The influence of flooding on the vegetation of the semi-arid floodplain of the lower Lachlan River

William Higgisson

2019

A thesis submitted in fulfillment of the requirements for the degree of Doctor of Philosophy in Applied Science

Institute for Applied Ecology

University of Canberra, Australia

Acknowledgements

Firstly, I want to thank my supervisors Fiona Dyer and Dianne Gleeson for giving me the opportunity to undertake this project, for allowing me the freedom to undertake research I found interesting and important, and for the time and energy you have invested throughout my PhD. I would like to acknowledge that undertaking field work associated with the Commonwealth Environmental Water, Long Term Intervention Monitoring Program (LTIM) within the Lower Lachlan Catchment gave me the opportunity to undertake this PhD that otherwise would not have been possible.

Thanks to Linda Broadhurst for the time and energy she invested into the initial stages of my PhD and later for manuscript revision. Thanks to Sue Briggs for her tireless effort in improving the quality of my research while improving my ability as a scientist. Thanks to Jane Roberts for her enthusiasm and for our many in-depth floodplain related discussions. I would like to thank Paul Packard for providing me with valuable and necessary knowledge throughout my PhD and answering my many phone calls and emails.

Thanks to Bernd Gruber, Arthur Georges, Peter Unmack and David Berman for advice on appropriate genetic analysis and help with R. Thanks to Richard Duncan and Elizabeth Wandrag for many discussions on study design and for teaching me about data analysis using R. Thanks to Dave Albrecht for assisting me with the identification of plant species I had put in the too hard basket. I would like to thank Alica Tschierschke for helping with ArcMap (often at very short notice) and with field work.

The field work and experimental work I conducted would not have been possible or (at least) not as enjoyable without the help from volunteers, so I would like to thank Luke Richmond, Rosie Harris, Teresa Gonzalez De Chavez Capila, Jonas Bylemans, Andrew O'Reilly Nugent, Rod Ubrihien, Mark Tupalski and Foyez Shams.

I would also like to thank the Institute for Applied Ecology for being a supportive, positive and fun place to work and study. Thanks to the past and present members of my office for providing a great atmosphere. I would like to thank my friends Jonas, Andrew, Foyez, Teresa, Rod, Kyle and Matt for all the fun we have had over the past few years. Most importantly, I thank my wife Yasmin for all her support, knowledge and enthusiasm. You have made this experience far easier and enjoyable.

Abstract

Floodplains are characterised by high biodiversity and productivity, providing habitat and refugia for a range of native species. These landscape features are also crucial for many aspects of human survival and quality of life. Inundation by flooding is the predominant factor that controls the observed ecological patterns and processes, and biological productivity on the floodplain. Semi-arid floodplains experience a highly variable flooding regime which has structured the vegetation communities.

This thesis addresses some of the current gaps in our understanding of floodplain plants and their responses to water, on the semi-arid floodplain of the lower Lachlan River, Australia. It characterises spatial and temporal differences in seed bank characteristics relative to hydrological gradients, investigates the life-history responses of two floodplain shrub species (tangled lignum (*Duma florulenta* (Meisn.), and nitre goosefoot (*Chenopodium nitrariaceum* (F.Muell.)) to watering, and investigates gene-flow and population genetic structure in tangled lignum and river cooba (*Acacia stenophylla* (A. Cunn. ex Benth.)). This study is framed within the context of the changes in river floodplain connectivity caused by water resource development.

I identified and compared the changes in floodplain-river connectivity characteristics that have occurred as a consequence of water resource development on four floodplain habitat types which occur on the lower Lachlan River, using a hydrological modelling approach. The results show that the floodplain of the lower Lachlan River connects to the river half as often under current flow conditions compared to flow conditions without water resource development. While all habitat types have been impacted by water resource development, the impact reduces away from the main channel of the river, with temporary floodplain lakes experiencing the largest change in connection regime and intermittent black box swamps which occur on the margins of floodplains experiencing the least.

A seedling emergence experiment was conducted using soil collected from a total of 25 locations from three typical floodplain habitat types (temporary floodplain lakes, intermittent river red gum swamps, and intermittent black box swamps) on the lower Lachlan River. The potted soil was subjected to four experimental treatments (rainfall, waterlogged soil, submergence, and submergence followed by recession). Areas on the floodplain that connect to the river irregularly had a higher seed bank density and species richness compared to areas

on the floodplain which connect more frequently. Sites collected in 2016 had significantly greater seed bank density and species richness than sites collected in 2017, following large-scale flooding. Comparatively lower numbers of terrestrial species were present at sites collected in 2017 than sites collected in 2016 compared to aquatic and semi-aquatic species.

I examined the effects of hydrological regime on germination of tangled lignum and nitre goosefoot, and the buoyancy of the seeds of the two species, and hence the ability of the seeds to disperse via water. Seeds of tangled lignum germinated best on soaked soil and on soil inundated for 20 days. Seeds of nitre goosefoot germinated best on soil inundated for five days and on soaked soil. The majority of tangled lignum seeds floated for at least seven days. The majority of nitre goosefoot seeds sank within seven days. Tangled lignum requires about 20 days of flooding, and wet soils following flood recession for optimal germination. Nitre goosefoot requires a few days of flooding, and wet soils following flood recession for optimal germination.

I investigated the effects of simulated rainfall and depth and duration of experimental flooding on mortality, leaf production, biomass and seed production of nitre goosefoot. The plants were subjected to 14 hydrological treatments: dry (no water applied), rainfall (simulating rainfall conditions at Hillston, New South Wales (NSW)) and 12 combinations of three water depths (10 cm, 50 cm, 75 cm) with four durations of inundation (5 days, 10 days, 20 days, 40 days). Nitre goosefoot plants survived flooding, providing plants were not totally submerged, leaf production increased during flooding and after drawdown, and leaf production, biomass and seeding were highest under shallow flooding for approximately one month. The results of this study allow the hydrological responses of nitre goosefoot to be considered in environmental watering programs.

I investigated and compared the population structure and dispersal patterns of tangled lignum and river cooba, across a large inland floodplain using a landscape genetics approach. Both species are widespread throughout flood prone areas of arid and semi-arid Australia but have differing distributions on rivers and floodplains. Leaves were collected from 144 tangled lignum plants across 10 sites and 84 river cooba plants across six sites, on the floodplain of the lower and mid Lachlan River Catchment, and lower Murrumbidgee River Catchment, NSW. DNA was extracted and genotyped using DArTseq platforms. Genetic diversity was compared with floodplain-river connection frequency, and genetic distance (F_{ST}) compared with river distance, geographic distance and floodplain-river connection frequency between

sites. It was found that genetic connectivity increased with increasing floodplain-river connection frequency in tangled lignum but not in river cooba. In tangled lignum, sites that experience more frequent flooding had higher levels of genetic diversity and were more genetically homogenous. There was also an isolation by distance effect where increasing geographic distance correlated with increasing genetic differentiation in tangled lignum, but not in river cooba. These data demonstrate how genetic patterns can highlight influential mechanisms over species distribution and persistence on floodplains.

Semi-arid floodplains such as the floodplain of the lower Lachlan River are temporally and spatially dynamic and complex. The organisation of vegetation on the floodplain is strongly related to the hydrological regime and the requirements and tolerances of each species. The dependency of floodplain vegetation on flooding means hydrological changes are likely to affect floodplain vegetation by reducing opportunities for life history processes such as growth, dispersal, seed production, and seed germination. The results suggest changes to the flow regime will influence the distribution and abundance of species, genetic diversity and genetic structure, and the distribution of populations and communities across floodplains. To maintain the diversity of vegetation and habitat types on the floodplain, environmental flows must consider the life-history strategies of the plants.

Contents

List of figures

recovery, and refuge-mediated functional redundancy. The extrinsic drivers at the ecosystem scale (arrows) that steer the prevalence of specific mechanisms (Van Looy *et al.* 2018).....159

Figures in appendices

List of tables

Tables in appendices

1. Introduction

Background: The vegetation communities which occur in Australia's floodplain landscapes are ecologically significant and among the most threatened ecosystems on the continent. This thesis addresses the impacts of water resource development on the hydrological regime to floodplain habitats of the lower Lachlan River Catchment and investigates relationships between the hydrological regime and the vegetation which occurs on the floodplain. The focus of this thesis is floodplain vegetation and its response to flooding. Chapter 1 is a literature review that provides context and background for this thesis. Chapter 1 introduces the floodplain landscape and its vegetation and the role of hydrology, with a focus on semi-arid environments, the impacts of water resource development on floodplains, and how we manage floodplains through the allocation of environmental flows. Chapter 1 then describes how the hydrological regime and the life-history traits of the vegetation structures the distribution and abundance of floodplain vegetation and provides the conceptual framework that underpins the research presented in this thesis. Chapter 1 then outlines the aims and structure of this thesis.

1.1   Background

Floodplains and their associated wetlands are some of the most biologically productive and diverse ecosystems on earth (Sparks 1995; Tockner and Stanford 2002). Floodplains are defined as 'low energy fluvial depositional environments which are periodically inundated by the lateral overflow of rivers and lakes and/or by direct precipitation or groundwater' (Junk *et al.* 1989). Floodplains consist of a range of distinct geomorphic regions including anabranches, billabongs, channels, backwaters, swamps, marshes and tributaries which are formed by a range of processes including river flow regime (Walker and Puckridge 1995; Ward 1998). The location of floodplains, on low-gradient alluvial shelves promotes periodic inundation, which transforms floodplains from terrestrial to aquatic systems (Junk *et al.* 1989; Tockner and Stanford 2002). Inundation by flooding facilitates the exchange of water, living organisms and resources (inorganic and organic matter) between the main channel, and the floodplain (Stanford and Ward 1993; Amoros and Bornette 2002), influencing the growth, reproduction and interaction of floodplain organisms (Poff 1997; Nicol and Ganf 2000). As such, inundation by flooding is the predominant factor that controls the observed ecological patterns and processes, and biological productivity on the floodplain (Junk *et al.* 1989; Poff and Ward 1989; Bayley 1995).

In temperate and tropical regions, annual (discharge) flooding is typically the primary environmental factor influencing floodplain plant community composition, structure and productivity (Menges and Waller 1983; Junk *et al.* 1989; Blom *et al.* 1990). On floodplains of semi-arid and arid riverine landscapes, flooding is no less important (Poff and Ward 1989; Walker *et al.* 1995; Baldwin *et al.* 2013; Reid *et al.* 2016). Rivers in arid and semi-arid regions exhibit extreme variability in flow, and inundation of floodplains relies on rainfall during particularly wet years (Walker *et al.* 1995; Kingsford 2000b). As such, floodplain inundation in these regions is usually temporally irregular or erratic (Nicholls 1991; Rogers and Ralph 2011), and although irregular, these flood pulses have ecological significance (Walker *et al.* 1995).

As ecotones or interfaces between aquatic and terrestrial environments (Naiman and Decamps 1997), floodplains are affected by the environmental drivers of ecosystem change that affect both terrestrial and aquatic systems, especially land conversion and degradation from agricultural land-use (Thoms 2003) and the overexploitation of freshwater (Millennium Ecosystem Assessment 2005). The ecosystem goods and services provided by floodplains,

such as water regulation, water supply, disturbance regulation, food production, and recreation, are of great value to human populations (Costanza *et al.* 1997). As such, floodplains have been centres of human development since early human civilisation (Welcomme 1979; Dugan 1990).

World-wide, the patterns of flow in rivers have been, and continue to be, affected by water resource development (Nilsson *et al.* 2005; Poff *et al.* 2007; Vörösmarty *et al.* 2010; Haddeland *et al.* 2014; Grill *et al.* 2015). The impacts of water resource development are particularly evident for rivers in dry regions (arid and semi-arid) which have seen pronounced changes under regulated flow conditions (Walker 1992; Walker *et al.* 1995; Thoms and Sheldon 2000).

The use of water to meet human needs is usually at the direct expense of water to floodplains, as water resource development, i.e. river regulation and extraction of water for irrigation and other purposes, reduces and even eliminates floodplain wetlands flow (Kingsford 2000a). Together with engineering structures on floodplains, these pressures have altered the natural flood regimes of floodplains by changing the lateral connectivity of floodplain-river systems (Thoms 2003), and by reducing the frequency, extent and duration of floodplain inundation (Driver *et al.* 2004b; Armstrong *et al.* 2009; Shilpakar and Thoms 2009; Leblanc *et al.* 2012).

These alterations to the natural flooding regime have had significant impacts on floodplains globally (Sparks 1995; Kingsford 2000a; Lemly *et al.* 2000; Poff and Zimmerman 2010; Junk *et al.* 2013). The reduced occurrence of flooding, reduces the formation of floodplain waterbodies, ultimately decreasing the spatiotemporal heterogeneity on the floodplain (Ward and Stanford 1995). Changes to the hydrological regime have resulted in declines in waterbird populations (Briggs *et al.* 1994; Kingsford and Johnson 1998; Roshier *et al.* 2002), widespread mortality and reduced condition of floodplain tree species (Wen *et al.* 2009), and reductions in floodplain biodiversity (Kuiper *et al.* 2014). Changes to the connection of the floodplain with the river obstructs organism dispersal, and can isolate local plant communities (Jansson *et al.* 2000; Merritt *et al.* 2010a). Reduced floodplain inundation has also impacted on the economic benefits provided by flooding on agriculture, fishing, and resource harvesting, which has led to substantial impacts on local communities (Dugan 1990; Barbier and Thompson 1998).

The distinct wet and dry phases of floodplains mean that floodplains provide habitat for terrestrial fauna during dry periods and aquatic and terrestrial fauna when flooded. Habitat

structure on floodplains is greatly enhanced by vegetation which provides both food and habitat. For example, the vegetation on floodplains is used by terrestrial fauna, particularly by terrestrial birds that colonise floodplain wetlands during flooding (Kingsford *et al.* 1999; McGinness *et al.* 2010). During flooding, the floodplain (including macrophytes) is used for spawning and food by riverine fish species (Junk *et al.* 1989; Copp *et al.* 1994). In the dry phase, floodplain vegetation provides important habitat, shelter and resources for small mammals (Briggs *et al.* 2000) and terrestrial arthropods (Rosado *et al.* 2015). While flooded, organic matter containing nutrients previously mineralized during the preceding dry-phase are dissolved (Bayley 1995), which provides an important food resource for aquatic detritivores that move on to the floodplain (Ward 1989). Changes to floodplain vegetation resulting from water resource development therefore has widespread implications. Consequently, a fundamental understanding of the relationship between floodplain vegetation and the hydrological regime is required to manage floodplain environments.

1.2   Environmental flows

It is recognised that to protect freshwater ecosystems and maintain the goods and services provided, components of the natural flow variability must be mimicked, including magnitude, frequency, timing, duration and predictability of flow events (Poff *et al.* 1997; Arthington *et al.* 2006). As such, providing water as an environmental flow to improve or maintain the health of a river system has occurred across many parts of the world (Postel and Richter 2012). Environmental flow encompasses both the determination of ecological water requirements and subsequent environmental flow allocations (Dollar 2004). Environmental flows are undertaken for a range of biophysical flow requirements, including channel management and in-stream sediment removal (Wilcock *et al.* 1996), conservation of waterbird populations (Galat *et al.* 1998), fish spawning and recruitment (King *et al.* 2010), and maintaining the condition of floodplain vegetation (SEWPaC 2011).

As environmental flows aim to improve or maintain biota and ecosystem processes (Lake 2000) by providing for the life stages of biota that depend on flooding, an in-depth knowledge of the needs of aquatic ecosystems and the life stages of biota that depend on flooding is required to manage floodplain environments. Additionally, forecasting the ecological consequences of water resource development on aquatic ecosystems is vital with

the increasing human demands for freshwater (Jackson *et al.* 2001) and climate change (Dunlop and Brown 2008).

1.3   The hydrological regime

Floodplains result from the episodic deposition or cut and fill of alluvial sediment during flooding (Daniels 2003). Floodplains experience pronounced dry and wet phases which are defined by the adjacent rivers flow rates; when at low flow, rivers flow in channel and the adjacent floodplains will likely be in a dry phase, but at high flow, floodplains will become inundated and the floodplain will be in a wet phase (Junk *et al.* 1989; Tockner and Stanford 2002). Once inundated, standing waters cover the floodplain and lentic habitats develop. Through evaporation and, to a lesser extent filtration, water bodies on the floodplain contract and eventually dry (Welcomme 1979). As the water level recedes, processes on the floodplain become less dependent on the river channel and more subject to local climatic events (such as the distribution and amount of local rainfall)(Junk *et al.* 1989).

The hydrological regime is seen as the maestro that orchestrates patterns and processes in rivers (Walker *et al.* 1995), and is determined by the climate of its up-stream catchment area (Junk *et al.* 1989). A number of important characteristics of the hydrological regime of a river contribute to 'the natural flow regime', including magnitude of discharge, the frequency, duration, and timing of a given flow condition (for instance floods, droughts or intermittent flows), and the rate of change from one flow magnitude to another (Poff *et al.* 1997). These flow characteristics contribute to the hydrological regime on floodplains via lateral connection between the river and its floodplain (Junk *et al.* 1989).

Hydrological connection provided by flooding is a key process in maintaining the ecological integrity, form and functioning of floodplains and their associated wetlands through the exchange of water, living organisms, and resources (inorganic and organic matter) between the main channel, and the floodplain (Junk *et al.* 1989; Ward 1989; Stanford and Ward 1993; Amoros and Bornette 2002; Tockner and Stanford 2002). As floodplains are disturbance-dependent ecosystems, flooding maintains this connectivity (Ward and Stanford 1995) and the expansion and contraction of floodwaters is the primary force that sustains connectivity between the river and floodplain (Tockner and Stanford 2002). While floodwater may persist

on lower lying parts of floodplains, hydrological connection between the river and floodplain provided by flooding only occurs while the river's discharge exceeds the channel capacity. Thus, connection between the river and its floodplain is the first stage of floodplain inundation. Floodplains are dynamic landforms and flooding builds and reworks floodplain surfaces, where suspended sediments are deposited in some areas while erosion and scouring occurs in others (Hupp and Bornette 2003). Thus, the hydrological connectivity between the river channel and floodplain is responsible for both the exchange between landscape patches and the kinetic energy of the running water responsible for fluvial dynamics (Amoros and Bornette 2002).

Both the dry phase and wet phase on floodplains play an important role in maintaining the health and functioning of the floodplain (Young 2001). Important characteristics of the flooding regime on floodplains include flood frequency, flood duration, number of days between floods, and flood predictability (Poff and Ward 1989). These characteristics of the flooding regime vary spatially across floodplains, with variation in surface topography (Scown *et al.* 2015), producing a spatial mosaic of habitat types (Welcomme 1979; Hughes 1988; Thoms 2003).

1.4   Floodplain plants

Variability in the hydrological regime on floodplains results in considerable unpredictability for plants (Junk *et al.* 1989; Ward *et al.* 1999; Colloff and Baldwin 2010). This is particularly evident on floodplains in semi-arid and arid environments which naturally experience a highly variable flooding regime (Puckridge *et al.* 1998; Thoms and Sheldon 2000). Both the drying-out (dry phase) and wetting (wet phase) conditions on floodplains can adversely affect plant growth, and if sustained can result in plant mortality (Auchincloss *et al.* 2012; Roberts *et al.* 2016). For example, flooding can cause an energy deficit because of a shortage of oxygen in the soil, which is stressful or lethal to plants (Armstrong 1979; Voesenek *et al.* 2006). Under these conditions, availability of nutrients for plants decreases and toxic substances accumulate in the soil (Blom and Voesenek 1996). When flooding extends to the submergence of the shoots of plants, photosynthesis becomes restricted by the reduced availability of carbon dioxide and shading, putting further stress on the plant (Jackson and Colmer 2005).

Plants which occur on floodplains have life-history traits which enable them to survive and exploit the hydrological conditions and subsequent ecological processes which occur (Lytle and Poff 2004). On floodplains where flooding is frequent and predictable, life-history traits (such as seed production and dispersal) are often synchronized to avoid or exploit flooding (Lytle and Poff 2004). On floodplains with unpredictable and variable flooding regimes, opportunistic and flexible life-history traits may be more common (Walker *et al.* 1995; Mommer *et al.* 2006).

