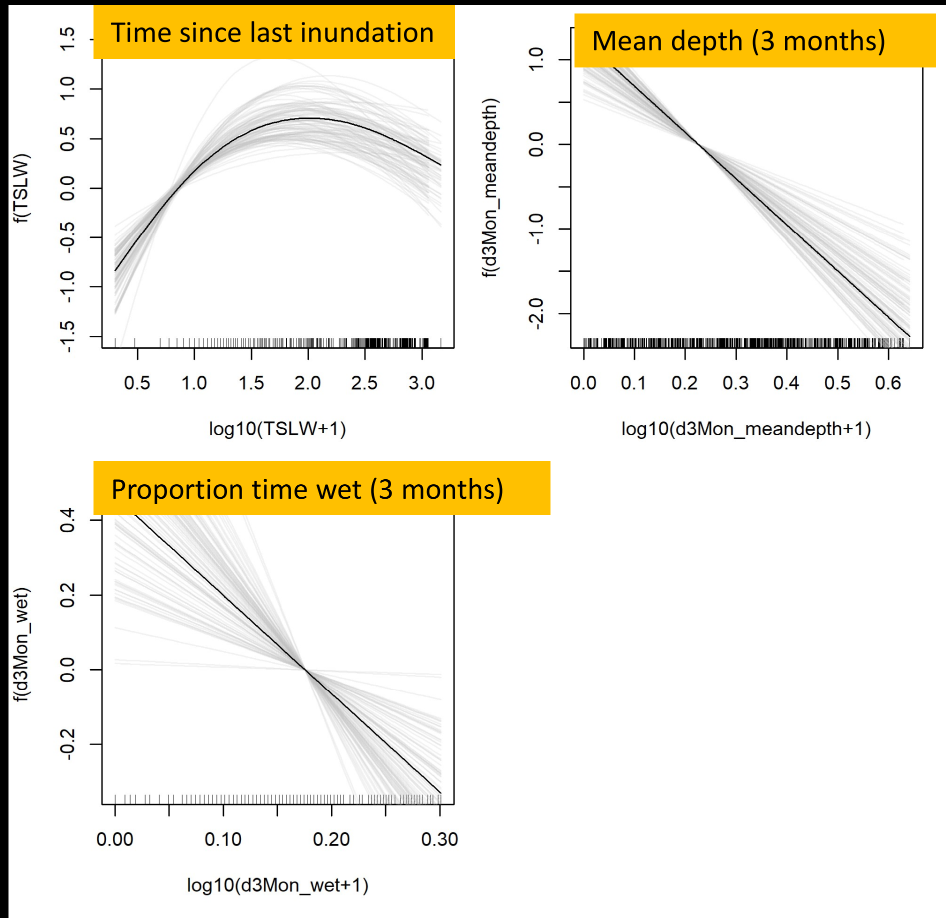
- Recent (3 months) and short-term regimes (3 years) most important



Do: non-woody vegetation response

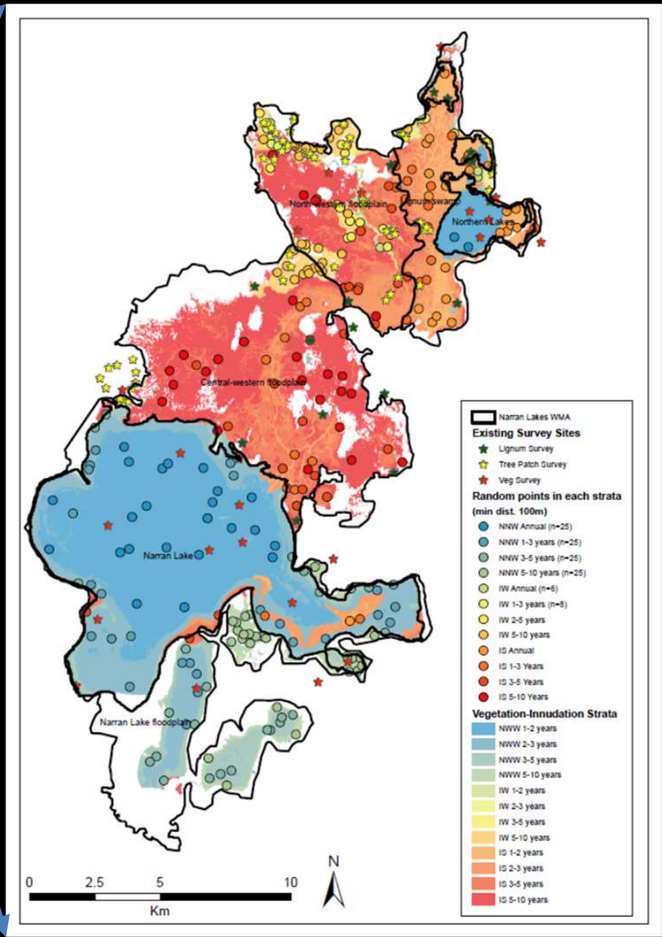
- Terrestrial plants
  - Strong negative influence of recent inundation
  - Non-linear relationship with time-since-last inundation