Plants which survive the stresses associated with flooding in a vegetative state can be broadly categorised as stress tolerant (Grime 1977). These plants have a range of morphological and physiological traits which enable survival during flooding conditions (Mommer *et al.* 2006; Voesenek *et al.* 2006), including elongation of shoots that maintain contact with the atmosphere under flooded conditions, transport of oxygen from leaves to roots, underwater photosynthesis, and adventitious roots (Kozlowski 1984; Blom *et al.* 1990; Armstrong *et al.* 1994; Kozlowski 1997). The capacity of these traits to mitigate the effects of flooding depends on the depth and duration of flooding (Laan and Blom 1990; Voesenek *et al.* 2006) and the age of the plant, with younger plants being less tolerant of flooding (Blom *et al.* 1990).

Many plants avoid the stresses associated with flooding or drying through the timing of important life-history stages. For example, species which are unable to survive through the dry or wet phases imposed on floodplain plants often produce long-lived dormant seeds as a means of avoiding unfavourable conditions (such as during a dry phase), where the population regenerates once appropriate conditions for seed germination occurs (such as during or following re-wetting) (Chesson and Huntly 1989; Leck and Brock 2000; Brock *et al.* 2003; Brock 2011). These ruderal species are often annual or short-lived perennial species, which are small and have a rapid growth rate (Grime 1977). A persistent soil seed bank is a common strategy of plants subjected to temporally unpredictable disturbance (Grime 1989).

Many plants which occur along rivers and on floodplains have buoyant fruits or seed which favours their dispersal by water (hydrochory) (Sculthorpe 1967). The dendritic nature, and unidirectional flow of rivers means hydrochory connects habitat patches linearly as propagules move downstream (Nilsson *et al.* 2010). On floodplains, inundation enables the dispersal of seeds, including through connection between otherwise disconnected habitat

patches (Nilsson *et al.* 2010). Waterbirds also play an important role in the dispersal of aquatic plants (Green *et al.* 2008). Kudoh and Whigham (1997) proposed that there are three possible models of pathways of dispersal in a hydrochorous wetland species. That is, a) a two-dimensional stepping stone distance model, where dispersal depends on the Euclidian distance between sites, b) a one-dimensional stepping stone distance model, where dispersal depends on the distance measured along the river network, and c) the stream accessibilty model, where dispersal depends on distance to the river channel and flooding frequency for populations not adjacent to the river (Figure 1.1). These pathways of dispersal, influence a species vulnerability to the effects of water resource development.
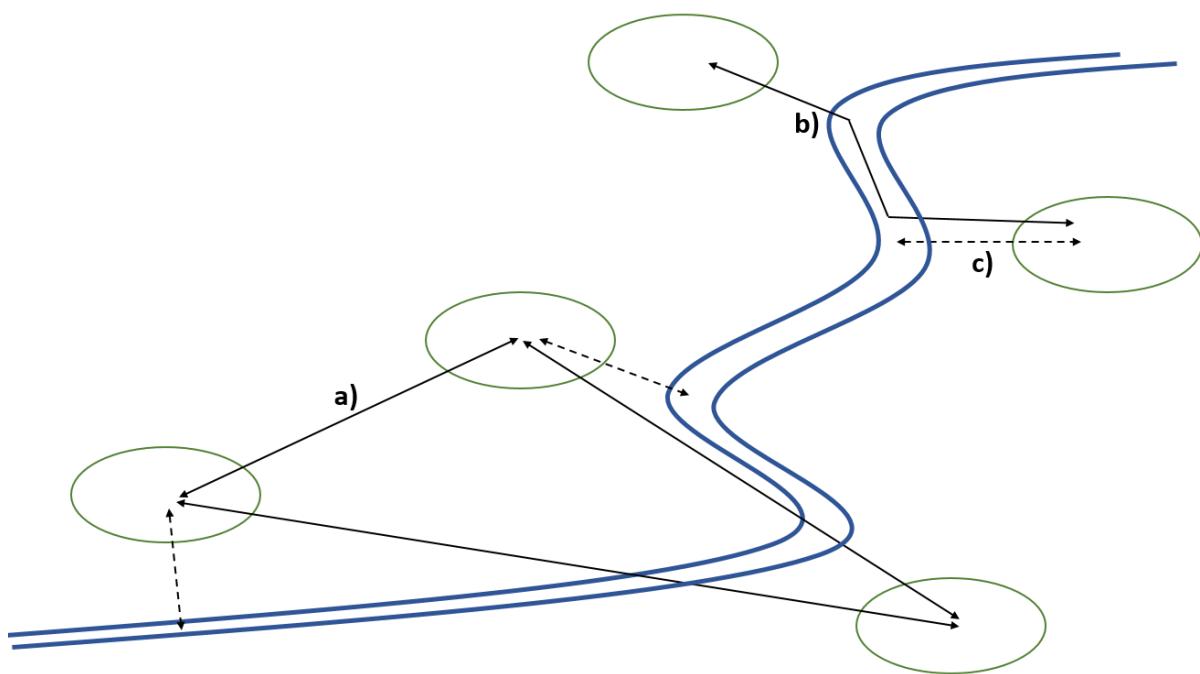
Figure 1.1 Three possible dispersal pathways in plants which occur on floodplains: a) TSD model (two-dimensional stepping stone distance model); where dispersal depends on the Euclidan distance between sites, b) OSD model (one dimensional stepping stone distance model); where dispersal depends on the distance between sites as measured along the river and the distance between the river and the study sites, and c) STA (stream accessibility model); where dispersal depends on the frequency of flooding at a site for sites not adjacent to the river (Adapted from Kudoh and Whigham 1997). Circles represent habitat patches.

Plants on floodplains often require flooding for survival, growth and reproduction as rainfall alone is often insufficient (Robertson *et al.* 2001; George 2004; Capon *et al.* 2009; Roberts and Marston 2011; Doody *et al.* 2014; Catelotti *et al.* 2015). Flooding maintains tree condition and can result in a flush of new growth in floodplain trees such as river red gum (*Eucalyptus camaldulensis*) and black box (*Eucalyptus largiflorens*) (Holland *et al.* 2009). Flooding has been observed to increase reproduction rates in two *Ranunculus* species (Mony *et al.* 2010). Flooding stimulates germination and growth responses in many aquatic and semi-aquatic species (Van der Valk and Davis 1976; Rea and Ganf 1994; Robertson *et al.* 2001), resulting in these species re-establishing during flooding or following flood recession (Casanova and Brock 2000; Brock *et al.* 2003; Nicol *et al.* 2003; Capon and Brock 2006; Nielsen *et al.* 2014). As such, variation in germination of plants which occur on floodplains and wetlands, results in temporal shifts in plant community composition and structure (Capon 2003; Nicol *et al.* 2003).

Organisms on floodplains respond to the effects of flooding (primarily changes in resource availability, landscape connectivity and habitat heterogeneity) by either benefiting from resource pulses (resource facilitation) or avoiding the stresses associated with flooding (through dispersal or resting stages)(Van Looy *et al.* 2018). As the life-history traits employed by each plant influences its success under certain hydrological conditions, both the life-history traits and the hydrological conditions strongly influence the distribution and abundance of plants on floodplains (Blom *et al.* 1990; Poff 1997). As such, there are three main filters which occur during flooding, which may be expected to ultimately determine the floristic composition of a site on a floodplain. These are: 1) the life-history traits and strategies of plants; 2) the ecological processes which occur during flooding (such as resource availability (primarily water and nutrients), connection between habitat patches via floodwaters, and stresses associated with flooding (soil anoxia, light deficiencies, abrasion)); and 3) the site flooding characteristics (such as frequency, duration, and depth of flooding) (figure 1.2). For example, species with long-lived seed banks will have propagules in the seed bank that can become established when suitable environmental conditions occur, while species without long-lived seed banks can only become established if their propagules arrive during a period when conditions are suitable for establishment (Van der Valk 1981). Terrestrial plants without adaptations to flooding that may have colonised the floodplain during the preceding dry phase, are likely to die during flooding (Figure 1.2).

Seed germination

Seedling establishment

Growth

Seed production

Stress avoidance (such as seed dormancy)

Mechanisms to cope with flooding (such as development of aerenchyma, adventitious roots, and rapid elongation of stems)

Dispersal

Life history traits

Site flooding characteristics

Frequency

Duration

Depth

Magnitude

Predictability

Timing

Rate of change

flood intolerant
flood respondent

flood intolerant
Flood tolerant

no hydrochory
hydrochory

Ecological processes created by flooding

Resource availability

The creation of habitat

Increased water and nutrient availability

Stresses

Soil anoxia

Light deficiencies

Abrasion

Accumulation of toxic materials in the soil

Increased connectivity

In-channel transport

Re-distribution of existing seed

Zoochory (primarily waterbirds)

Distribution, composition and structure of floodplain vegetation

Figure 1.2 A conceptual model of the factors that influence the distribution, composition and structure of vegetation on the floodplain.

As plants on floodplains are adapted to certain hydrological attributes (such as depth, duration, and frequency of flooding), the hydrological regime on the floodplain, influences their distribution (Van der Valk and Davis 1976; Junk *et al.* 1989; Froend and McComb 1994) related to variations in tolerances and requirements for flooding and drying among species (Blom *et al.* 1990; Walker *et al.* 1995; Naiman and Decamps 1997; Ward *et al.* 1999). This results in vegetation communities on floodplains often being observed in distinct lateral zones relating to spatial variation in flooding characteristics and elevational gradients (Hughes 1988; Brock and Casanova 1997; Eamus *et al.* 2006; Roberts *et al.* 2016). These zones are distinguished by structural characteristics (e.g. density and height) and the dominant species (Roberts *et al.* 2016).

In systems such as floodplains which are maintained by natural disturbance (i.e. the flooding regime) (Ward and Stanford 1995), the success of a life-history trait or strategy is dependent on the temporal heterogeneity of the disturbance regime, that is the length of the favourable period (permits reproduction) and the length of the unfavourable period (permits existence) (Southwood 1977) and the variability and unpredictability with which these occur. Traits common in species which occur in habitats with a high frequency of disturbance include a short generation time, small size, high fecundity, and high levels of dispersal (Southwood 1977). In plants, an annual or short-lived perennial life-cycle is a specialization adapted to exploit environments intermittently favourable for rapid plant growth (Grime 1977). As the frequency of the disturbance reduces, habitat durational stability increases and a different suite of life-history traits may be favoured (such as long-generation time and larger size) (Southwood 1977; Southwood 1988). As the frequency and duration of flooding is related to elevation on the floodplain, life-history traits have been observed to vary with elevation (Menges and Waller 1983).

Frequently inundated, near-channel sites on the floodplain are typically treeless and dominated by flood-tolerant annual species, exhibiting strategies characterised by fast turnover rates (i.e., completing their life-cycle between flooding events), while the higher elevated parts of floodplains which are flooded less frequently are dominated more so by (flood tolerant) perennial species (Hughes 1988; Blom and Voesenek 1996; Capon 2003; Capon 2005; Mommer *et al.* 2006) (Figure 1.3). The dominant control of species diversity has also been observed to shift from predominantly abiotic (driven by hydrology) in frequently flooded parts of the floodplain to biotic factors (such as competition) on higher elevated parts of floodplains which are flooded less frequently (Blom and Voesenek 1996; Lenssen *et al.* 1999) (Figure 1.3).
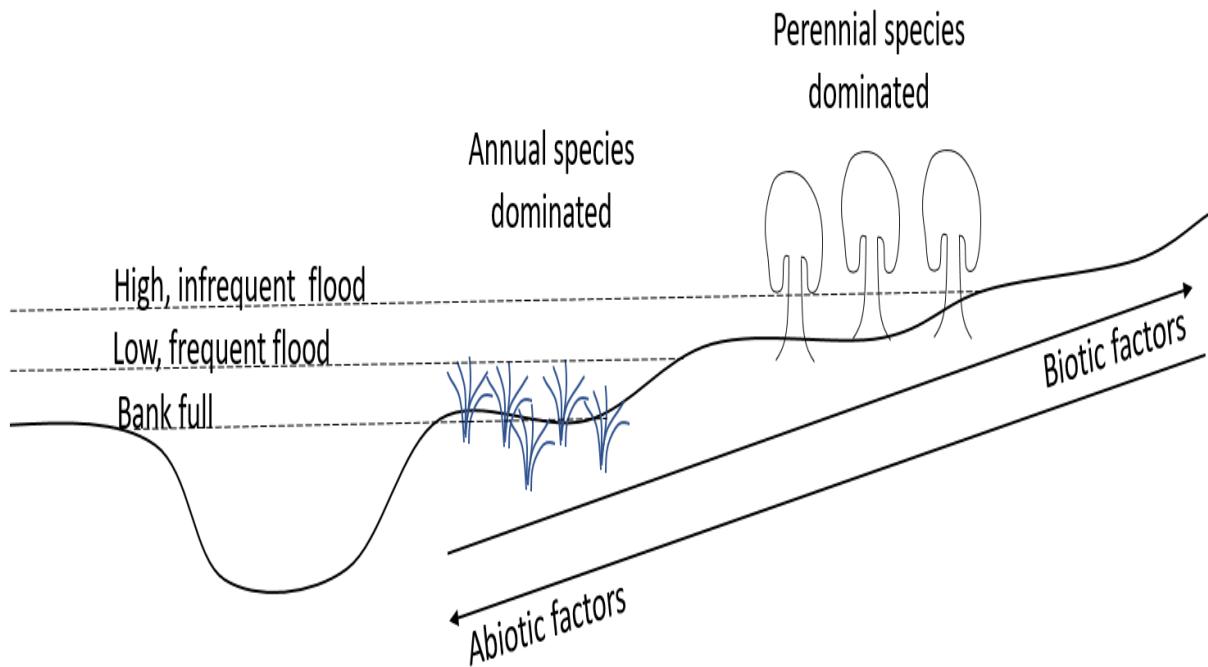
Figure 1.3 Diagrammatic representation of the zonation of floodplain vegetation in relation to flood frequency.

1.5   Research aims

While a range of studies have examined the influence of the hydrological regime on floodplain vegetation in semi-arid and arid regions (Hughes 1988; Higgins *et al.* 1997; Costelloe *et al*. 2008; Dunkerley 2008; Capon *et al.* 2009; Rodríguez-González *et al.* 2010). Very few studies are framed within the context of the flow requirements of the life-history stages of the vegetation (such as dispersal, germination and growth), and the ecological implications of changes to the hydrological regime caused by water resource development on the vegetation. This is of great importance when considering the possible consequences of changes to the way in which water is managed within the landscape or the consequences of changes in climate.

This thesis addresses some of the current gaps in our understanding of floodplain plants and their requirements for water, by exploring 1) the changes to floodplain-river connection by flooding which have occurred as a result of water resource development on floodplain habitat types in the lower Lachlan River Catchment, New South Wales, and 2) investigating life-

history traits seed production, dispersal and germination, and plant growth, and the hydrological responses of these life-history traits.

Studying the soil seed bank provides an informative way of studying the spatial and temporal distribution of plant species on the floodplain and their responses to the hydrological regime (Casanova and Brock 2000; Capon 2007; James *et al.* 2007; Porter *et al.* 2007). As such, this thesis includes a soil seed bank germination experiment on soil collected from the floodplain of the lower Lachlan River. The soil seed bank typically comprises annual non-woody species which establish in response to certain hydrological conditions (Middleton 2003; Greet 2016). Shrubs which occur on the floodplains of the Murray-Darling Basin are an important component of the landscape (Brandis *et al.* 2009; Roberts and Marston 2011). These species typically do not rely on a soil seed bank (Middleton 2003; Greet 2016) There is limited information available on the shrubs which occur on the floodplains of the Murray-Darling Basin and their response and requirements to the hydrological regime. As such, this thesis tackles the response of four shrub species common in the Murray-Darling Basin to the hydrological regime through undertaking two glasshouse experiments and a population genetics study.

This thesis is primarily focused on the floodplain of the lower Lachlan River Catchment, which has a semi-arid climate (370 mm mean annual rainfall at Hillston Airport) (Bureau of Meteorology 2017). Semi-arid floodplains, which occupy areas with a mean annual rainfall of 250 to 500 mm, make up the majority of floodplains in the Murray-Darling Basin (Colloff and Baldwin 2010). While this thesis is primarily focused on floodplain vegetation of the Murray-Darling Basin, many of the concepts and findings are relevant to semi-arid floodplain vegetation more broadly.

This thesis aimed to:

1) Assess the impacts of water resource development on the hydrological connectivity to different floodplain habitat types which are common in the southern parts of the Murray-Darling Basin, Australia.

2) Investigate the influence of depth, duration and frequency of flooding on the growth, germination, and dispersal of plants which occur on semi-arid floodplains, by:

    a) Investigating the spatial and temporal variability in the germinable soil seed bank in relation to variation in floodplain-river connection and recent and large-scale flooding.

    b) Determining relationships between germination of tangled lignum (*Duma florulenta* (Meisn.)) and nitre goosefoot (*Chenopodium nitrariaceum* (F.Muell.)) and hydrological regime; and determining the buoyancy of seeds of tangled lignum and nitre goosefoot.

    c) Determining relationships between hydrological regime and mortality, biomass, leaf production, and seed set of nitre goosefoot plants.

    d) Identifying and comparing the population structure and dispersal patterns of two plant species with varying distribution on rivers and floodplains: tangled lignum and river cooba (*Acacia stenophylla* (A. Cunn.)) using a landscape genetics approach.

3) Provide a greater understanding of the ecology of floodplain plants, including the possible threats related to water resource development and recommendations in the allocation of environmental flow.

1.6   This thesis

Given the importance of the hydrological regime to the composition and structure of the vegetation (as outlined in chapter 1), it is likely that changes to the hydrological regime as a result of water resource development have significant consequences for floodplain vegetation. For this reason, this thesis commenced with an analysis of the hydrology and changes to the hydrological regime which have occurred under current water resource development conditions on different floodplain habitat types on the lower Lachlan River, New South Wales.

A soil seed bank germination experiment was conducted on soil collected from the floodplain of the lower Lachlan River as an exploratory study to understand the spatial and temporal distribution of plants on floodplains and the influence of the hydrological regime. While the results showed that the germinable soil seed bank is diverse, very few woody perennial plants (especially shrubs) germinated during the seedling emergence experiment with the vast majority consisting of herbaceous annual or short-lived perennial species. This raised questions about the role of hydrology for certain species that were then investigated in more detail in subsequent studies in this thesis.

Only a few species of shrubs, especially large shrubs (i.e. >2 m tall), occur on the floodplains of the lower Lachlan River or the Murray-Darling Basin more broadly (Cunningham *et al.* 1981; Keith 2004; Benson *et al.* 2006). As such, the few species which do occur provide important habitat for terrestrial fauna, particularly for terrestrial birds (McGinness *et al.* 2010; McGinness *et al.* 2013). In the Murray-Darling Basin, habitat structure is generally provided by tangled lignum and nitre goosefoot (McGinness *et al.* 2013). These species were not present in the seedling emergence experiment and like most woody perennial plants which occur on the floodplains of the Murray-Darling Basin are unlikely to rely on a persistent soil seed bank (Middleton 2003; Chong and Walker 2004; Greet 2016). As such, these species may exhibit traits which maximise survival in hydrologically variable habitats common to riverine trees and shrubs, such as opportunistic germination and seedling growth, flood tolerance, and timing reproduction, dispersal, germination and growth around flooding events (Capon *et al*. 2016). To understand their distribution in the landscape and role of hydrology, I investigated the seed germination characteristics of these two important and widely distributed shrub species in relation to duration of inundation and local rainfall conditions, to understand their reproductive requirements and the role of hydrology.

To persist on the floodplain, woody perennial plants must tolerate the stresses related to flooding and often require flooding for survival, growth, and seed production (Roberts and Marston 2011). Nitre goosefoot has not generally been considered a floodplain species (despite its distribution on the margin of floodplains) and its requirements and tolerances to flooding are unknown. As such, I conducted a further experiment on nitre goosefoot plants, where I put the plants under a range of depths and durations of inundation, to investigate the species response to flooding.