- Recent regime (3 months) most important



Do: non-woody vegetation response

- 4 wetland systems
  - 180 sites
  - Autumn 2017, 2018
- 4 flood frequencies
  - Near annual (Cat 1)
  - 1.5-3 years (Cat 2)
  - 3-5 years (Cat 3)
  - 5-10 years (Cat 4)
- 3 vegetation structural types
  - Non-woody wetlands (NWW)
  - Inland shrublands (IS)
  - Inland woodlands (IW)

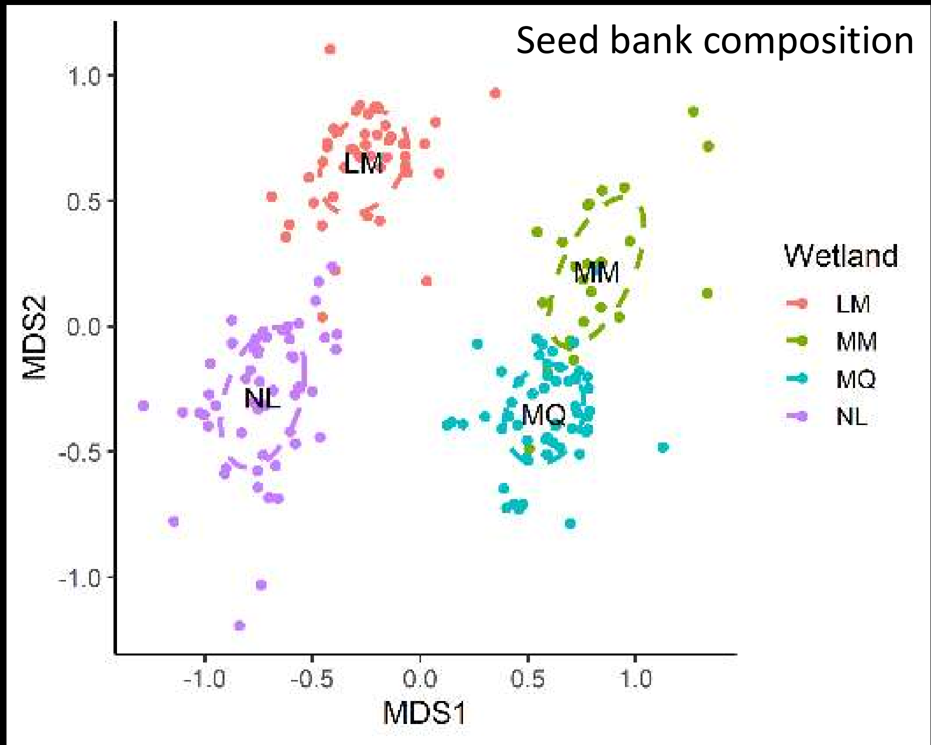


Do: non-woody vegetation response

Do: non-woody vegetation response

Field work

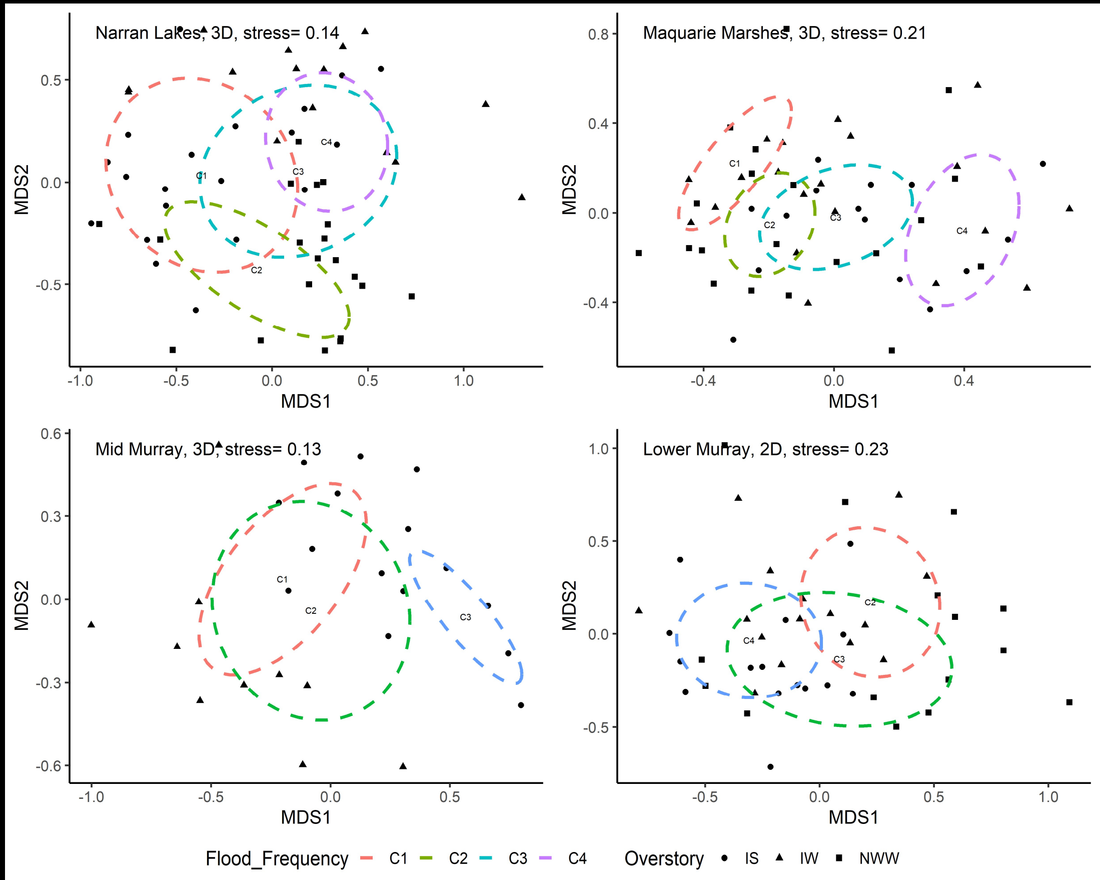
- Outcomes
  - Overwhelming influence of location



Do: non-woody vegetation response

- Outcomes
  - Within locations there are different influences

Location	Recent conditions	Flood Frequency	Vegetation structure
Macquarie Marshes	Complete flooding	Strong	Weak
Narran Lakes	No recent flooding	Weak	Strong
Mid Murray	Partial flooding	Moderate	Strong
Lower Murray	Partial flooding	Weak	Weak