The seed germination experiment demonstrated that tangled lignum seeds can float on water for at least 7 days, with some seeds floating for 30 days, while most seed of nitre goosefoot only floated for a few days. Others have identified the ability of the seed of tangled lignum to float and have assumed that this species disperses primarily by hydrochory (Chong and Walker 2005). This has implications for the species population genetic structure and the possible role of hydrology in connecting patches on the floodplain.

A second woody perennial species, river cooba is one of the few small tree species to exist along the lower Lachlan River and its floodplains and other inland catchments of the Murray-Darling Basin and provides important nesting habitat for waterbirds (Roberts and Marston 2011). River cooba is also assumed to disperse by hydrochory owing to its occurrence along rivers and as it has been observed to germinate following the recession of floodwaters (Cunningham *et al.* 1981). To investigate the ability of these species to disperse by hydrochory, and the role of floodplain-river connectivity in connecting patches on the floodplain, I conducted a population genetic study on tangled lignum and river cooba. A genetic approach can identify spatial genetic variation and how this is associated with landscape features such as river networks and ecological features such as river-floodplain connection by flooding.

1.7   Thesis structure

Following the introductory chapter (chapter 1) which introduced the floodplain landscape, the impacts of water resource development on floodplains, and the importance of the hydrological regime to floodplain vegetation, chapters 2-6 are data chapters and chapter 7 is a synthesis of the main findings of this thesis. Chapter 2 identifies and compares changes in floodplain-river connectivity by flooding that have occurred in the lower Lachlan River as a consequence of water resource development on four key floodplain habitat types using a hydrological modelling approach. Chapter 3 describes the results from a seedling emergence experiment undertaken on soil collected from locations across the floodplain of the lower Lachlan River in order to investigate the temporal and spatial variability of the soil seed bank and the influence of flooding. Chapter 4 describes a seed germination experiment conducted on two shrub species which occur in semi-arid and arid Australian floodplains, tangled lignum and nitre goosefoot in relation to flooding duration. Chapter 5 describes an inundation

experiment conducted on nitre goosefoot, to investigate the species tolerance and requirements for flooding on growth, seed production and mortality. Chapter 6 describes a population genetics study looking at genetic diversity levels, pathways for gene-flow and possible dispersal mechanisms in tangled lignum and river cooba.

Each of the data chapters (chapters 2-6) is presented as a scientific paper and the literature is reviewed in the introduction of each of these chapters. As such, a traditional literature review is not presented in this thesis. I have used common names when describing plant species because the three papers that are published use common names and this retains a consistent approach throughout the thesis. Appendices contain additional figures and tables that were not included within the main text. Figures and tables included in appendices are labelled and referred to in the text with the letter of the appendix heading.

DECLARATION OF CO-AUTHORED PUBLICATION CHAPTER

For use in theses which include publications. This declaration must be completed for each co-authored publication and to be placed at the start of the thesis chapter in which the publication appears.

Declaration for Thesis Chapter 2

Declaration by candidate

In the case of Chapter 2 the nature and extent of my contribution to the work was the following:

Nature of Contribution	Extent of contributions
I designed the study, conducted all field work, the majority of the modelling and data analysis and led the writing of the manuscript.	90%

The following co-authors contributed to the work:

Name	Nature of Contribution	Contributor is also a student at UC Y/N
Fiona Dyer	Study design, data analysis and manuscript writing	N
Brian Higgisson	Hydrological modelling	N
Megan Powell	Data collection from Landsat imagery and manuscript writing	N
Patrick Driver	Manuscript writing	N

Candidate's Signature: Date: 01/02/2019

Declaration by co-authors

The undersigned hereby certify that:

1) the above declaration correctly reflects the nature and extent of the candidate's contribution to this work, and the nature of the contribution of each of the co-authors.

2) they meet the criteria for authorship in that they have participated in the conception, execution, or interpretation, of at least that part of the publication in their field of expertise;

3) they take public responsibility for their part of the publication, except for the responsible author who accepts overall responsibility for the publication;

4) there are no other authors of the publication according to these criteria;

5) potential conflicts of interest have been disclosed to (a) granting bodies, (b) the editor or publisher of journals or other publications, and (c) the head of the responsible academic unit; and

6) the original data are stored at the following location(s) and will be held for at least five years from the date indicated below:

Location(s):	Institute for Applied Ecology, University of Canberra, Canberra, Australia

Signatures	Date
	24/01/2019
	31/01/2019
	29/01/2019
	28/01/2019

2. Impacts of water resource development on hydrological connectivity to different floodplain habitats in a highly variable system

Background: Chapter two describes changes in river-floodplain connectivity characteristics; connection frequency, connection duration (mean, median and maximum), dry spell duration (mean, median and maximum), and timing of connection that have occurred as a consequence of water resource development on four floodplain habitat types common to the Murray-Darling Basin: temporary floodplain lakes, intermittent river red gum (*Eucalyptus camaldulensis*) swamps; intermittent black box (*E. largiflorens*) swamps, and terminal wetlands (wetlands along distributary creeks). This chapter focuses on river-floodplain connectivity to different floodplain habitat types. The initial selection of sites were those used in an existing vegetation monitoring program (14 sites). These were augmented with a further five sites that were selected on the basis of my ability to access sites. As such an equal number of each habitat type was not possible. It is acknowledged that there are other hydrological factors such as hydrological variability, as well as landscape features such as groundwater, soil type, and topography which were not analysed as part of this study which are likely to play an important role in the distribution of floodplain habitat types and vegetation communities that inhabit them. However, this chapter specifically focused on river-floodplain connectivity by flooding as this is likely to be influenced by changes in flow regime related to water resource development. The results of this chapter provide context for the other chapters in this thesis in regard to the effects of hydrological changes on the vegetation and the hydrological data developed for this chapter is used throughout this thesis. This chapter contains a manuscript that has been accepted for publication in the Journal of **River Research and Application** as:

Higgisson, W., Higgisson, B., Powell, M., Driver, P., and Dyer, F. (2019) Impacts of water resource development on hydrological connectivity to different floodplain habitats in a highly variable system

2.1   Abstract

Floodplains and their associated wetlands are important features of semi-arid and arid landscapes, providing habitat and refugia for a range of native species as well as contributing to the human requirements for freshwater. These landscape features naturally experience a highly variable flooding regime which has resulted in the development of unique biological communities that are both spatially and temporally variable. Globally, floodplain habitats are some of the most modified ecological communities as a result of water resource development and land-use changes. However, the hydrological changes which have occurred in highly variable semi-arid and arid systems are rarely quantified in a way that will help us to understand the consequences of water resource development across floodplain habitat types. This study investigated changes in river-floodplain connectivity that have occurred as a consequence of water resource development on four floodplain habitat types in the Lachlan River Catchment, Australia: 1. temporary floodplain lakes, 2. intermittent river red gum (*Eucalyptus camaldulensis*) swamps; 3. intermittent black box (*Eucalyptus largiflorens*) swamps, and 4. terminal wetlands (wetlands along distributary creeks). Changes to river-floodplain connectivity characteristics on these habitat types were calculated using their Commence to Fill (CTF) thresholds and flow scenarios derived from a river hydrology model (the Integrated Quantity and Quality Model (IQQM)), enabling comparison of a long-term data set (120 yrs.) encompassing a range of climate conditions. All habitat types have been impacted by flow regulation. Temporary floodplain lakes have experienced the greatest reduction in number of connection events, followed by intermittent river red gum swamps. Intermittent black box swamps have experienced the least change in number of connection events. The connection regime has significantly changed in all floodplain habitats except intermittent black box swamps. The nature of the change in connection suggests a change in vegetation communities will occur in response to long-term hydrological change.

2.2   Introduction

Floodplains and their associated wetlands are important features of semi-arid and arid riverine landscapes (Hughes 1988). They are typically highly productive environments compared with the surrounding landscape as a result of water and nutrient inputs from flooding (Ballinger and Lake 2006; Mac Nally *et al.* 2011). As a consequence they support a

unique and diverse water dependent biota (Sabo *et al.* 2005; Brock *et al.* 2006), provide areas of refuge for many terrestrial species (Selwood *et al.* 2015; Blakey *et al.* 2017) and are critical for many aspects of human survival and quality of life, including use of water for agricultural production and recreational uses (Costanza *et al.* 1997). Despite their ecological and economic importance, floodplains are globally one of the most modified and threatened ecosystems (Tockner and Stanford 2002).

Floodplains are characterised by highly variable inundation regimes which drives the form, function, and ecological character of floodplains and their associated wetlands (Junk *et al.* 1989; Poff and Ward 1989). Thus, floodplains display a diversity of habitats which are caused by spatial variation in flooding characteristics (i.e. frequency, duration, and depth) (Junk *et al.* 1989; Blom and Voesenek 1996). This natural variability creates challenges for the detection of significant human-caused hydrological changes and the subsequent ecological consequences. This is particularly challenging in systems which have highly variable and unpredictable hydrology, such as many of the inland arid and semi-arid river systems of Australia (Puckridge *et al.* 1998; Thoms and Sheldon 2000).

World-wide, the patterns of flow in rivers have been, and continue to be, affected by water resource development (Nilsson *et al.* 2005; Haddeland *et al.* 2014; Grill *et al.* 2015). These changes disrupt the natural flood regime of rivers and their associated floodplains (Ward and Stanford 1995), changing the lateral-connectivity of floodplain-river systems (Thoms 2003), and reducing the frequency, extent and duration of floodplain inundation (Kingsford 2000a; Shilpakar and Thoms 2009). Water resource development has led to a decline in the ecological condition of floodplains globally (Lemly *et al.* 2000) with floodplain vegetation communities changing in structure and composition as a result (for example Stevens *et al.* 1995; Bunn and Arthington 2002).

In naturally highly variable and unpredictable systems, such as those of many Australian inland rivers (Puckridge *et al.* 1998; Thoms and Sheldon 2000) the consequences of altered flow regimes for vegetation communities is hard to predict. Central to making predictions is understanding how the character of the hydrology has changed (Poff *et al.* 2010) and many studies have characterised the changes in river flow as a consequence of water resource development (Richter *et al.* 1996; Thoms and Sheldon 2000; Poff *et al.* 2010). Few studies have characterised the changes in river-floodplain connectivity (Dyer 2002; Driver *et al.*

2004b; Driver *et al.* 2005) and this work generally hasn't been used to identify or predict possible ecological consequences (except see Overton and Doody 2008).

Changes in flooding regimes as a consequence of water resource development are unlikely to be uniform across the flow related gradients of flood frequency and duration. Water resource development has tended to remove small to intermediate sized floods from the hydrograph of inland Australian rivers (Thoms and Sheldon 2000). Thus, it is likely that the inundation of floodplains and their associated habitat types will not be uniformly affected. Therefore, I hypothesize that for inland Australian rivers, floodplain habitat types which are closer to the river which naturally would have been flooded by small frequently occurring flood events, are affected by water resource development to a greater extent than communities on the extremities of floodplains which require a far larger, less frequently occurring flood event to become inundated. This subsequently defines the vulnerability of floodplain vegetation to the effects of water resource development.

The objectives of this study were to test this hypothesis by: (i) characterising the floodplain-river connection regime of floodplain habitat types in an Australian river system, the lower Lachlan River Catchment, (ii) identifying and comparing changes in connection characteristics that have occurred as a consequence of water resource development, and (iii) identifying the implications of these changes for floodplain habitat types, generating hypothesis for future testing. This study focused on four floodplain habitat types (temporary floodplain lakes, intermittent river red gum (*Eucalyptus camaldulensis*) swamps, intermittent black box (*Eucalyptus Largiflorens*) swamps, and terminal wetlands) in the lower Lachlan River Catchment, of the Murray-Darling Basin, Australia.

2.3   Study area

The Lachlan River Catchment forms a part of the Murray-Darling Basin, covering an area of 84,700 km^2 (Driver *et al.* 2010; Figure 2.1). The climate is broadly described as temperate in the eastern upper catchment and semi-arid in the western lower catchment (Peel *et al.* 2007). The Lachlan River is more than 1500 km long, rising in the Great Dividing Range and terminating in the Great Cumbung Swamp, in the southwest of the catchment, where it joins the Murrumbidgee River during floods which occur in 15 to 20% of years (O'Brien and Burne 1994). Rainfall across the catchment is extremely variable both spatially, with annual

rainfall in the catchment ranging from 1,100 mm per year in the east of the catchment to 200 mm in the far west, and temporally, with large inter-annual variability in rainfall (ranging from 101 mm to 820 mm annually at Hillston Airport, data from 1881 to 2018) (Bureau of Meteorology 2017). Consequently the Lachlan River exhibits extreme variability in flow (SEWPaC 2011).

In the Murray-Darling Basin, and specifically the lower Lachlan River Catchment, important floodplain habitat types include temporary floodplain lakes, river red gum forests and woodlands, black box woodlands, and lignum (*Duma florulenta*) shrublands. These habitat types are distributed across floodplains in relation to flow-related gradients in flood frequency and duration (Roberts *et al.* 2016). For example, river red gum forests occur in the lower, more frequently flooded parts of the floodplain, while river red gum woodlands and, even more so, black box woodlands occur higher up on the floodplain where flooding is less frequent (Roberts and Marston 2011).

The Lachlan River epitomises the river regulation which is widespread across the Murray-Darling Basin. River regulation and irrigation activities have intensified across the Lachlan River Catchment since the construction of Wyangala dam in 1935 (Kingsford 2000b). There are 10 large dams and 323 weirs within the Lachlan River Catchment (Armstrong *et al.* 2009). These structures assist in diverting water to the various anabranches and creeks for stock and domestic supply as well as irrigation activities (Armstrong *et al.* 2009). Regulation and flow extraction of the Lachlan River has altered the behaviour and distribution of floodwaters (Driver *et al.* 2004b; Driver *et al.* 2010).

The lower Lachlan River Catchment (below the junction of the Lachlan River and Willandra Creek, Figure 2.1) is characterised by numerous distributary channels, anabranches, and tributaries, many of which do not return to the main branch of the Lachlan River (Green *et al.* 2011). At the junction with Willandra Creek (north of Hillston), the Lachlan River spreads in to a low gradient alluvial plain (O'Brien and Burne 1994), which experiences low and highly variable rainfall (370 mm annual rainfall at Hillston Airport) (Bureau of Meteorology 2017). These geological and climatic conditions have resulted in vast areas of irregularly filled floodplain wetlands (445,794 ha) (Kingsford *et al.* 2004), including some of national importance (Environment Australia 2001; SEWPaC 2011).
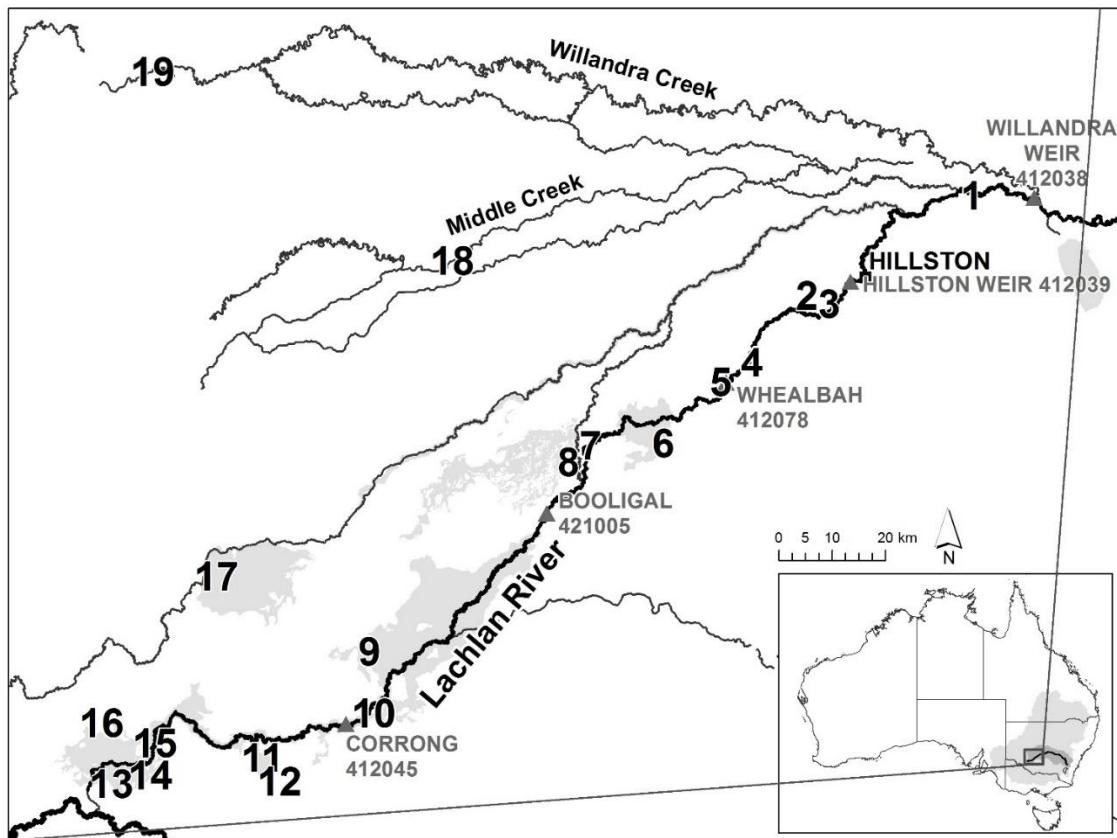
Figure 2.1 Floodplain wetland sites used in this study and the inset shows the location of the Lachlan River and its Catchment (shaded dark grey), Murray-Darling Basin (shaded light grey), and Australia. Numbers coincide with site name and site information described in table 2.1.

2.4 Methods

A variety of approaches have been used to identify changes in floodplain-river connectivity. These include hydrodynamic modelling (Tockner *et al.* 1999), analysing long-term flow data in relation to floodplain connection thresholds (Dyer 2002; Thoms 2003; Driver *et al.* 2004b), and analysing satellite imagery (Townsend and Walsh 1998; Overton and Doody 2008). In this study, I analysed and compared modelled long-term flow data under current and 'without development' flow scenarios in relation to floodplain connection thresholds.

A total of 19 floodplain wetland sites were assessed across the lower Lachlan River Catchment, NSW, Australia (Table 2.1 and Figure 2.1) during 2017. At each site, field surveys were conducted to confirm the floodplain habitat type based on the dominant perennial vegetation, geomorphological features and landscape position. Following the

Australian National Aquatic Ecosystem (ANAE) classification of Brooks *et al.* (2013), three floodplain habitat types were identified: 1. temporary floodplain lakes, being depressions devoid of trees or shrubs, 2. intermittent river red gum swamps, with river red gum the dominant tree species, including both woodland and forest forms, and 3. intermittent black box swamps, with black box the dominant tree species. These floodplain habitats are common on the Lachlan River. Another four sites were situated at the end of distributary channels of the Lachlan River. These four sites comprise Lake Tarwong and Lake Bullogal which are river red gum channel mound wetlands and Middle Creek and Kilfera which are Lignum Shrublands. These four floodplain wetland sites were grouped based on the extended duration of flooding they require to be connected to the river by floodwaters.

The commence to fill (CTF) of a floodplain wetland is the flow in the river when a floodplain wetland site starts to fill and is typically referenced to a nearby gauging station (Driver *et al.* 2004b; Catford *et al.* 2011). CTF values were determined using two lines of evidence: 1. field observations provided by the NSW OEH Environmental Water Manager for the Lachlan River Catchment and Driver *et al.* (2004b), as well as through analysis of Landsat imagery. The CTF values provided by the NSW OEH Environmental Water Manager for the Lachlan River Catchment were derived from numerous field observations, and observations by landholders, along with observations from near real time Landsat imagery during high flow events. The CTF values derived from Driver *et al.* (2004b) were developed based on numerous field observations and informed by Landsat imagery.

Using Landsat images to establish CTF values

Landsat imagery has been compared to river flow data to examine connection by others (Thoms 2003; Driver *et al.* 2004b) and this study similarly included analyses of Landsat datasets to assist with the characterization of floodplain habitat connectivity. An archive of 30m resolution Landsat 5 TM, Landsat 7 ETM+ and Landsat 8 OLI imagery for 2 scenes (path 093 row 083, and path 094 row 084) was included for analysis. The acquisition dates of the images spanned the period from 26/08/1986 to 11/02/2016 and included all available images for the relevant scenes. This imagery was provided by the USGS and corrected to surface reflectance (Flood *et al.* 2013) by the NSW Government's Imagery and Remote Sensing Facility. A water index WI_{2015} developed for Eastern Australia (Fisher *et al.* 2016) was then applied. To identify an optimal threshold value and classify wet and dry pixels, all

images were first thresholded at values of 5, 0, -5, -10, and -15. The thresholded Landsat images were visually interpreted and compared with single date ADS-40 50cm RGB images collected from 2008 to 2014, as well as known water storage areas and known areas of semi-permanent to permanently-wet and vegetated floodplain habitats within the lower Lachlan River Catchment. This visual assessment was completed independently by image analysts without knowledge of the study site locations. A threshold value of -10 was identified to provide the optimal trade-off between commission and omission error across all dates and the two Landsat tiles covering the study sites, and the -10 threshold value was subsequently applied to identify wet and dry pixels across all image dates and both scenes. In this case, the selected threshold is below the value of 0.63 identified as optimal by Fisher *et al.* (2016). However Fisher *et al*. (2016) developed a threshold optimized for all of continental Eastern Australia, including wetter coastal regions and drier inland regions, while this study required selection of a threshold for the smaller area and corresponding semi-arid climate of the lower Lachlan River Catchment. Further, the visual assessment found the -10 value correctly identified more wet pixels in areas of mixed water and vegetation and in this study omission error in areas of mixed pixels was of greater concern than commission error across the broader landscape. Following the identification of wet/dry pixels, a final image processing step was applied to identify cloud affected pixels and classify these as no data pixels (Zhu and Woodcock 2012).

The Landsat imagery data sets were compared to historic (observed) river flow records over the 30-year period (1986-2016) from the nearest river gauge (https://realtimedata.waternsw.com.au/). For each date within the Landsat imagery dataset with a wet pixel, the highest daily river flow (ML/per day) prior to the wet date and after the previous known dry date was recorded. In these instances, I could only estimate the flow volume in the river at the time when a given site had water present. This flow volume may not necessarily represent the CTF, and as such, this estimate was only calculated for sites with a close proximity to the Lachlan River (i.e., not terminal wetlands).

As multiple CTF values were derived for each site (over the 30 years), the lowest river flow (ML/day) value, where no rainfall was recorded (Bureau of Meteorology 2017) over the periods between wet and dry Landsat images was used. These CTF estimates were verified with the NSW OEH Environmental Water Manager for the Lachlan River Catchment.

Establishing flow scenarios

Long-term data sets are required to describe the characteristics in river flow patterns (Poff *et al.* 1997) and variability in flows and the consequent magnitude of uncertainty in bias and precision reduces with length of flow record (i.e. sample size) (Kennard *et al.* 2010). The considerable inter-annual variability in flows of the Lachlan River and the extended period over which water resources were developed make it challenging to evaluate differences to the hydrological regime related to water resource development through examination of historic flow data (i.e. comparing before and after regulation). The use of modeled flow data from different water resource development scenarios allows comparison between long term data sets. Further, the use of modelled data (in contrast to observed data) accounts for changes in climatic conditions and land-use effects on flow conditions. Comparing modeled flow scenarios during a common period is a widely used approach to identify hydrological changes to river flow patterns (Poff *et al.* 2010).

Modeled daily river flow data for river gauges on the lower Lachlan River (from 1895 to 2015) were obtained from New South Wales Department of Primary Industries under current and 'without development' flow scenarios. Modeling was undertaken using the Integrated Quantity and Quality Model (IQQM) designed to examine long-term flow behavior under different management regimes (Hameed and Podger 2001). The current flow conditions represent current water resource development conditions, including current water supply infrastructure and licensed extractions while 'without development' flow conditions have water management infrastructure and water extraction activities removed.

Modeled daily river flow data for the five river gauges used in this study, under current and 'without development' flow scenarios were partitioned according to their average recurrence interval (ARI) and compared (Table 2.2).

Calculating connection statistics

Using the calculated CTF values for each floodplain wetland site and the (IQQM) modelled river flow data from the nearest river gauge, the determination of daily connectivity to the river was calculated for 'without development' and current flow scenarios. A floodplain wetland site was assumed to be connected when the flow in the river exceeded the CTF (threshold) for that site. Our statistics are based around connection of the floodplain wetland

with the main channel and are thus framed in terms of connection and disconnection. It is acknowledged that floodwater is likely to persist for longer than the duration of connection as water is often retained within lower lying areas of floodplains. While duration of flooding relates to floodplain topography, duration of floodplain-river connection and local climatic conditions, I specifically set out to address the effects of river regulation which are likely to be directly attributable to changes in floodplain-river connection.

Using the CTF values and modelled current and 'without development' flow records I calculated seven connection metrics for each site (Appendix Table A1). These were: number of connection events, connection duration mean, connection duration median, connection duration maximum, dry spell mean, dry spell median, and dry spell maximum. These connection metrics represent important components of the flow regime of intermittent streams (Olden and Poff 2003).

The number of connection events, being the total number of times where the daily flow continuously exceeded the CTF (threshold), which occurred during the 120 years of modelled current and 'without development' flow records was modelled. Connect duration mean and connect duration median were the mean and median connection durations calculated from the total connection events that occurred in the 120 years of modelled current and 'without development' flow records. Connect duration maximum was the connection event with the maximum number of days of all connection events from the 120 years of modelled flow records. The dry spell is the number of days between connection events, i.e., a dry spell commences when the flow in the river drops below the CTF (threshold) and ends when the CTF is exceeded. The dry spell mean and median were the mean and median duration of a dry spell from the total number of dry spells from the 120 years of modelled flow records. Dry spell maximum was the dry spell event with the largest number of days that occurred in the 120 years of modelled flow records. The mean ($\pm$ s.e.) of each of the seven metrics within each habitat type and for all sites combined are presented in table 2.3. Proportion of change between the current and 'without development' scenarios was calculated for three of the connection metrics, the number of connection events (N), connect duration median (D), and dry spell median (T) for the floodplain wetland sites, habitat types, and for all sites combined. Change was calculated as the ratio of each of these connection metrics under current flow conditions from 'without development' flow conditions, where an increase will be >1 and a decrease will be <1.

Timing of connection was analysed by calculating the percentage of connection days (i.e. days > CTF) within each month from the 120 years of modelled current and 'without development' flow records. The mean (± s.e.) monthly percentage connections were plotted for each habitat type.

A permutational multivariate analysis of variance (perMANOVA) using distance matrices was undertaken comparing the seven metrics outlined in Appendix Table A1, comparing each habitat type (temporary floodplain lakes, intermittent river red gum swamps, intermittent black box swamps, and terminal wetlands) between current and 'without development' flow scenarios using adonis2 function in the R package vegan (Oksanen *et al.* 2013). This analysis makes no explicit assumptions. The perMANOVA analysis for all sites combined was corrected for variation between habitat types, and the analysis within individual habitat types was corrected for variation between sites. P-values were obtained using 999 permutations.

Table 2.1 Site names, floodplain habitat type, river gauge name, and commence to fill (CTF, ML/Day), and the information used to derive CTF estimates for floodplain wetland sites on the lower Lachlan River. three sources of CTF estimates are identified: [A] field estimate from Driver et al. (2004b); [B] estimate from satellite imagery; [C] field estimate from the NSW OEH Environmental Water Manager for the Lachlan River. Code coincides with the numbers used on the map of the study area (Figure 2.1).

Sites (Code -see Figure 2.1)	River Gauge Name (Number)	CTF (ML/day) estimate	Sources of CTF estimates
Temporary floodplain lake			
Whealbah Billabong (4)	Lachlan @ Whealbah (412078)	2700	3062-3584 [A]; 2700 [B] [C]
Booligal Swamp (7)	Lachlan @ Booligal (412005)	2500	2500 [A]
Lake Ita (11)	Lachlan @ Corrong (412045)	650	650 [C]
Lake Marool (15)	Lachlan @ Corrong (412045)	730	275-661 [A]; 730 [B] [C]
Clear Lake (13)	Lachlan @ Corrong (412045)	600	600 [B] [C]
Lignum Lake (16)	Lachlan @ Corrong (412045)	900	713 [A]; 900 [C]
Intermittent river red gum swamp			
Moon Moon Swamp (6)	Lachlan @ Whealbah (412078)	1600	2000 [A]; 1600 [B] [C]
Whealbah Billabong (5)	Lachlan @ Whealbah (412078)	3600	3600 [B] [C]
Clear Lake (14)	Lachlan @ Corrong (412045)	1500	1500 [B] [C]
Hazelwood (3)	Lachlan @ Hillston Weir (412039)	3000	2691 [A]; 3000 [C]
The Ville (10)	Lachlan @ Corrong (412045)	950	950 [B] [C]
Intermittent black box swamp			
Hunthawang (1)	Lachlan @ Willandra Weir (412038)	10000	10000 [B] [C]
Hazelwood (2)	Lachlan @ Hillston Weir (412039)	5700	5700 [C]
Lake Ita (12)	Lachlan @ Corrong (412045)	2000	2000 [B] [C]
Booligal Swamp (8)	Lachlan @ Booligal (412005)	3400	3400 [B] [C]
Terminal wetland			
Lake Bullogal (9)	Lachlan @ Booligal (412005)	1250 for 7 weeks	> 1250 for 7 weeks [C]
Lake Tarwong (17)	Lachlan @ Hillston Weir (412039)	1500 for 7 weeks	> 1500 for 7 weeks [C]
Willandra @ Kilfera (18)	Lachlan @ Willandra Weir (412038)	45 consecutive days flow >2600	> 2600 for 45 days [C]
Middle Creek @ Cobb HW (19)	Lachlan @ Willandra Weir (412038)	35 consecutive days flow >2600	> 2600 [A] and 35 days [C]

2.5   Results

Modelling results for the 'without development' and current flow scenarios show that the hydrological character of the Lachlan River has changed (Figure 2.2). The flow of the Lachlan River has not been uniformly affected by water resource development (Figure 2.3). Periods of no flow, which occurred approximately 12% of the time 'without development' now do not occur. Flows on the Lachlan River at Booligal Gauge that occurred 40% of the time 'without development' now occur 16% of the time, while flows that occurred 30% of the time 'without development' now occur approximately 15% of the time, and this difference continues to reduce with increasing flow (ML/day).
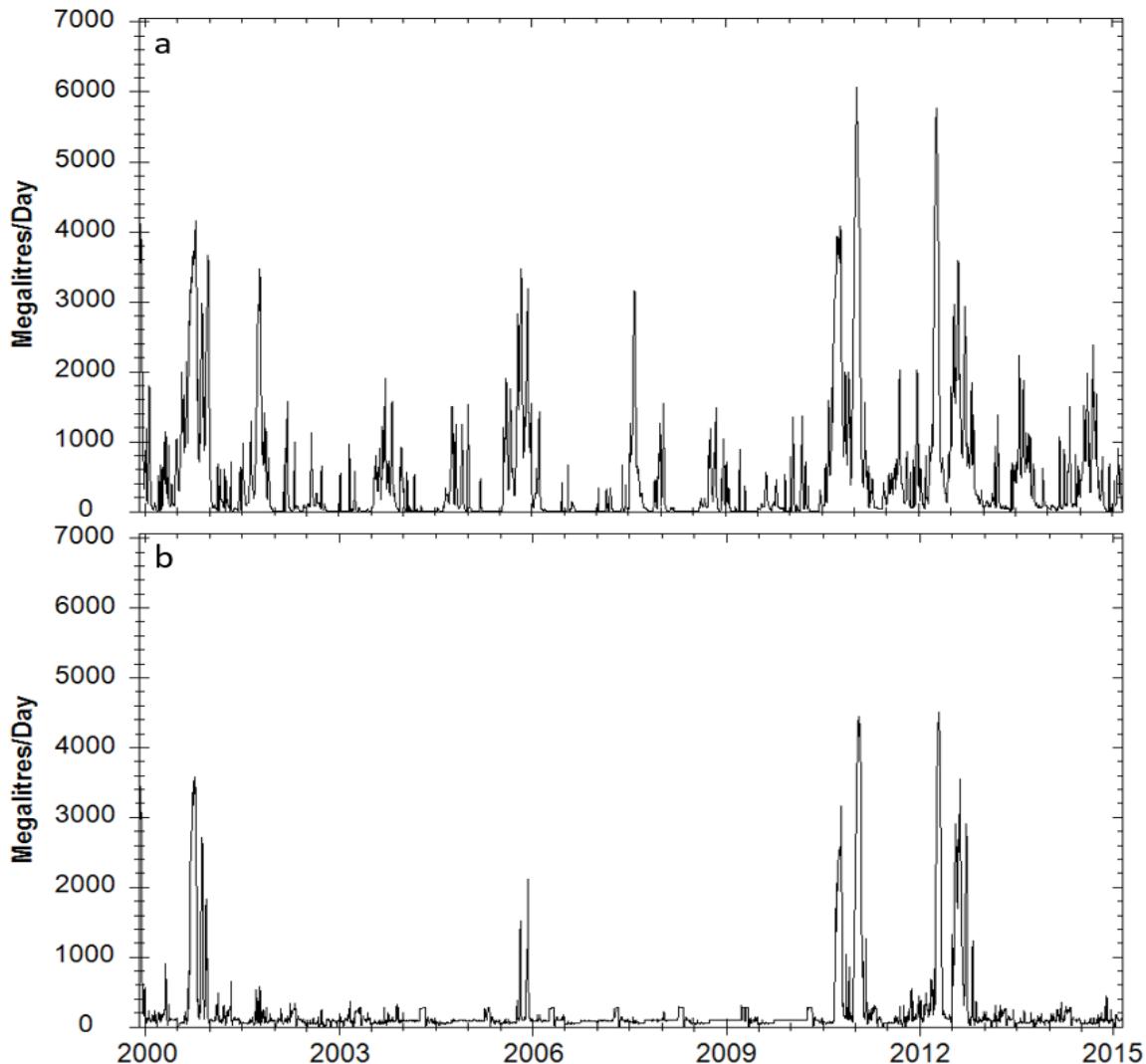
Figure 2.2 Modelled flow (Megalitres/day) of the Lachlan River at Booligal river gauge 'without development' (a) and under current flow rates (b) from 2000 to 2015.
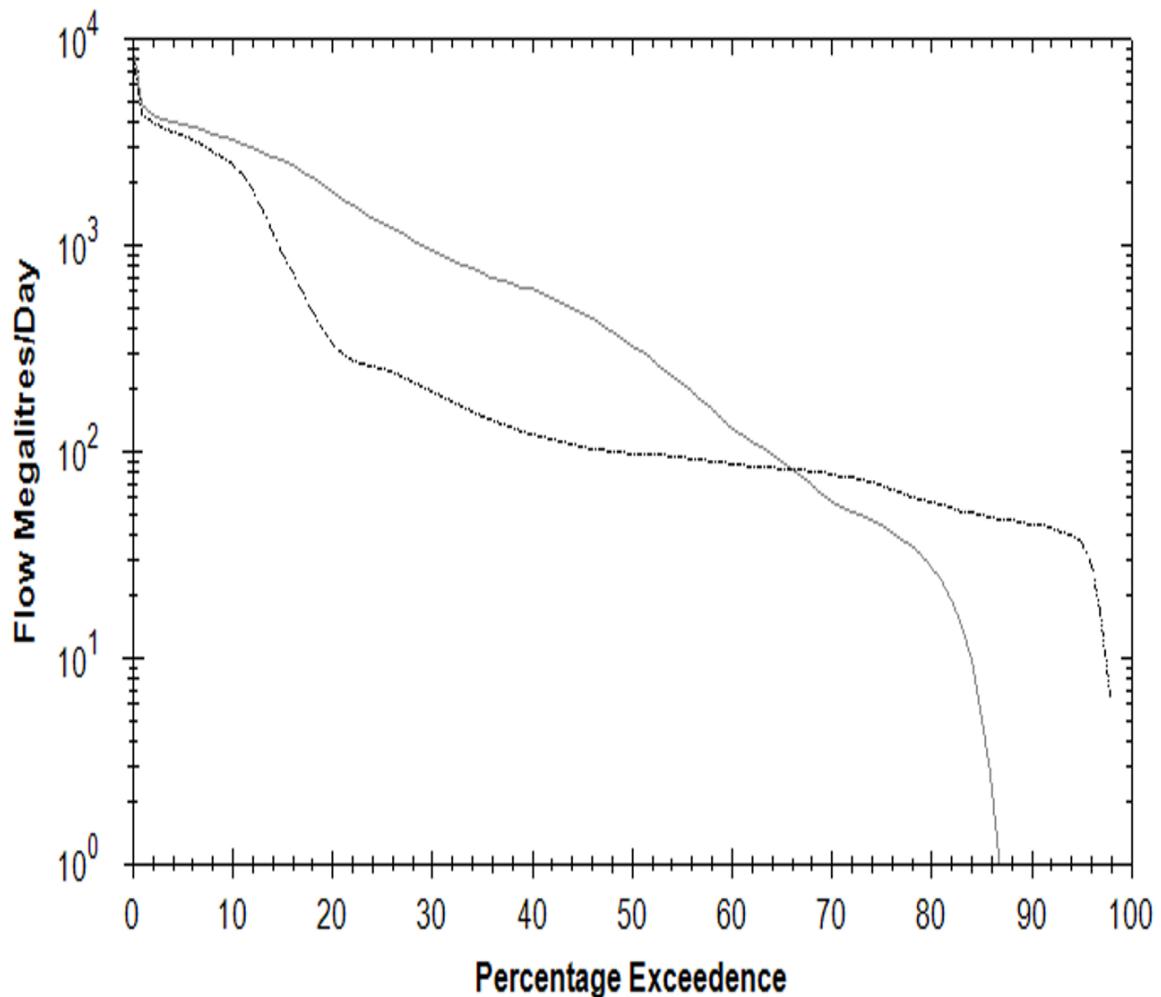
Figure 2.3 Percentage of time for a given discharge (ML/day on a logged scale) on the Lachlan River at Booligal Gauge for the river flow under the (modelled) current flow scenario (black dashed line) and 'without development' flow scenario (grey solid line).

At all (five) river gauging stations, flows with an ARI of 1.11 experienced the greatest reduction, having been reduced from 77 to 168%. Flows with an ARI of between 2 and 10 years were also impacted but to a lesser extent, reducing from 7 to 32%. Flows with an ARI of 24 years or greater, were impacted to a much lesser extent (Table 2.2).

Table 2.2 modelled current flow (ML/day) and the percentage change from 'without development' conditions at five river gauges on the lower Lachlan River. Average recurrence interval = ARI (yearly).

ARI	Willandra	Hillston	Whealbah	Booligal	Corrong
1.01	573 (-21)	408 (-35)	403 (-36)	268 (-37)	203 (-41)
1.11	1132 (-152)	810 (-168)	684 (-159)	302 (-80)	252 (-77)
2	7208 (-25)	4644 (-18)	3858 (-31)	3139 (-20)	1659 (-31)
5	11230 (-20)	5950 (-14)	5561 (-12)	3908 (-12)	2651 (-10)
10	17196 (-25)	7207 (-32)	6343 (-28)	4431 (-18)	2861 (-7)
24	26080 (-2)	9795 (-14)	8419 (-10)	5449 (-9)	3250 (-10)
60	29536 (-3)	10334 (-13)	9153 (-9)	5936 (-7)	3913 (-8)
120	62905 (7)	17932 (-8)	13551 (-6)	9183 (-7)	6042 (-13)

As expected, the floodplain habitat types on the floodplains of the lower Lachlan River varied in their connection characteristics (Table 2.3). Temporary floodplain lakes experience the most connection events and have the shortest dry spell (under both flow scenarios), intermittent river red gum swamps experience the second most connection events, followed by intermittent black box swamps, and terminal wetlands. Not surprisingly the number of connection events reduced with increasing CTF. The floodplain wetlands of the lower Lachlan River typically commence connection in spring (September and October), although the habitat types varied in the proportion of connection days per month (Figure 2.4). Although connection is most common between August to November, sites with a lower CTF value (such as many temporary floodplain lakes) can connect throughout the year, while sites with a higher CTF value like intermittent black box swamps only connect in spring (Figure 2.4).

The connection regime of the floodplain wetlands of the lower Lachlan River has been substantially modified under current flow conditions. The number of connection events under current flow conditions has been halved compared to flow conditions 'without development'.