Do: non-woody vegetation response

- Outcomes
  - Within locations there are different influences

Location	Recent conditions	Flood Frequency	Vegetation structure
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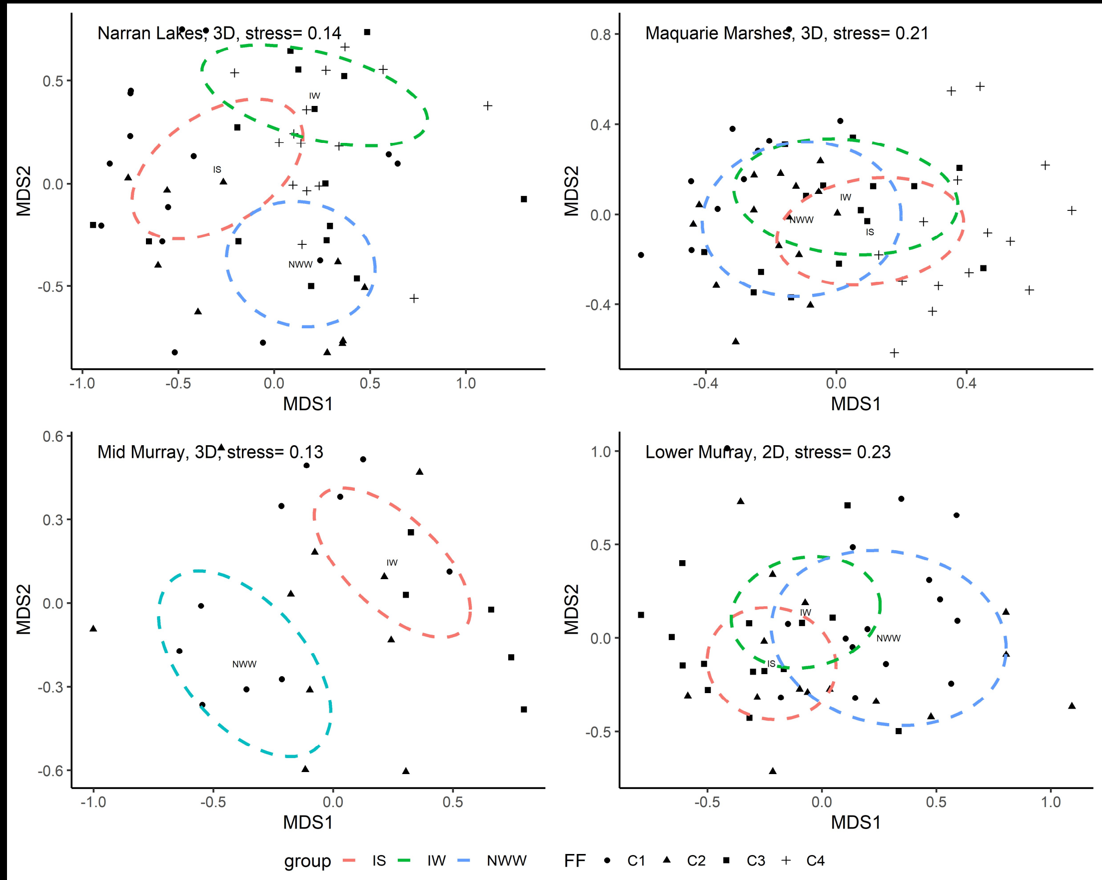
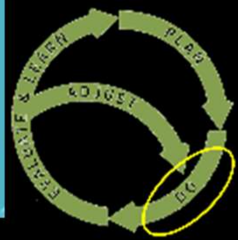


Do: non-woody vegetation response

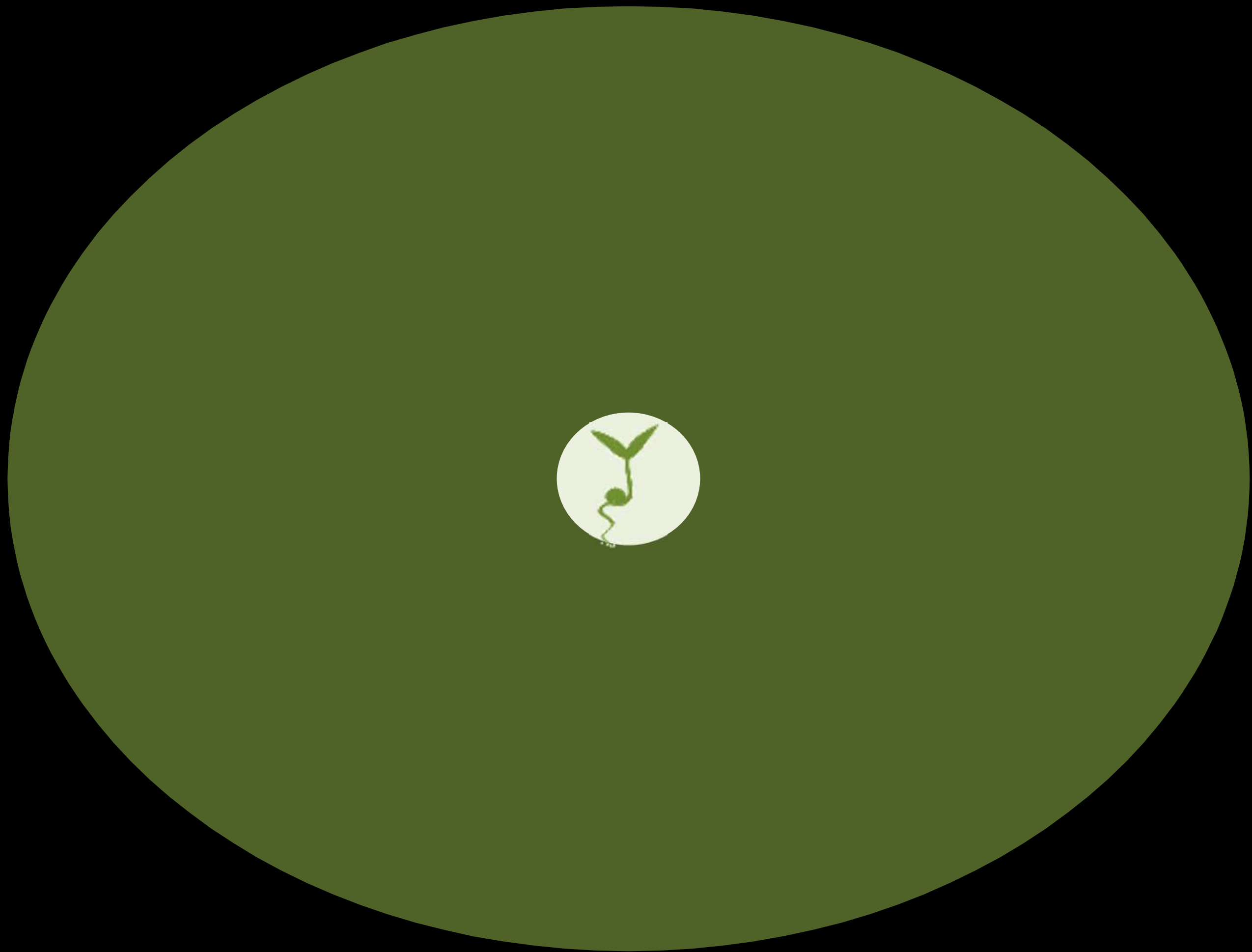
Basin

Do: non-woody vegetation response

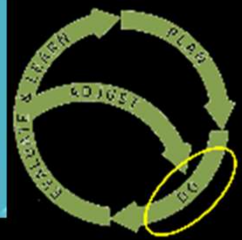


Basin

Local



Do: non-woody vegetation response



Basin

Local

Location

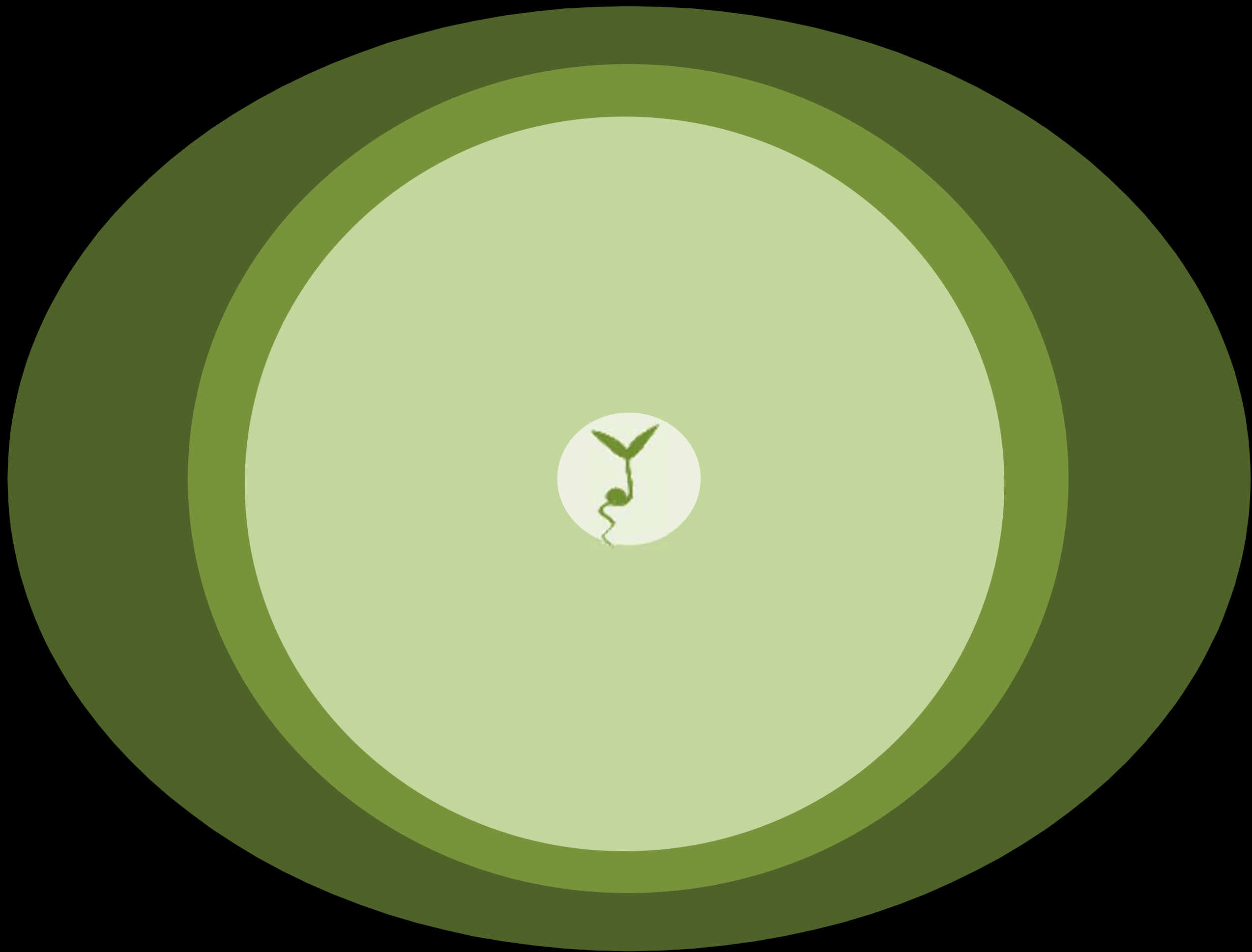
Do: non-woody vegetation response

Basin

Local

Location

Recent flow regime



Do: non-woody vegetation response

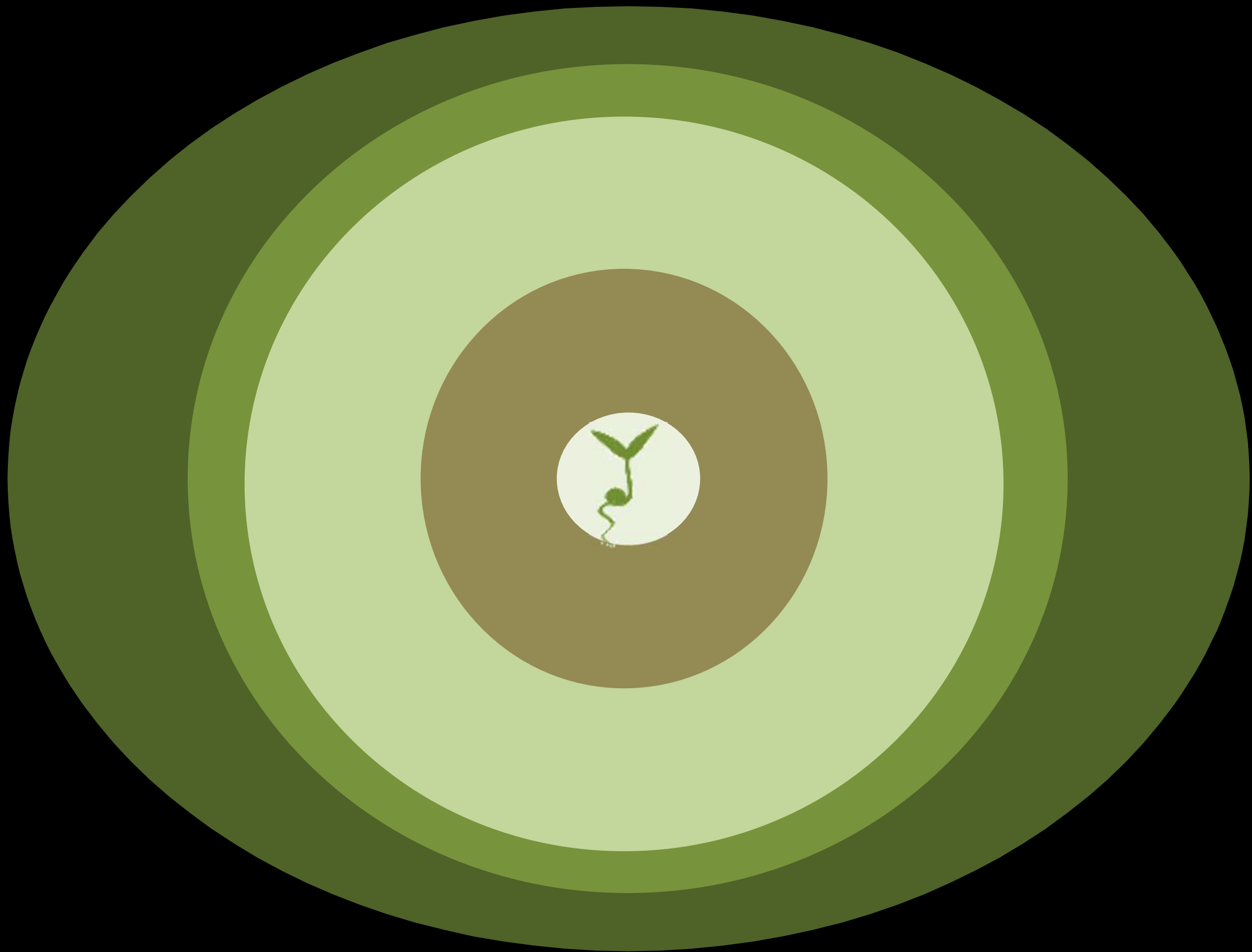
Basin

Local

Location

Recent flow regime

Vegetation structure