The connection duration (median) has increased by 28% and the duration of the dry spell (median) has almost doubled (Table 2.3). No change in the timing of connection has occurred under current flow conditions (Figure 2.4).

Temporary floodplain lakes demonstrated the largest reduction in number of connection events (59%) of all habitat types, the change in connection duration (median) increased by around 20%, and the change in dry spell (median) increased by nearly two and half times. Intermittent river red gum swamps had the second largest reduction in number of connection events (52%) and a substantial increase in dry spell (median). Intermittent black box swamps demonstrated the least change in number of connection events of all habitat types, although the number of connection events did still reduce by 37%. Intermittent black box swamps also demonstrated the least change in connection duration (median) and dry spell (median) (Table 2.3).

Table 2.3 Connection metrics for floodplain habitat types on the lower Lachlan River under 'without development' (W) and current (C) flow scenarios for the Lachlan River (over 120 years) (± s.e.), and the change in the number of connections (N), change in the connection duration (median) (D), and change in the dry spell (median) (T). All connection duration and dry spell duration metrics are in number of days.

Sites	Number of connections (mean)		Connection duration (mean)		Connection duration (median)		Connection duration (max)		Dry spell (mean)		Dry spell (median)		Dry spell (max)		Change		
	W	C	W	C	W	C	W	C	W	C	W	C	W	C	N	D	T
All sites combined (n = 19)	272 ± 49	125 ± 17	29 ± 2	34 ± 2	14 ± 2	18 ± 2	248 ± 15	239 ± 9	336 ±79	574 ± 114	123 ± 34	236 ± 63	2196 ± 370	3815 ± 441	0.46	1.28	1.93
Temporary floodplain lakes (n = 6)	480 ± 74	197 ± 14	26 ± 1	31 ± 1	10 ± 2	12 ± 2	304 ± 10	271 ± 4	81 ± 20	196 ± 15	24 ± 6	40 ± 6	889 ± 180	2375 ± 385	0.41	1.25	1.65
Intermittent river red gum swamps (n = 5)	327 ± 63	158 ± 20	27 ± 3	32 ± 3	13 ± 3	15 ± 1	269 ± 13	261 ± 4	130 ± 29	266 ± 46	30 ± 8	108 ± 40	1199 ± 226	2521 ± 449	0.48	1.18	3.60
Intermittent black box swamps (n = 4)	113 ± 33	71 ± 24	22 ± 3	25 ± 4	14 ± 2	13 ± 3	175 ± 36	183 ± 24	478 ± 145	769 ± 196	171 ± 76	270 ± 40	3403 ± 552	5430 ± 810	0.63	0.93	1.58
Terminal Wetlands (n = 4)	51 ± 6	32 ± 3	41 ± 4	50 ± 2	23 ± 3	37 ± 2	211 ± 17	222 ± 2	836 ± 114	1331 ± 123	337 ± 45	655 ± 160	4193 ± 548	5980 ± 49	0.61	1.59	1.95
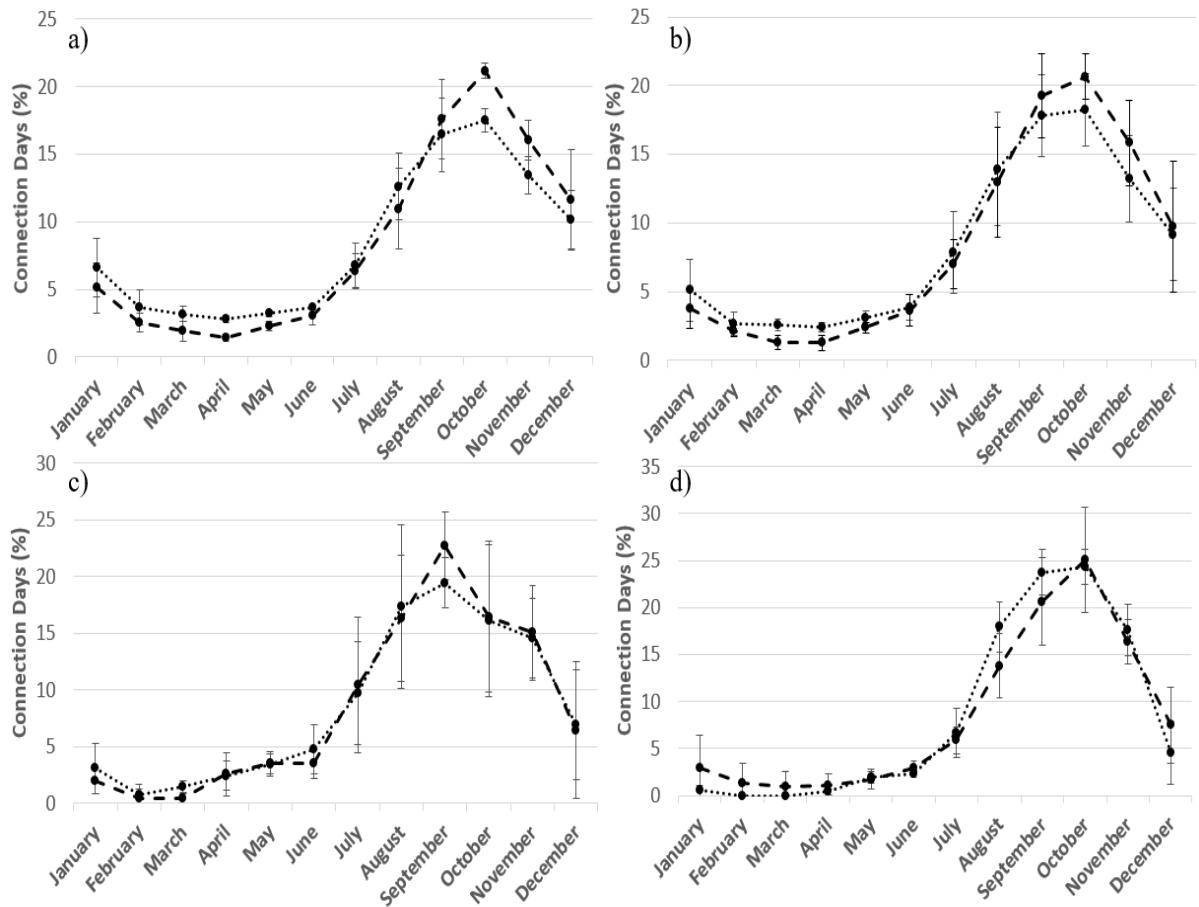
Figure 2.4 Modelled average number of days a site is connected to the river (i.e. days > CTF) for each month under 'without development' (dotted line) and current flow conditions (thick dashed line) for each floodplain habitat type. a) temporary floodplain lakes, b) intermittent river red gum swamps, c) intermittent black box swamps, and d) terminal wetlands.

The perMANOVA analysis based on dissimilarities between current and 'without development' flow scenarios combining the seven floodplain-river connection metrics (outlined in the Appendix Table A1) as a measure of connection regime, demonstrated a significant difference in connection regime between current and 'without development' flow scenarios for all sites combined, temporary floodplain lakes, intermittent river red gum swamps and terminal wetlands. No significant difference was observed in connection regime for the intermittent black box swamp sites (Table 2.4).

Table 2.4 perMANOVA results based on Bray Curtis dissimilarities between current and "without development" flow scenarios using the seven connection metrics presented in Table 2.2.

	Df	Sum Sq	R_2	F	P
All sites combined					
Compartment	1	0.484	0.135	5.633	0.018
Residuals	36	3.090	0.865		
Total	37	3.573	1.000		
Temporary floodplain lakes					
Compartment	1	0.401	0.584	14.043	0.002
Residuals	10	0.286	0.416		
Total	11	0.687	1.000		
Intermittent river red gum swamps					
Compartment	1	0.211	0.440	6.293	0.018
Residuals	8	0.268	0.560		
Total	9	0.478	1.000		
Intermittent black box swamps					
Compartment	1	0.085	0.297	2.528	0.178
Residuals	6	0.201	0.704		
Total	7	0.285	1.000		
Terminal wetlands					
Compartment	1	0.077	0.653	11.271	0.038
Residuals	6	0.041	0.347		
Total	7	0.117	1.000		

2.6 Discussion

The flow of the Lachlan River and subsequent floodplain connection naturally experiences a high degree of variability. This natural variability is vital to the rivers capacity to sustain biodiversity and ecological integrity (Stanford *et al.* 1996; Poff *et al.* 1997). The flow of the Lachlan River has been significantly modified by flow regulation by dams such as Wyangala, and the harvesting of water for stock, domestic and irrigation purposes (Driver *et al.* 2005; SEWPaC 2011). This study characterises the change between the Lachlan River 'without development' and under current flow scenarios and shows that changes in river flow pattern have substantially altered the connection regime of the floodplain wetlands of the lower Lachlan River. The number of connection events across the floodplain of the lower Lachlan River has been halved. This is consistent with work of Driver *et al.* (2005) who, applying slightly different modelling, calculated a 57% loss in wetland inundation frequency for the aggregation of temporary lakes and terminal wetlands that form the lower Lachlan swamps.

The loss of connection events, means that flow regulation has substantially increased the median number of days between connection events (dry spell), from four months (123 days) to more than seven months (236 days). Similar modelled reductions in the time between wetting events have been shown for other wetlands on the lower Lachlan River under different flow regulation scenarios (Driver *et al.* 2010). Further, the maximum dry spell has increased from six to 10 years across all sites. Water resource development has amplified the impacts of drought conditions on river flow, substantially reducing the frequency of flood events during drought conditions (van Dijk *et al.* 2013) and the results of this study confirm this, showing that under the current flow scenario, drought conditions are prolonged on floodplains. This means that the strategies aquatic and amphibious vegetation use to survive dry periods must include the ability to cope with the increased maximum dry period. A species ability to persist on the floodplain reduces with increasing length of a dry spell, for example the number of species germinating from the soil seed bank (Brock *et al.* 2003) and the ability of a floodplain shrub species (tangled lignum) to regenerate (Freestone *et al.* 2017) reduces during extended dry periods. Species that were at their limits of survival with a more natural drying regime are thus likely to have been lost from the system.

The median duration of connection events has increased slightly at most sites under current flow conditions. This slight increase is a result of the lesser number of frequently occurring

(ATI: 1 to 10-year interval, ARR, book 1 Chapter 2.2.5 http://book.arr.org.au.s3-website-ap-southeast-2.amazonaws.com/) small and medium flow events in the Lachlan River (Table 2.2 and Figure 2.3), which would have produced shorter periods of connection. Such changes to the flooding regime are a common theme across the Murray-Darling Basin (Bren 1991; Maheshwari *et al.* 1995; Dyer 2002; Catford *et al.* 2011).

I found no major changes in the monthly timing of connection. A seasonal shift in river flow has occurred in the Lachlan River resulting from water being released from dams such as Wyangala, for downstream irrigators and other uses of water, which is often required in periods which would have historically been periods of low or no flow (McMahon and Finlayson 2003; SEWPaC 2011). Water delivery for human requirements is primarily within channel and would very rarely exceed the bank full height. Thus, the results of this study show that these with-in channel changes to the flow of the Lachlan River have not resulted in changes to the timing of floodplain connection. The floodplain still typically connects to the river between August and November. These results differ from a similar study on the Barmah-Millewa forest, on the River Murray, where water resource development was found to have resulted in a seasonal shift in connection, and connection now occurs more frequently in summer (Bren 1988).

While the connection regime of all floodplain habitat types has been affected by the changed flow pattern of the Lachlan River, there are notable differences in the degree to which the different habitat types are affected. Temporary floodplain lakes have experienced the largest reduction in number of connection events and the intermittent black box swamps the least. This result is attributable to the loss of frequently occurring (ATI: 1 to 10-year interval) small and medium sized floods under current flow conditions. Larger, rarely occurring (ATI: 10 to 100 year intervals, ARR, book 1 Chapter 2.2.5 http://book.arr.org.au.s3-website-ap-southeast-2.amazonaws.com/) floods have been affected to a lesser degree. In the absence of water resource development, a greater number of small to medium sized flood events would have connected lower lying areas (i.e. temporary floodplain lakes and intermittent river red gum swamps) but would not have reached the extremities of the floodplain (i.e. intermittent black box swamps). A similar change has occurred in other parts of the Murray-Darling Basin, such as on the floodplain of the River Murray (Overton and Doody 2008) and the Barwon-Darling (Thoms and Sheldon 2000). The results support my hypothesis that habitat types which are closer to the river and which naturally would have connected more frequently are affected by

water resource development to a greater extent than communities on the extremities of the floodplain.

The physical environment of river ecosystems, including the floodplain is largely driven by the movement of water (Poff *et al.* 1997) and the composition and structure of the vegetation is a direct result of the hydrological regime (Poff and Ward 1989). It is well accepted that changes in flow regime has adverse consequences for a range of ecosystem processes and attributes, including vegetation communities (Stanford *et al.* 1996). Elsewhere changes to the flooding regime of floodplains has resulted in shifts in the spatial arrangement of vegetation communities within riparian areas and floodplains, with the migration of communities toward the channel (Bren 1992; Sankey *et al.* 2015), or a change in the vegetation community to one which is more adapted to the new hydrological regime (Caskey *et al.* 2015). This analysis gives some insight to possible changes in floodplain vegetation communities and habitat types that may occur, or possibly have already occurred, as a result of long-term hydrological changes. The changes to floodplain connection resulting from water resource development observed in this study can be linked to observed changes in ecological conditions to help understand the impacts which have already occurred, and predict future ecological changes (Poff *et al.* 2010).

Temporary floodplain lakes are areas on the floodplain which are most frequently connected and floodwaters generally sit in these lower-lying areas of the floodplain for extended periods (Roberts *et al.* 2016). While I acknowledge that this study focuses on connection and disconnection and not flooding duration river red gums may encroach/establish (or may have encroached) in to temporary floodplain lakes, as they may have the opportunity to establish as a result of the reduced connection frequency and duration (as seen in other areas in the Murray-Darling Basin (Bren 1992; Roberts and Marston 2011)). To the same end, landholders who maintained connections with the lower Lachlan River from the 1840s to the 2000s describe the conversion of much of the Cumbungi (*Typha* spp.) dominated habitat along the main channel of the Great Cumbung Swamp into more Common Reed (*Phragmites australis*) dominated habitat over time (Patrick Driver from pers. comm. c. 2000 with Robert 'Bob' McFarland). Changes to river flow patterns, have resulted in changes to the distribution of submergent macrophytes in other parts of the world, such as in the Grand Canyon, Arizona (Stevens *et al.* 1995).

While the results of this study demonstrated that the connection regime experienced by intermittent black box swamps has been least affected by water resource development, they have still been impacted. Studies on black box show that while it is resilient to drought, extended dry periods reduce tree health and opportunities for seedlings to establish (Moxham *et al.* 2018). As seeds do not persist in the soil, periodic flooding is required to ensure the species' resilience through dry periods.

Environmental flows have been delivered to certain sites in the lower Lachlan Catchment to maintain or improve vegetation condition as well as achieve other environmental outcomes (SEWPaC 2011). The delivery of environmental water is often constrained by the availability of water, the ramping impacts of human-induced climate change (van Dijk *et al.* 2013) and requirements to not flood private-land and infrastructure, and as a result inundation of much of the floodplain relies on unregulated flow events in wet years. The delivery of purchased environmental flow is constrained to areas with low CTF (i.e. temporary floodplain lakes) or sites where flow can be manipulated using weirs and levees.

The results presented here indicate that without active management over time, the current connection regime will result in loss of some temporary floodplain lake habitat, which are located predominantly in lower CTF areas, and a potential loss of aquatic respondent species with replacement by more drought tolerant species. The challenge for prioritisation of environmental water will be to maintain the health of representative floodplain habitat types within the area defined by lower CTF wetlands. This may mean allowing some migration of forest and woodland communities into temporary floodplain lakes and the change in the composition of wetland vegetation communities, while actively increasing connections and limiting encroachment of temporary floodplain lakes in other areas. Much of the floodplain and associated wetlands of the lower Lachlan River Catchment are unable to receive environmental water, particularly those on the margins of the floodplain such as the intermittent black box swamps. These are reliant on the boom periods for survival. The modifications to the connection regimes caused by water resource development for these wetlands are likely to result in changes to the species composition, with perhaps the loss of flood dependent species in some areas. Maintaining connectivity to allow migration of communities between higher CTF wetlands and the outer floodplain to areas closer to the river channel or with lower CTF values is an important and additional management challenge. Dealing with the impacts of water resource development on floodplain communities, is challenging, and maybe beyond what is achievable with environmental flow

alone. Further research is recommended on the importance of small and medium sized floods and the impacts of losing these from the system.

3. Differences in floodplain soil seed bank dynamics across hydrological gradients

Background: To understand the spatial and temporal distribution of plant species on floodplains, the role of hydrology, and the implications of changes to the hydrological regime because of water resource development (chapter 2) requires knowledge of the potential species. Chapter 3 explores the spatial and temporal variation in the germinable soil seed bank across a large inland floodplain and investigates the influence of floodplain-river connection frequency as well as recent and large-scale flooding on the density, diversity and composition of the soil seed bank. This study was initially interested in differences in the soil seed bank between sites within different floodplain habitat types, and as such, site selection was undertaken to maximise the number of sites within each floodplain habitat type. Field sampling occurred across two years: 2016 and 2017, and sites sampled in 2017 differed from those sampled in 2016. A major flood occurred between sampling years, and the decision to sample additional sites made it challenging to disentangle the effects of time and space between sites sampled in 2016 and 2017. However, the study provided important insight that contributed to the design of the rest of the study. This chapter is written in the style of a manuscript, to retain consistency with the thesis chapter formats. It was an exploratory study, undertaken at the start of the PhD that has informed the development of the broader study.

3.1   Abstract

Many (particularly annual) plant species which occur on floodplains produce a long-lived soil seed bank as a means of persisting through the temporally unpredictable hydrological regime. I investigated the effects of hydrological conditions on the characteristics of the soil seed bank of the floodplain of the lower Lachlan River. A seedling emergence experiment was conducted using soil collected from 25 sites from three typical floodplain habitat types (temporary floodplain lakes, intermittent river red gum swamps, and intermittent black box swamps) on the lower Lachlan River, which were subjected to four experimental treatments (rainfall, waterlogged soil, submerged, and receding). These experimental treatments represented typical conditions on a floodplain in a dry phase and during and following flooding. Soil was collected from 17 sites in May-June 2016 and a further eight sites in May-June 2017. During late 2016 and early 2017, the lower Lachlan River experienced the fourth largest flood event on record, and as such, soil collected in 2017 had been recently inundated. Soil seed bank density and species richness were compared to the number of floodplain-river connection events using 30 years of historic river flow data and commence to fill thresholds for each site. The density and species richness of seedlings was significantly higher on waterlogged soil than all other treatments. Seed bank density and species richness were significantly greater at sites which were connected less often compared to more regularly connected sites. Few woody perennial species germinated during the experiment. Sites collected in 2016 had significantly greater seed bank density and species richness than sites collected in 2017. Comparatively lower numbers of terrestrial species were present at sites collected in 2017 than sites collected in 2016, compared to aquatic and semi-aquatic species.

3.2   Introduction

The hydrological regime of floodplain landscapes structures and sustains the vegetation communities and habitat types occurring on them (Brock and Casanova 1997; Naiman and Decamps 1997; Roberts *et al.* 2016). Flooding provides connectivity, and increases productivity, and available habitat and resources (water and nutrients) on the floodplain and consequently, plays an overarching role in shaping floodplain communities (Porter *et al.* 2007; Reid *et al.* 2016; Van Looy *et al.* 2018).

Variability in the hydrological regime on floodplains results in a great deal of unpredictability for plants (Junk *et al.* 1989; Ward *et al.* 1999; Colloff and Baldwin 2010). As such, many plant species which occur on floodplains and in wetlands produce long-lived seeds as a means of persisting through unfavourable conditions (such as during a dry phase). This enables the population to regenerate once appropriate conditions for seed germination occurs (such as during or following re-wetting) (Chesson and Huntly 1989; Leck and Brock 2000; Brock *et al.* 2003; Brock 2011). These long-lived dormant seeds contribute to the soil seed bank (the bank of living seeds stored in the soil) (Templeton and Levin 1979).