Dry in the medium term regime

Do: non-woody vegetation response

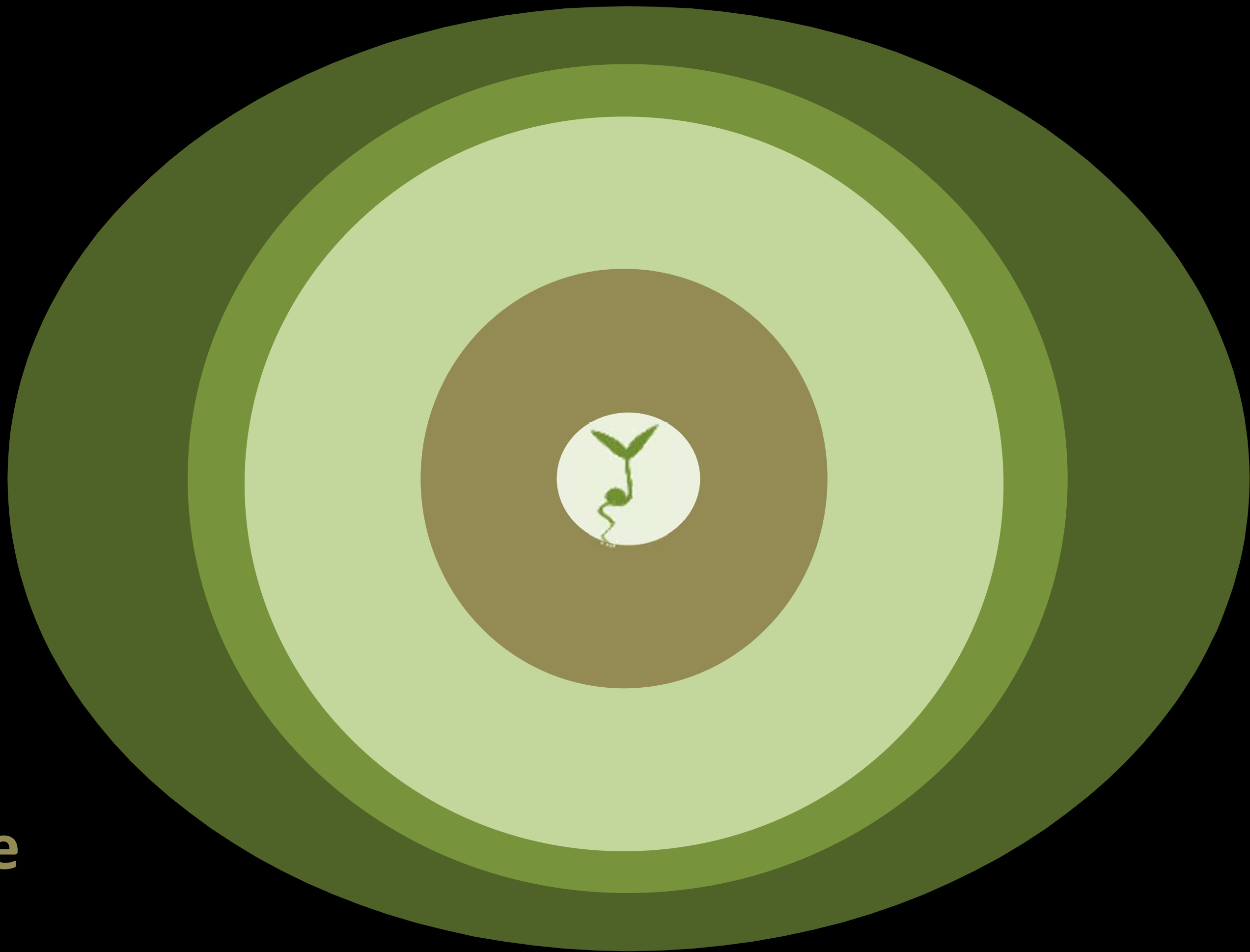
Basin

Local

Location

Recent flow regime

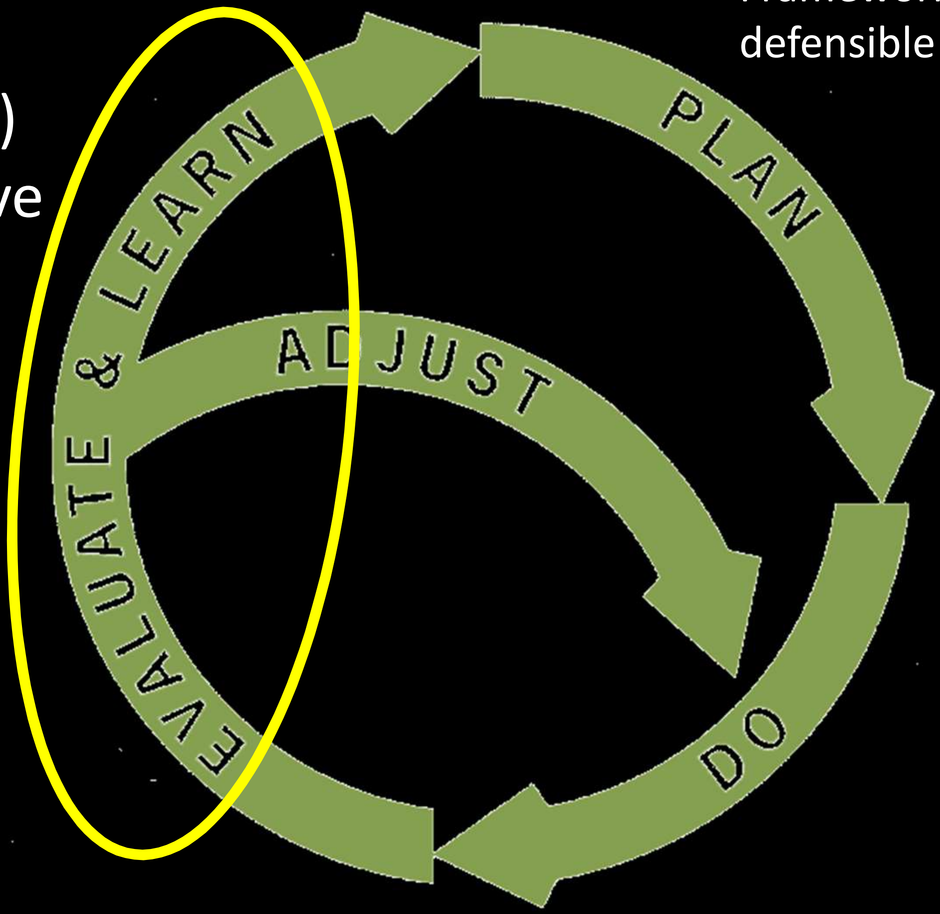
Medium to long term flow regime



Wet in the medium term regime

How can we best monitor and evaluate (collect and analyse data) to inform adaptive management?

How can we learn more from existing data?



Framework to develop robust and defensible objectives

How to identify SMART indicators

What drives vegetation responses to watering actions?

- Flow regime (pulse, short to long-term)
- Climate
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- Soil seed banks

Evaluate and learn

- Consistent classification: e.g. species, communities, vegscape
- Align and develop response indicators

- Complementary data
- Analytical know-how
- Data management

- Traits and strategies

- Consistent approach to data collection
- Sampling protocols

EWKR babies

Acknowledgements:

- The support and inspiration provided by the broader project teams
- Numerous administrative, technical and academic staff, agency staff, and land managers for assistance with: site selection, property access, field data collection, experimental trials, data entry, data analysis, access to complementary data, write-up and communication and project management

Thankyou

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NSW GOVERNMENT Office of Environment & Heritage

Griffith UNIVERSITY

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SARDI SOUTH AUSTRALIAN RESEARCH AND DEVELOPMENT INSTITUTE

UNSW SYDNEY

Future questions

- Test the framework with a diversity of water decision makers
- Develop decision support tools
- Better understand relationships between structure, function and values for different vegetation responses
- Basin-wide inundation mapping (what Rachael does)
- Transferability of predictive relationships (DISC)
- Traits and strategies for a range of wetland-floodplain plant species
- Limits to resilience and key vulnerabilities (e.g. climate change)