The composition of the soil seed bank is a consequence of seed produced by past and present extant vegetation as well as seeds that have been contributed by various dispersal processes (Welling *et al.* 1988). Aquatic plants primarily disperse via hydrochory and, on floodplains, floodplain-river connection by flooding facilitates hydrochory, resulting in the introduction of seed to a site, while redistributing existing seeds (Nilsson *et al.* 2010). Waterbirds have also been identified as important disperses of aquatic plants (Green *et al.* 2008). Terrestrial species which may occur on floodplains often rely on wind or animals to disperse but may also disperse by hydrochory during flooding (Nilsson *et al.* 2010; Catford and Jansson 2014).

The germination of plants which occur on floodplains has been observed in response to specific hydrological conditions, which results in temporal shifts in plant community composition and structure (Casanova and Brock 2000; Capon 2003; Nicol *et al.* 2003). For example, flooding stimulates germination and growth responses in aquatic and semi-aquatic species (Van der Valk and Davis 1976; Rea and Ganf 1994; Robertson *et al.* 2001), resulting in these species re-establishing during flooding or following flood recession (Casanova and Brock 2000; Brock *et al.* 2003; Nicol *et al.* 2003; Capon and Brock 2006; Nielsen *et al.* 2014).

Seeds may be lost from the soil seed bank by germination, predation, and aging processes that reduce viability (Murdoch and Ellis 2000). While the seed of many floodplain species can remain viable for decades and over successive wetting and drying cycles (Brock and Rogers 1998; Brock 2011), during flooding, seeds not adapted to anaerobic conditions may be eliminated (as observed by Poiani and Dixon 1995). However, inputs in to the seed bank generally exceed losses, resulting in an accumulation of seed in the soil seed bank (Porter *et al.* 2007).

Infrequently flooded parts of floodplains have been observed to have a greater soil seed bank density and species richness than more frequently flooded parts of floodplains (Seabloom *et al.* 1998; Casanova and Brock 2000; James *et al.* 2007; Porter *et al.* 2007). This maybe attributed to the loss of seeds not adapted to anaerobic conditions (Poiani and Dixon 1995) as well as the reduced opportunity for terrestrial species to reproduce between flooding events. As observed by Capon (2007) and Casanova and Brock (2000), it may also be related to the large number of seeds which germinate in response to hydrological conditions which are typical during flooding and following flood recession (i.e., waterlogged soil), which would occur more frequently on areas more frequently flooded. Capon (2005) observed that frequently flooded parts of floodplains had a similar species composition to each other, consisting of a lower number of more flood tolerant species compared to the infrequently flooded parts of the floodplain. Seed bank density and species richness has also been observed to decrease following flooding on flood meadows on the Rhine River (Hölzel and Otte 2004). Additionally, prolonged drying (at least ten years) has been observed to reduce species richness in the seed bank compared to sites more recently flooded (Brock *et al.* 2003; Nielsen *et al.* 2013).

Components of the vegetation on floodplains have been observed to have varying levels of representation in the soil seed bank. For example, woody perennials such as trees and shrubs have been observed to be poorly represented in the soil seed bank compared to the extant vegetation (Middleton 2003; Greet 2016). Conversely, annual species especially forbs have been found to be represented in the soil seed bank while absent in the extant vegetation (Williams *et al.* 2008). As such, the soil seed bank is more likely to contribute to the restoration of annual species than woody perennial species during or following inundation (O'Donnell *et al.* 2016).

Casanova (2011) classified wetland species into functional groups to enable prediction of their responses to watering events. Water plant functional groups (WPFG) can provide a useful approach for considering the temporal and spatial variation of the germinable soil seed bank on floodplains (Capon and Brock 2006).

I investigated the influence of hydrological conditions on the soil seed bank of a large inland floodplain, on the lower Lachlan River. I examined how the hydrological conditions (dry, waterlogged, submerged, and receding), which are typical on floodplains, influence what germinates from the soil seed bank, and how the hydrological regime on the floodplain

influences what is available to germinate. A seedling emergence experiment was conducted on soil samples collected from a large semi-arid floodplain in three floodplain habitat types (temporary floodplain lakes, intermittent river red gum swamps and intermittent black box swamps) with varying hydrological regime. Seventeen sites were sampled during 2016. A further eight sites were sampled opportunistically in 2017 following a very large and extensive flood event, providing a comparison of the soil seed bank before and after a large flood event. It was predicted that the soil seed bank density and diversity would be greater at sites which are connected to the river less frequently compared to sites connected more frequently and that soil collected in 2017 following large and extensive flooding would have a lower density and diversity compared to sites sampled in 2016.

3.3 Methods

3.3.1 Data collection

The Lachlan River and its catchment form part of the Murray-Darling Basin (Figure 3.1). The lower Lachlan River Catchment is a very low gradient, alluvial plain made up of large areas of irregularly flooded floodplains (Page 1994; Kingsford *et al.* 2004; Meredith and Beesley 2009; Chapter 2).

Soil cores were collected from 25 locations at 12 sites across the floodplain of the lower Lachlan River, in May-June 2016 (17 locations) and May-June 2017 (8 locations) (Figure 3.1). For this study, a site was an area within the lower Lachlan River floodplain that comprises a number of different floodplain habitat types. Locations are transects along which soil cores were taken at a site and within a given floodplain habitat type. The 12 sites used as part of this study, were already established vegetation monitoring sites as part of a long-term monitoring project (Dyer *et al*. 2015) thus facilitating site access. At each site, there were one or more floodplain habitat types present, and thus, some sites have more than one sampling location (Figure 3.1). Locations were classified as occurring within three typical floodplain habitat types which occur on the lower Lachlan River (temporary floodplain lakes, intermittent river red gum swamps and intermittent black box swamps). At each location, the floodplain habitat type was determined using the Australian National Aquatic Ecosystem (ANAE) classification of Brooks *et al.* (2013). These floodplain habitat types vary in their connection frequency with the river. Temporary floodplain lakes connect to the main channel

of the river most frequently, intermittent river red gum swamps less frequently, and intermittent black box swamps the least frequently (Chapter 2; Table 3.1).

No standing water was present at the time of soil collection at all locations. At each site (14) soil cores (7 cm wide by 6 cm deep) were collected and aggregated from two randomly selected 50 metre transects spaced 20 metres apart. Surface leaf litter was removed from the soil prior to extracting each soil core. Following collection, the soil samples were taken to the University of Canberra glasshouse and sifted to remove large debris and stored in open containers for two weeks prior to the commencement of a seedling emergence experiment. During this two-week period the soil samples all dried to a similar soil moisture level.
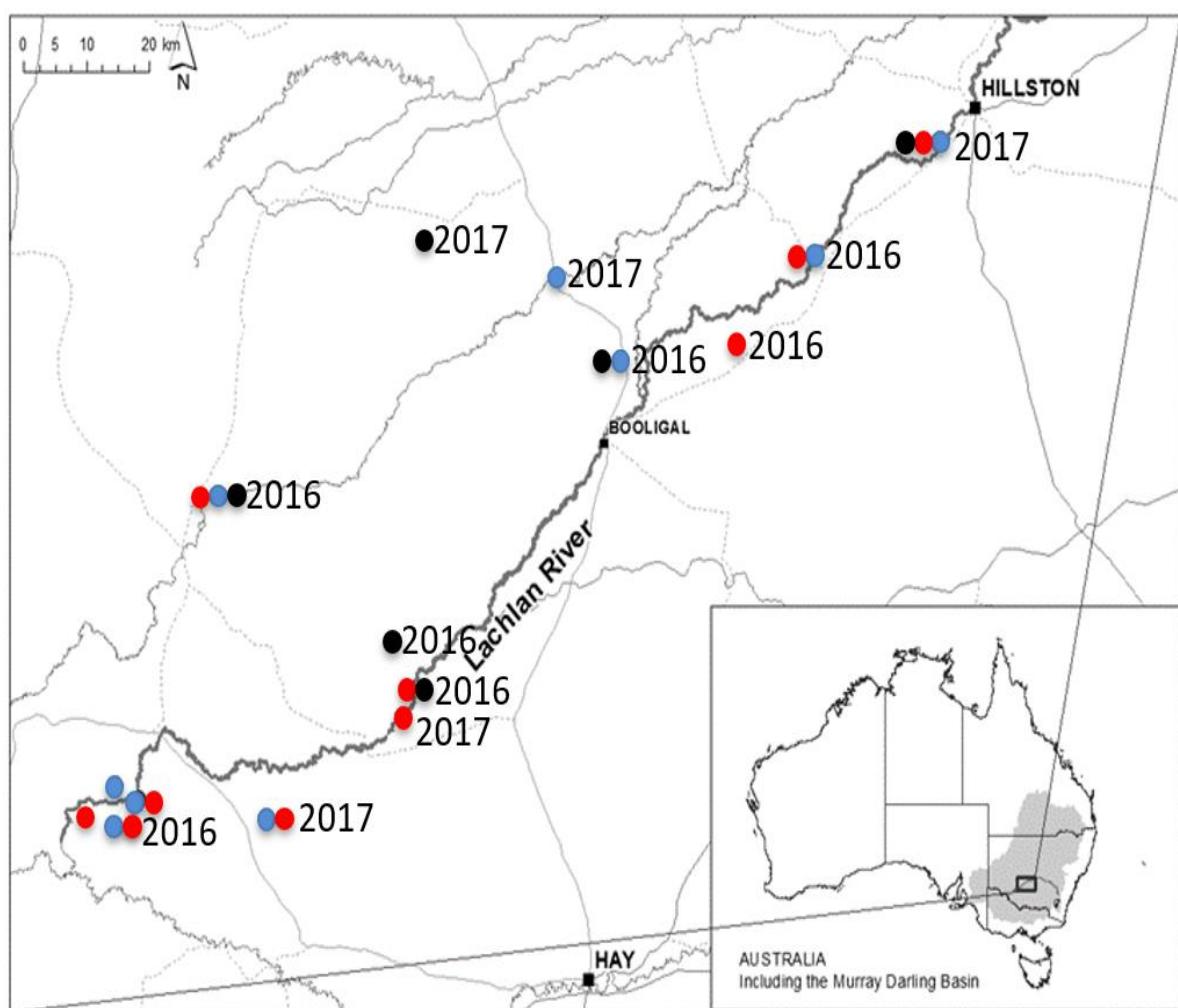
Figure 3.1 The study area on the lower Lachlan River, and the locations where soil samples were collected and year of sampling. Blue circles represent temporary floodplain lakes, red circles represent intermittent river red gum swamps and black circles represent intermittent black box swamps.

The number of floodplain-river connection events which have occurred over the past 30 years were calculated for 11 of the locations where soil was collected in 2016 and five of the locations where soil was collected in 2017. Hydrological data for the remaining nine locations were not available or the sites were excluded from the analysis because they operate under very different hydrological conditions to sites closer to the main channel of the river (see Chapter 2). The number of floodplain-river connection events were calculated using 30 years of historical river flow data (https://realtimedata.waternsw.com.au/) and the commence to fill (CTF) required to connect each site to the river (chapter 2; Table 3.1).

Each site was assumed to be connected to the river by floodwater when the flow in the river exceeded the CTF threshold and disconnected when the flow in the river dropped below the CTF threshold. The number of floodplain-river connections was the total number of connection events that occurred during the 30 years of flow records (Table 3.1).

Table 3.1 Sampling locations, floodplain habitat type (BB = black box swamp, RRG = river red gum swamp, mixed = river red gum, black box and river cooba, and TL = Temporary Lake) , floodplain/ wetland complex, land use type, year of sampling, and number of connection events which have occurred over the past 30 years. Sites with an Asterisk (*) were included in comparison between years, including all (eight) sites sampled in 2017 and eight sites from 2016 based on a number of criteria (page 57). NA, not available.

Location name	Floodplain habitat type	Floodplain /Wetland Complex	Land use	Sampling Year	Number of connections 30 yrs.
Lake Ita *	BB	Lachlan River floodplain	Nature conservation	2016	3
Lake Tarwong *	BB	Merrowie/Box Creek	Grazing (sheep)	2016	NA
Booligal Swamp *	BB	Booligal Wetlands	Nature conservation	2016	5
The Ville *	RRG	Lachlan Swamp	Nature conservation	2016	15
Lake Bullogal *	RRG	Lachlan Swamp	Grazing (sheep)	2016	NA
Nooran Lake	RRG	Great Cumbung Swamp	Grazing (cattle)	2016	9
Clear Lake	RRG	Great Cumbung Swamp	Grazing (cattle)	2016	9
Lake Marrool	RRG	Great Cumbung Swamp	Grazing	2016	13
Lake Tarwong	RRG	Merrowie/Box Creek	Grazing (sheep)	2016	NA
Moon Moon	RRG	Booligal Wetlands	Grazing (cattle)	2016	43
The Ville2	Mixed	Lachlan Swamp	Nature conservation	2016	NA
Tom's Lake *	TL	Booligal Wetlands	Grazing (cattle)	2016	NA
Lignum Lake *	TL	Great Cumbung Swamp	Grazing	2016	17
Lake Marool *	TL	Great Cumbung Swamp	Grazing	2016	28
Nooran Lake	TL	Great Cumbung Swamp	Grazing (cattle)	2016	33
Lake Tarwong	TL	Merrowie/Box Creek	Grazing (sheep)	2016	NA
Whealbah	TL	Lachlan River floodplain	Grazing (sheep)	2016	28
Murphy's Lake*	BB	Booligal Wetlands	Grazing (sheep)	2017	NA
TheVille*	BB	Lachlan Swamp	Nature conservation	2017	NA
Hazelwood*	BB	Lachlan River floodplain	Grazing (sheep)	2017	4
Whealbah*	RRG	Lachlan River floodplain	Grazing (sheep)	2017	21
Hazelwood*	RRG	Lachlan River floodplain	Grazing (sheep)	2017	28
Booligal Swamp*	TL	Booligal Wetlands	Nature conservation	2017	17
Hazelwood*	TL	Lachlan River floodplain	Grazing (sheep)	2017	NA
Lake Ita*	TL	Lachlan River floodplain	Nature conservation	2017	32

The fourth largest flood on record occurred in the Lachlan River system between August 2016 and March 2017, caused by rainfall in the upper Lachlan Catchment in 2016 (Bureau of Meteorology 2017). This event inundated the floodplain of the lower Lachlan River and flood waters had receded on most parts of the floodplain by April 2017 (W. Higgisson and F. Dyer pers. obs). A further eight locations which had not been sampled in 2016 were sampled following the flooding. This flooding meant that the eight locations sampled in 2017 were not directly comparable with those collected in 2016.

Two seedling emergence experiments were conducted at the University of Canberra glasshouse, Canberra. The first seedling emergence experiment commenced on 11 June 2016 using soil cores collected from 17 sites in 2016. The second seedling emergence experiment commenced on 19 June 2017 using soil cores collected from eight sites in 2017. The sifted soil from each site was divided evenly in to 20 (85 mm wide) square pots. Each pot contained 20 mm of washed river sand at the base of the pot and 20 mm of soil.

Five pots from each sampling site were allocated to each of four experimental treatments. The experimental treatments were designed to represent the range of hydrological conditions that might be experienced on the floodplain: (i.e., rainfall, waterlogged soil, flood recession, and submergence), in combination, these conditions optimise the potential for germination (Table 3.2). Five control pots, comprising 20 mm of washed river sand and no soil, were included in each treatment.

Table 3.2 Experimental treatments used in the soil seed bank seedling emergence experiment.

Treatment	floodplain conditions	Experimental conditions
Rainfall	Dry phase (wet only by rainfall)	Pots kept on trays allowing water to drain and watered approximately once to twice per week.
Waterlogged	Waterlogged/soaked soil typical of areas surrounding inundated parts of floodplains	Pots kept in plastic trays with the water level maintained at the top of the soil level (approximately 40 mm depth) throughout the experiment.
Receding	During flooding and flood recession	Pots kept in plastic tubs and flooded to a depth of 15 cm (above the soil surface), with recession of water at 2.5 cm per week, followed by soaked soil for the remainder of the experiment.
Submerged	During flooding and on areas of floodplains which experience extended inundation	Pots kept in plastic tubs and flooded to a depth of 15 cm for the length of the experiment.

Each seedling which germinated was counted weekly (providing seed bank density) and identified to species level (providing seed bank species richness and composition) once possible. at the end of the experiment, seedlings which were unable to be identified to species level were re-potted individually in sterilised potting mix to promote growth and the production of identifying features (flowers, seeds and fruit) and identified once possible. The seedling emergence experiments went for a total of 16 weeks.

During the first experiment from 11 June to 1 October 2016, the average maximum daily temperature was 25.4 °C and average minimum night-time temperature was 10.8 °C in the glasshouse. During the second experiment from 19 June to 9 October 2017, the average maximum daily temperature was 22.6 °C and average minimum night-time temperature was 8.6 °C in the glasshouse. These glasshouse temperatures were similar to the mean annual daily maximum (24.4 °C) and nightly minimum (10.9 °C) temperatures at Hillston (airport) (Bureau of Meteorology 2017), which is within close proximity to the study locations.

3.3.2 Data analysis

All statistical analysis was conducted in R (R Core Team 2013). The total number of seedlings (seed bank density) and number of species (species richness) at each location was calculated by combining all data from the four experimental treatments for each location. These data were converted to number of seedlings or number of species per square metre by multiplying each value by the number of times the total sampled area per site (14 soil cores each with a surface area of 38.48 cm^2) of 538.78 cm^2 fits in to one m^2 (18.56). Seed bank density and species richness within each experimental treatment and within each habitat type were analysed using a one-way ANOVA using locations as replicates and Tukey's HSD test to make pairwise comparisons. The data met the assumptions of ANOVA. Preliminary analysis demonstrated a weak effect of habitat type on the germinable soil seed bank and as such, interactions between habitat type and experimental treatment were not assessed. To investigate the influence of the number of floodplain-river connections (over the past 30 years) on the soil seed bank, a regression analysis was undertaken between the number of connection events and the seed bank density and the species richness. Regression analysis was done separately for data from the locations collected in 2016 and 2017. The land-use (i.e., nature conservation or grazing) at each site was not statistically analysed because of the low numbers of nature conservation locations used.

To compare the seed bank density and species richness of the soil seed bank of locations sampled before and after the flood event (in 2016 and 2017) a sub-sample of eight locations were used from the 17 locations sampled in 2016. These locations were randomly selected to have the same number of each habitat type (three temporary floodplain lakes, two intermittent river red gum swamps, and three intermittent black box swamps) as the eight locations sampled in 2017, while ensuring they were from different sites to avoid any local effect of site (table 3.2). The locations sampled in 2016 and 2017, were compared using a two samples T-test. The data met the assumptions of a T-test. Species observed in the sites used in comparing between years were grouped in to WPFGs defined by Casanova (2011), based on information compiled and used by the NSW Office of Environment and Heritage (NSW OEH, pers. Comm.). These data were plotted to compare differences in number of species within each WPFG and between years. These data were not statistically analysed as sites sampled in 2016 were different to those sampled in 2017 and as such were not directly comparable. Species were also classified as native or non-native using information provided by PlantNET (http://plantnet.rbgsyd.nsw.gov.au/).

3.3.3 Extant vegetation surveys

Extant vegetation data were obtained from a Commonwealth Government funded monitoring program (Dyer et al 2015) for 10 sites (Appendix B, Figure B1) in the lower Lachlan catchment. The data were collected in spring and Autumn during 2016, 2017 and 2018 from field surveys of sites that were near to those at which soil was collected for this thesis. Field collection methods are outlined in Dyer et al 2015, but in short, at each site, two 100 m transects were extended from the fringing woodland into the deeper section of the wetland. Species were recorded in 1 m^2 quadrats placed at 10 m intervals along the transect. During field surveys it was noted if the site was in a dry, partially wet, or wet condition. All plants were identified to species level during field surveys or using specimens.

This study was primarily designed to describe the spatial and temporal variation in the soil seed bank on the floodplain of the lower Lachlan River. However, it was also used to begin to explore the role of the hydrological regime and the extant vegetation in controlling the spatial and temporal variations observed. At some sites, the transects used during extant vegetation surveys were extended over two habitat types, transects were not positioned at the exact locations where soil was collected, and there were some sites where soil was collected and where extant vegetation surveys were not conducted. For these reasons, the extant vegetation

data for all 10 sites and from all six surveys were combined and compared to the total list of species from the soil seed bank experiment as presence/absence data, without statistical analysis.

3.4   Results

A total of 4481 seeds germinated during both seedling emergence experiments, consisting of 83 different species from 41 families. The species comprised 47% annual species, 38% perennial species, and 15% annual/perennial or bi-annual species. Nearly three quarters (73%) of the species were native, compared to 27% introduced species. Of the species present, the majority (80 species) were flowering plants, as well as two species of Charophytes (*Chara* sp. and *Nitella furcata*), and one fern species (*Marsilea drummondii*). Asteraceae was the most commonly represented family with 11 species. *Centipeda cunninghamii* was the most commonly occurring species with 275 seedlings from 18 of the 25 locations. 30 species were only represented by one or two individuals. No seeds germinated within the control pots during the experiment.

The mean germinable soil seed bank density per location was 3449 $m^2 \pm$ 470 m^2, ranging from 787 germinable seeds per m^2 to 8064 germinable seeds per m^2. A mean of 13 ± 1.23 species germinated per location, but species richness varied considerably between locations (from 3 to 24 species).

3.4.1 Effect of treatment type

The germinable soil seed bank density (both years combined) differed significantly between experimental treatments ($F(3,104) = 10.48$, $P = <0.001$; Figure 3.2a). The waterlogged treatment had a significantly larger number of seedlings germinate per location than all other treatments (i.e., rainfall $P = 0.022$, receding $P <0.001$, and submerged $P <0.001$). The species richness in the experimental treatments also varied significantly between treatment ($F(3,91) = 20.24$, $P = <0.001$; Figure 3.2b). The waterlogged treatment had significantly more species germinate per location than that in all other treatments ($P = <0.001$ in all treatments; Figure 3.2b). The waterlogged treatment had 64 species germinate across all locations during the experiment, compared to the receding treatment with 36 species, rainfall treatment with 32 species, and submerged treatment with 10 species.
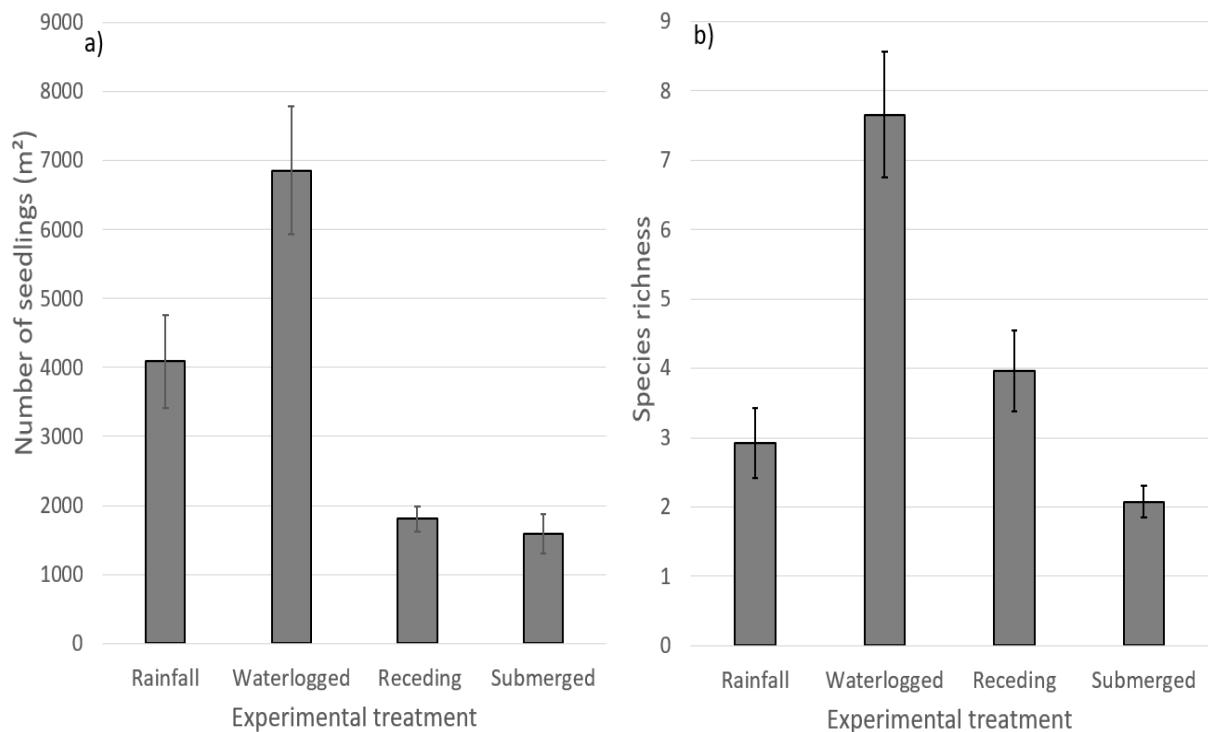
Figure 3.2 a) Mean ± s.e.m. germinable soil seed bank density (number of seedlings per m^2), and b) the mean ± s.e.m. number of species which germinated (species richness) per location, within each of the four experimental treatments.

Some species demonstrated a strong preference for an experimental treatment. A total of eight species occurred solely in the rainfall treatment, including native species; *Atriplex suberecta*, *Poa fordeana* and *Zygophyllum apiculatum*, and five introduced species (*Cirsium vulgare*, *Hordeum leporinum*, *Lolium rigidum*, *Stellaria media* and *Schismus barbatus*). A total of 22 species occurred solely in the waterlogged treatment, including native species such as *Eucalyptus camaldulensis* and *Rorippa laciniata* and introduced species such as *Veronica peregrina* and *Oxalis corniculata*. A total of four species occurred solely in the receding treatment, three native species (*Ammannia multiflora*, *Goodenia heteromera*, and *Triglochin procera*), and one introduced species (*Heliotropium europaeum*). Three species occurred solely in the submerged treatment (*Marsilea drummondii*, *Myriophyllum variifolium* and *Nitella furcata*), all of which are native. Another three species *Chara* sp.*, Potamogeton tricarinatus* and *Lemna disperma* (all of which are native) occurred in both submerged and receding treatments. No species were observed across all four treatments (see Appendix Table B1 for a full list of species, and the likelihood of them occurring within each experimental treatment).

3.4.2 The seed bank within each floodplain habitat type

There was no significant difference in germinable soil seed bank density per location between the three floodplain habitat types ($F(2,23) = 0.703$, $P = 0.506$) (both years combined), although the mean germinable soil seed bank density was higher at intermittent black box swamp sites (4102 ± 1201 m^2), than intermittent river red gum swamp sites (3078 ± 667 m^2), and temporary floodplain lake sites (2745 ± 540 m^2), which had the lowest mean germinable soil seed bank density per site. The species richness in the germinable soil seed bank also did not vary significantly between habitat types ($F(2,23) = 1.827$, $P = 0.183$). The mean number of species in the germinable soil seed bank was 14.83 ± 3.94 in intermittent black box swamp sites, 14.55 ± 1.45 in intermittent river red gum swamp sites, and 9.89 ± 1.41 in temporary floodplain lakes (Appendix B2).

3.4.3 Number of floodplain–river connection events

The number of floodplain-river connection events at each location (over 30 years) had a significant effect on germinable seed bank density ($R^2 = 0.50$, $F(1, 9) = 9.07$, $P = 0.01$) for locations sampled in 2016, with locations with fewer connection events having a greater germinable seed bank density compared to those with a greater number of connection events (Figure 3.3). The number of floodplain-river connection events had no significant effect on seed bank density ($R^2 = 0.44$, $F(1, 3) = 2.36$, $P = 0.22$) for locations sampled in 2017.
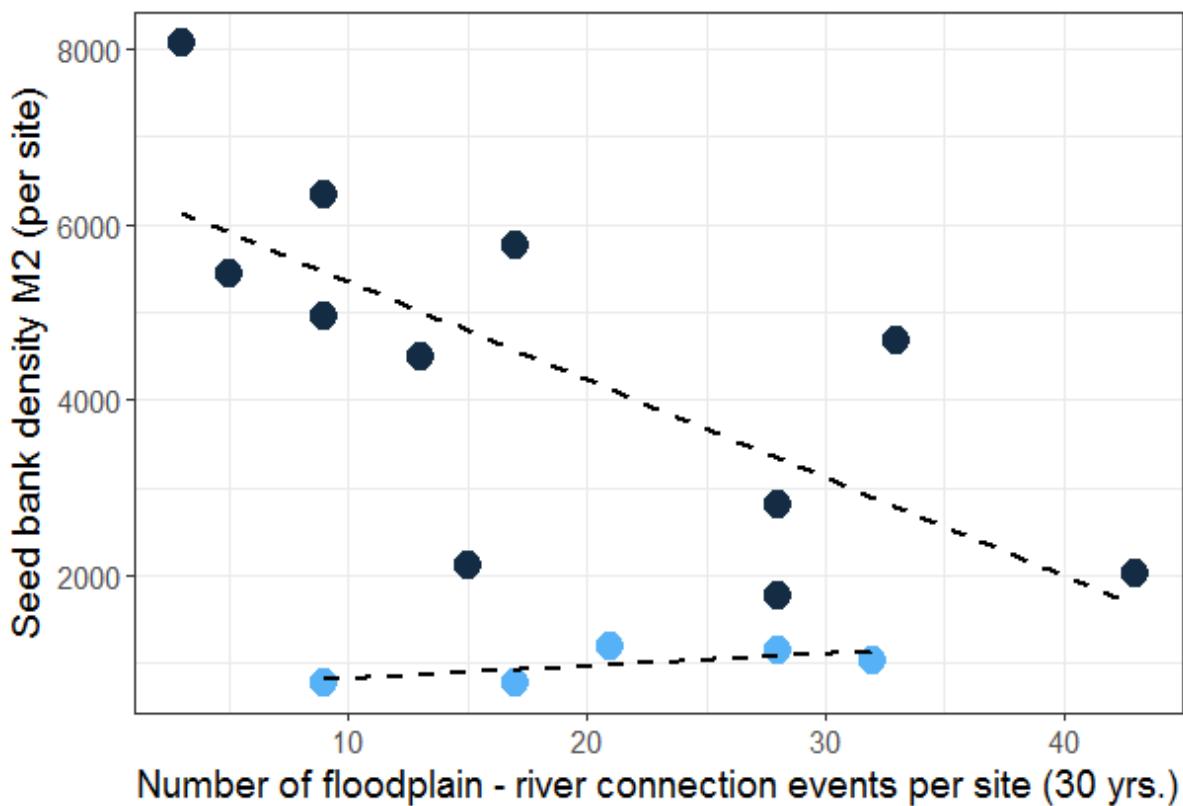
Figure 3.3 Linear regression analysis of seed bank density (per m^2) per location and the number of floodplain-river connection events per location from 30 years (1986 - 2015) of historical river flow data. Black dots represent data from locations sampled in 2016 and blue dots represent data from locations collected in 2017. Note that different locations were sampled in 2016 and 2017.

The number of floodplain-river connection events at each location also had a significant effect on the number of species (species richness) germinating from the soil seed bank ($R^2 =$ 0.85, $F_{(1, 9)}$ =51, P = <0.001) for locations sampled in 2016, with locations with fewer connection events having a greater number of species (Figure 3.4). Using the five locations from 2017, the number of floodplain-river connection events at each location had no significant effect on number of species in the germinable soil seed bank (R^2 = 0.17, $F_{(1, 3)}$ = 0.62, P = 0.49) for locations sampled in 2017.
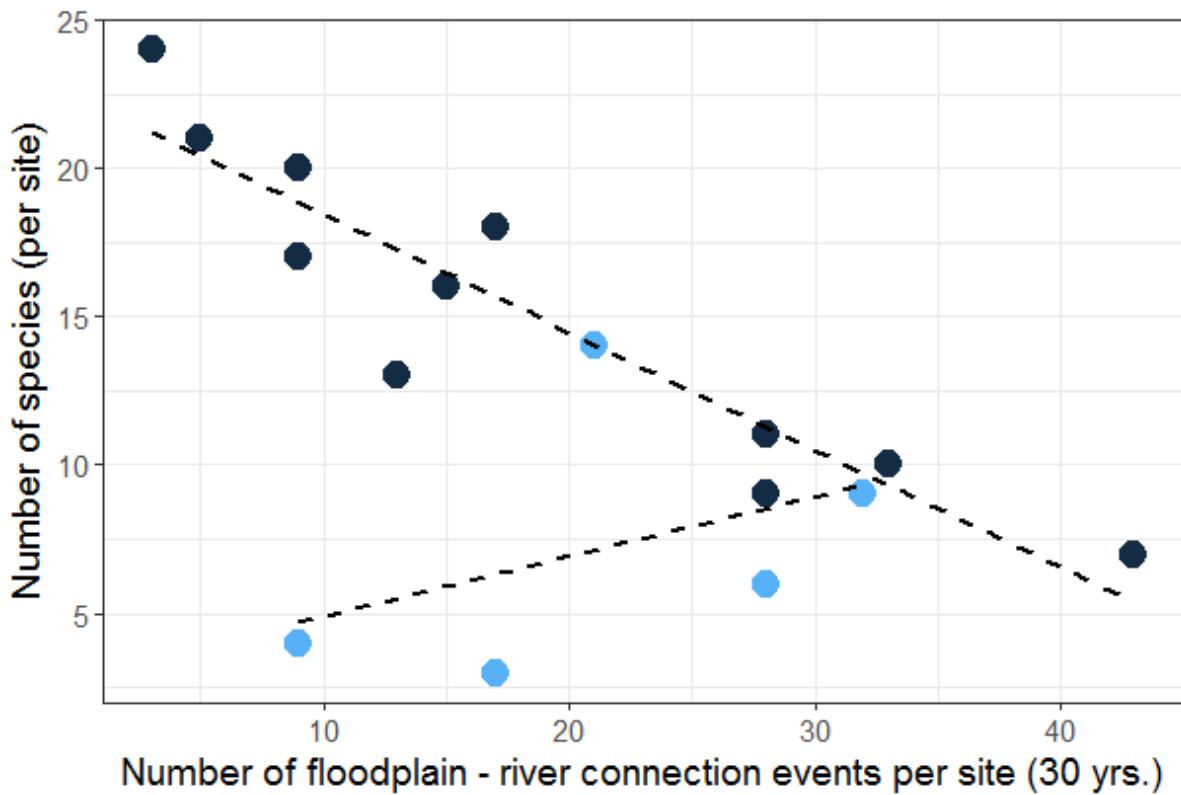
Figure 3.4 Linear regression analysis of the number of species (species richness) which germinated from the soil seed bank at each location, and the number of floodplain-river connection events per site from 30 years (1986 - 2015) of historical river flow data. Black dots represent data from locations sampled in 2016 and blue dots represent data from locations collected in 2017. Note that different locations were sampled in 2016 and 2017.

3.4.4 The effect of a major flood

The germinable soil seed bank varied considerably between sampling years (2016 and 2017) comparing the eight sub sampled locations in 2016 and eight locations in 2017. The soil samples which were collected in 2016 had a significantly larger germinable seed bank density (t = 3.233, df 8.25, p = 0.0115) compared to those collected in 2017, with a mean germinable soil seed bank density in 2016 of 4487 ± 895 m^2 compared to 1465 ± 269 m^2 (Figure 3.5a). The soil seed bank samples which were collected in 2016 also had a significantly greater mean number of species germinating per site (t = 4.505, df 13.49, p = <0.0005) than those

collected in 2017, with a mean species richness in 2016 of 18 ± 1.87 compared to 2017 with 7 ± 1.53 species (Figure 3.5b).
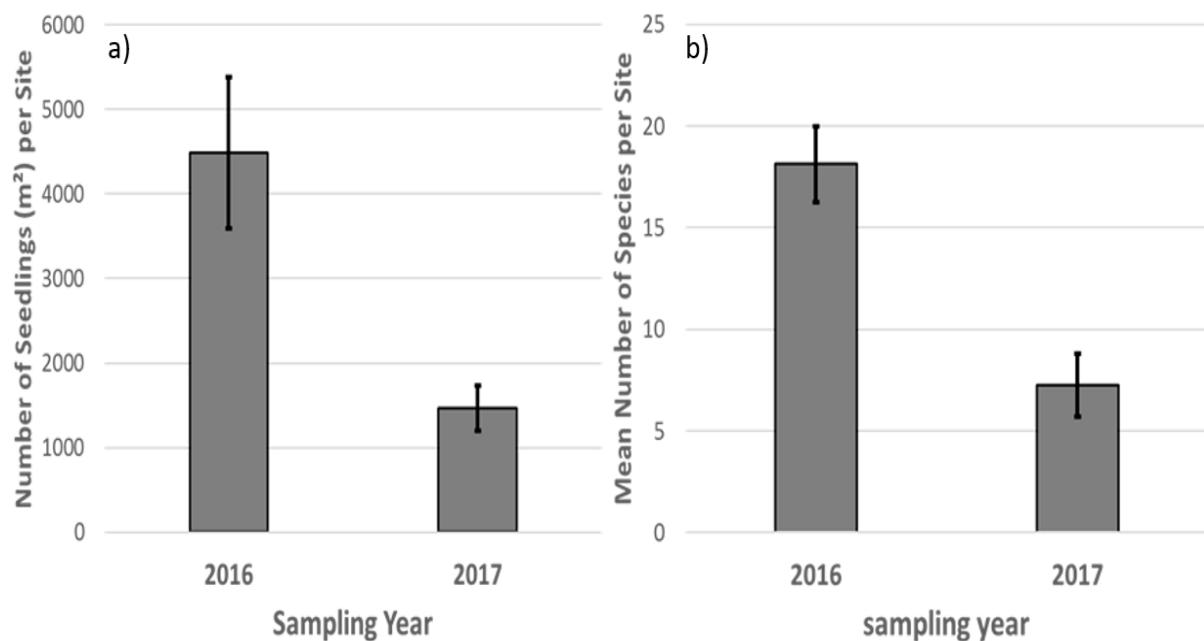
Figure 3.5 a) Germinable seed bank density (per m^2) per location, and b) mean number of species (species richness) per location, from samples collected from eight locations collected in 2016 and eight different locations collected in 2017. Sites selected for analysis were from three temporary floodplain lakes, two river red gum wetland, and three black box woodland sites in each year (table 3.2).

A total of 65 species germinated from the eight sub sampled locations in 2016 and eight locations in 2017. A total of 27 species occurred in both years of sampling, 31 species only occurred at locations collected in 2016, and seven species only occurred at locations collected in 2017. The two most common WPFGs in both years were Terrestrial dry (Tdr) and Terrestrial damp (Tda). Fewer species were observed in all other WPFGs in both years (Figure 3.6). The number of species from each WPFG varied between sampling years (Figure 3.6). Fewer species were observed in WPFGs Tdr (27 species in 2016 and 12 species in 2017) and Tda (16 species in 2016 and 9 species in 2017) in locations sampled in 2017. Functional groups' Amphibious fluctuation responder – floating (ARf) (one species *Marsilea drummondii*), and Perennial – emergent (Se) (species *Poa fordeana* and *Triglochin procera*) were only observed at sites sampled in 2017. There was one Amphibious fluctuation tolerator – low-growing (ATl) species (*Limosella australis*) and two Submerged – r-selected (Sr) species (*Nitella furcata* and *Chara* sp.), and these species were present in both years. Five

Amphibious fluctuation tolerator – emergent (Ate) species germinated, four species in both years (*Cyperus difformis*, *Eleocharis acuta*, *Juncus flavidus*, and *Ranunculus pumilio*) and one species only in 2017 (*Juncus articulatus*). A total of five Amphibious fluctuation responder-plastic (Arp) species germinated, three species in both years (*Damasonium minus*, *Myriophyllum crispatum Myriophyllum veruccosum*) and two species only in 2016 (*Elatine gratioloides,* and *Potamogeton tricarinatus*).
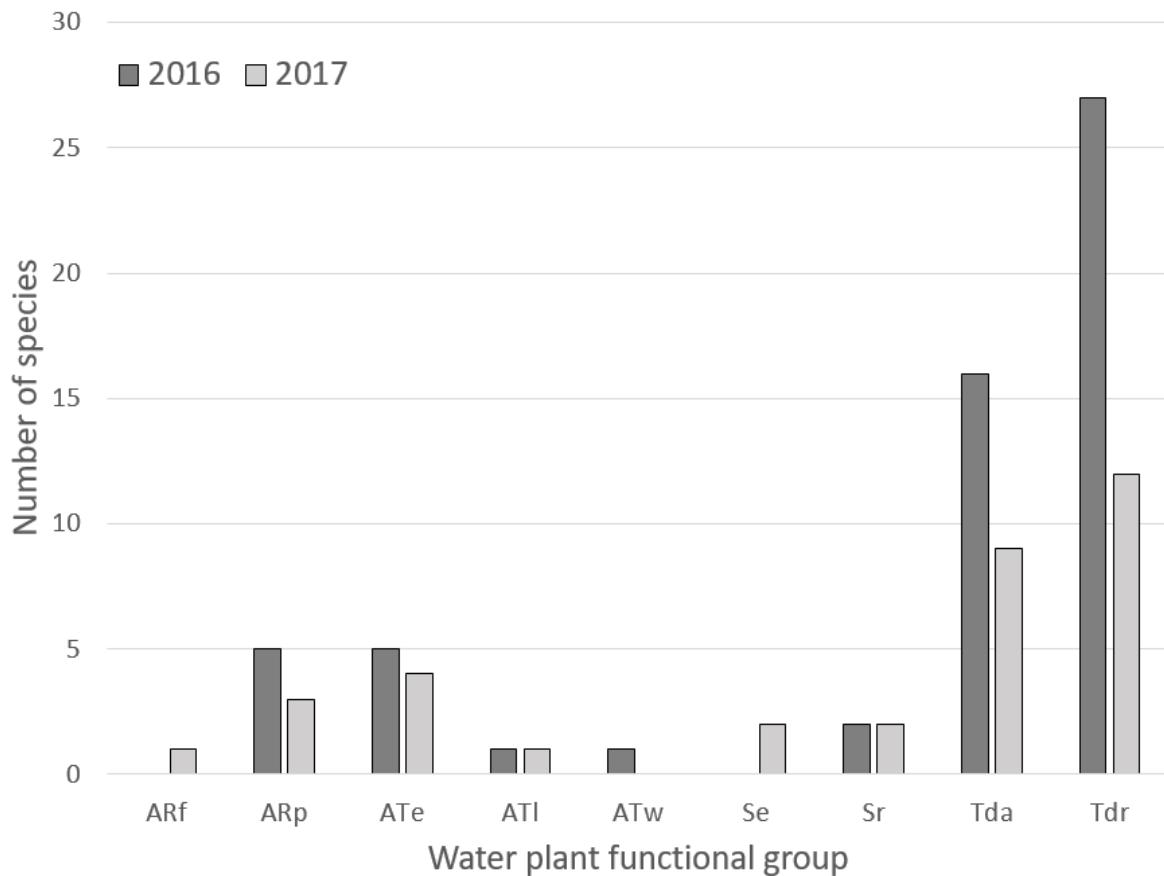
Figure 3.6 Total number of species that germinated from the seedling emergence experiment from the (eight) sites collected in 2016 and eight sites collected in 2017 from each water plant functional group (defined in Casanova 2011). ARf = Amphibious fluctuation responder – floating, Arp = Amphibious fluctuation responder – plastic, Ate = Amphibious fluctuation tolerator – emergent, ATl = Amphibious fluctuation tolerator – low growing, ATw = Amphibious fluctuation tolerator – woody, Se = Perennial - emergent, Sr = Submerged – r selected, Tda = Terrestrial damp, Tdr = Terrestrial dry.

3.4.5 Comparison between extant vegetation and germinable seed bank

The combined list of species from the extant vegetation surveys and the soil seed bank germination experiment included a total of 182 species. This included 154 species which were recorded during field surveys. A total of 54 species occurred in both the extant vegetation surveys and the seedling emergence experiment. A total of 27 species occurred solely in the seedling emergence experiment, these included both algae species (*Chara* sp. and *Nitella furcata*) and submerged aquatic species (such as *Potamogenton tricarinatus*, *Myriophyllum crispatum*, *Myriophyllum variifolium*, and *Elatine gratioloides*). A range of long-lived species were present in the extant vegetation and absent from the soil seed bank. A total of 99 species occurred only in the extant vegetation surveys, including perennial species *Acacia stenophylla, Enchylaena tomentosa, Chenopodium nitrariaceum* and *Duma florulenta*. Over half of the 100 species (53 species) which were absent from the seedling emergence experiment were observed only during field surveys in dry conditions (many of which are in the *Chenopodiaceae* family, including four species of *Sclerolaena*, four species of *Atriplex*, two species of *Maireana*, and *Rhagodia spinescens*). In contrast, only four species were observed solely in field surveys and absent from the seed bank only during the partially wet surveys (*Brassica tournefortii*, *Calotis scapigera*, *Raphanus raphanistrum*, *Euphorbia planiticola*) and eight in wet surveys (see appendix Table B3 for a full list of species observed during field surveys). The extant vegetation had a slightly lower proportion of native species identified (60%) than the germinable seed bank (73%).

3.5   Discussion

The germination numbers from the soil seed bank of the floodplain of the lower Lachlan Catchment, were low ranging from 787 to 8064 germinable seeds per m^2, compared to the germinable soil seed bank reported from other floodplains, such as the Paroo and Warrego floodplains in Queensland (7678 per m^2) (Porter *et al.* 2007) and Bool and Hacks Lagoons in South Australia (>20000 per m^2) (Nicol *et al.* 2003). The lower germination numbers may be a result of the combined experimental treatments reducing the overall mean germination number. The different experimental treatments resulted in very different germinable seed bank densities. Thus, the seed bank densities reported in this study are likely to be an underestimate of the total soil seed bank.

The species richness germinating from the soil seed bank (total 83 species), was comparable to other soil seed bank studies on floodplains and in wetlands, such as Yellingbo Nature Reserve (80 species) (Greet 2016), Cooper Creek Catchment (36 species) (Capon 2007), Goulburn Catchment (55 Species) (Williams *et al.* 2008), and New England Tablelands (73 species) (Brock *et al.* 2003). It should be acknowledged that sampling effort varies between these studies and this will likely influence the number of species observed.

3.5.1 The effect of treatment type

The results of this study demonstrate that a large number of floodplain species germinate in response to flooding, flood recession, and on waterlogged soil, thus, demonstrating the importance of flooding conditions in the re-establishment of floodplain vegetation from the soil seed bank following a dry phase. Waterlogged soil was found to have the most seedlings germinate of all treatments. The number of germinants has been found to be much higher on waterlogged soil compared to inundated or rainfall conditions by others (Casanova and Brock 2000; Capon 2007). The fact that waterlogged soil conditions had significantly more seeds of a greater number of species germinate than all other treatments reflects that a large number of species which occur on floodplains germinate during very large rainfall events and on waterlogged soil which would occur on higher parts of floodplains during floodplain inundation and on lower parts of floodplains following flood recession. Thus, demonstrating that these species capitilise on the increased availability of habitat and resources which occurs during floodplain-river connection (Reid *et al.* 2016) and have evolved to tolerate and exploit the hydrological variabilty to which they are exposed (Poff *et al.* 1997).

Many species had a strong association with an experimental treatment, confirming that the vegetation on floodplains and wetlands shifts in response to the hydrological regime (Van der Valk and Davis 1976; Seabloom *et al.* 1998; Capon 2003), and species persist in the soil seed bank and contribute to the extant vegetation when conditions permit. The wet and dry phases on floodplains, thus, have selected for wetting and drying as germination cues, as discussed by Leck and Brock (2000). Colloff and Baldwin (2010) proposed that floodplains shift between two alternative stable states (wet and dry), each state with characteristic dominant species. This study supports this, demonstrating that around half (40/83) the total number of species only occurred within a single treatment.

3.5.2 Spatial variation in the soil seed bank

While the soil seed bank density and species richness did not vary considerably between floodplain habitat types, both were strongly correlated with the number of connection events for locations collected in 2016. Parts of floodplains which are flooded infrequently were shown to have a higher germinable seed bank density and diversity in the seed bank compared to frequently flooded parts of floodplains (in line with Brock and Rogers 1998; James *et al.* 2007; Porter *et al.* 2007). Prolonged drying (at least ten years) has been observed to reduce species richness and number of individuals compared to sites more recently flooded (Brock *et al.* 2003; Nielsen *et al.* 2013).

The average recurrence interval (ARI) of floodplain-river connection at locations used as part of this study ranged from (six locations with) an ARI at approx. 1 year, five locations with an ARI at 2 years, three locations with an ARI at 3 years, and two locations with an ARI at 7 to 10 years, over the past 30 years. Consequently, on the floodplain of the lower Lachlan River, flooding frequency varies from frequent (1:1 years) to infrequent (1:10 years). Thus, while the infrequently flooded locations in the present study demonstrated the highest species richness and germinable seed bank density, these locations have been flooded at least once in the last 10 years and prolonged drying may reduce this trend. Interestingly, this trend was not observed at locations where soil was collected in 2017 following large scale flooding.

3.5.3 Temporal variation in the soil seed bank

The soil collected in May/June 2017, following the fourth largest flood on record in the Lachlan River had significantly lower germinable seed bank density and species richness than soil collected in 2016. A similar finding to that of Hölzel and Otte (2004) who found a 50% reduction in seedling density and 30% decrease in species richness following flooding on flood meadows on the Rhine River, Germany. In the present study, species with different functional responses were observed to have a different response to flooding. Terrestrial species (functional groups Tdr and Tda) were substantially lower in 2017, while all aquatic respondent functional groups (all other groups) were either present or in greater numbers at locations collected in 2017. These species are tolerant of flooding conditions. This result suggests that during flooding a large portion of the terrestrial species are removed from the soil seed bank while flood tolerant species remain viable or are replenished. This result

relates to Capon's (2005) spatial observation that the species composition of the soil seed bank is more divergent on infrequently flooded parts of floodplains and more similar on frequently flooded parts of floodplains.

3.5.4 Comparison between extant vegetation and seed bank

The germinable soil seed bank consisted of mostly annuals or short-lived perennial species. This was an expected result (see Williams *et al.* 2008; O'Donnell *et al.* 2016). Comparison between the soil seed bank and extant vegetation, showed that the germinable soil seed bank was lacking in long-lived perennial shrubs, many of which were observed during field surveys. These included a range of species in the *Chenopodiaceae* family as well as *Acacia stenophylla* and *Duma florulenta*. Seedlings of these species were observed in the field following flood recession (F. Dyer, W. Higgisson pers obs.). Long-lived perennial species were also poorly represented in the soil seed bank in a study by Capon and Brock (2006) and Greet (2016), who describe that these species do not appear to depend on a soil seed bank for persistence. Seedling establishment of trees and shrubs on floodplains has been observed to be extremely spatially patchy (Capon *et al.* 2012).

While other studies have observed only a subset of the extant vegetation within the soil seed bank (Nielsen *et al.* 2014), there were 29 species, including a range of submerged aquatic species which germinated from the soil seed bank that were not recorded in the extant vegetation including *Potamogeton* sp. and two *Charophytes*. Brock and Rogers (1998) also observed *Potamogeton* sp. and *Chara* sp. only from the seed bank and not in the extant vegetation.

3.6   Synthesis

Floodplain inundation stimulates productivity on floodplains (Kingsford *et al.* 1999; Thapa *et al.* 2016), and the germination from the soil seed bank is no exception. A range of (mostly annual or short-lived perennial) species germinated in response to submergence and flood recession, with the highest density and diversity of seedlings establishing on waterlogged soil. The soil seed bank and local hydrological conditions on floodplains results in spatial and temporal variation in the soil seed bank and subsequently the extant vegetation.

The results of this study show that the soil seed bank density and diversity are greater at sites which are connected to the river less frequently compared to sites connected more frequently. This is likely related to the increased frequency of flooding resulting in a greater loss of seeds which are not adapted to anaerobic conditions (which are likely to consist of species from WPFGs Tdr and Tda) from the soil seed bank as well as the reduced opportunity for these species to reproduce between flooding events. The author acknowledges that the 2016 and 2017 data sets are not directly comparable, as they were collected from different locations and germinated at different times and therefore potentially different conditions. The results, however, appear to be consistent with the literature and the density and species richness in the soil seed bank initially depletes following flooding in response to germination and scouring but are then replenished once germinated plants reproduce and contribute more seeds, and the species that remain viable in the soil seed bank are those most tolerant of flooding conditions. The shape of the curve may differ but these results support the establishment of a conceptual model that may be tested and further refined (Figure 3.7). The sites where soil was collected as part of this study are all flooded at least every 10 years and seed bank density and diversity may reduce at very low flood frequencies.
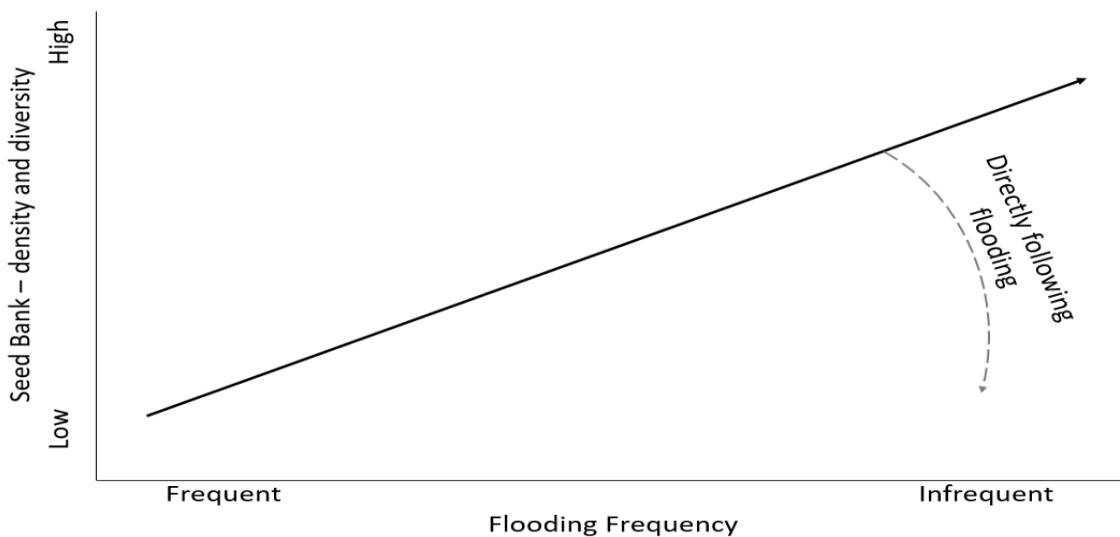
Figure 3.7 Conceptual model of the spatial and temporal variability in the soil seed bank of semi-arid floodplains.

This study used a large number of sites across a relatively large spatial scale within the lower Lachlan River Catchment and demonstrated spatial variation in seed bank density and species richness and this was related to the hydrological regime. It is recommended that further research occur, and the locations used in this study are resampled so as the model developed

67

in this study (Figure 3.7) can be tested. It is also recommended that research be conducted to investigate the effect of depth of flooding on establishment of seedlings on floodplains.

A range of long-lived woody species including *Duma florulenta*, *Chenopodium nitrariaceum* and *Acacia stenophylla* were absent from the soil seed bank, while known to occur on the floodplain of the lower Lachlan River (Cunningham *et al.* 1981; AVH 2016) and being present during the extant vegetation surveys. These species provide important habitat on floodplains in the Murray-Darling Basin (McGinness *et al.* 2010; McGinness *et al.* 2013). For these reasons, chapters 4-6 of this thesis investigated the life history traits of these long-lived woody species, to better understand their requirements for flooding and their distribution on floodplains.

DECLARATION OF CO-AUTHORED PUBLICATION CHAPTER

For use in theses which include publications. This declaration must be completed for each co-authored publication and to be placed at the start of the thesis chapter in which the publication appears.

Declaration for Thesis Chapter 4

Declaration by candidate

In the case of Chapter 4 the nature and extent of my contribution to the work was the following:

Nature of Contribution	Extent of contributions
I designed the study, conducted all field work and experimental work, analysed the data and led the writing of the manuscript.	90%

The following co-authors contributed to the work:

Name	Nature of Contribution	Contributor is also a student at UC Y/N
Fiona Dyer	Study design and manuscript writing	N
Sue Briggs	Study design and manuscript writing	N

Candidate's Signature: Date: 01/02/2019
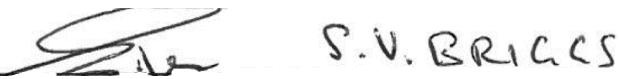
Declaration by co-authors

The undersigned hereby certify that:

7) the above declaration correctly reflects the nature and extent of the candidate's contribution to this work, and the nature of the contribution of each of the co-authors.

8) they meet the criteria for authorship in that they have participated in the conception, execution, or interpretation, of at least that part of the publication in their field of expertise;

9) they take public responsibility for their part of the publication, except for the responsible author who accepts overall responsibility for the publication;

10) there are no other authors of the publication according to these criteria;

11) potential conflicts of interest have been disclosed to (a) granting bodies, (b) the editor or publisher of journals or other publications, and (c) the head of the responsible academic unit; and

12) the original data are stored at the following location(s) and will be held for at least five years from the date indicated below:

Location(s):	Institute for Applied Ecology, University of Canberra, Canberra, Australia

Signatures	Date
Fiona Dyer	24/01/2019
S.V. BRIGGS	24/01/2019

4. Seed germination of tangled lignum (*Duma florulenta*) and nitre goosefoot (*Chenopodium nitrariaceum*) under experimental hydrological regime

Background: As described in Chapter 3, few woody perennial species germinated during the seedling emergence experiments, including tangled lignum and nitre goosefoot, while both these shrubs were observed in the extant vegetation community and are common and widely distributed on floodplains in the Murray-Darling Basin. Hence, chapter 4 explores the hydrological requirements for seed germination and seed dispersal in tangled lignum and nitre goosefoot. Tangled lignum and nitre goosefoot can occur as part of a range of floodplain habitats, including river red gum swamps, black box swamps and lignum shrublands which have all experienced changes to hydrological connectivity related to water resource development (chapter 2). As part of this study seed of both species were collected from within or surrounding a black box swamp. This chapter contains a manuscript that has been published in the journal of **Marine and Freshwater Research** as:

Higgisson, W., Briggs, S., and Dyer, F. (2018) Seed germination of tangled lignum (*Duma florulenta*) and nitre goosefoot (*Chenopodium nitrariaceum*) under experimental hydrological regimes *Marine and Freshwater Research,* **69**, 1268-1278.

4.1   Abstract

The distribution of plants on floodplains depends on the hydrological regime on the floodplain, and the hydrological requirements of the plants. The goals of this study were to determine: (i) the relationships between germination of tangled lignum (*Duma florulenta*) and nitre goosefoot (*Chenopodium nitrariaceum*) and hydrological regime, (ii) the buoyancy of the seeds of the two species, and hence the ability of the seeds to disperse via water, and (iii) to inform environmental flow requirements for the two species. Seeds of tangled lignum germinated best on soaked soil and on soil inundated for 20 days. Seeds of nitre goosefoot germinated best on soil inundated for five days and on soaked soil. The majority of tangled lignum seeds floated for at least seven days. The majority of nitre goosefoot seeds sank within seven days. The results of the study are consistent with the observed distributions of the species on floodplains. Tangled lignum requires about 20 days of flooding, and wet soils following flood recession for optimal germination. Nitre goosefoot requires a few days of flooding, and wet soils following flood recession for optimal germination. The inundation requirements for germination of tangled lignum and nitre goosefoot should be considered in the management of environmental flows.

4.2   Introduction

Floodplains are characterised by large changes in water availability driven by episodic flood pulses (Junk *et al.* 1989; Ward *et al.* 1999). Inundation by flooding maintains the ecological integrity, form and functioning of floodplain ecosystems by providing water to the floodplain and enabling the exchange of water, living organisms and resources (inorganic and organic matter) between the main channel of the river and the floodplain (Stanford and Ward 1993; Johansson *et al.* 1996; Amoros and Bornette 2002).

The flow regime of Australian rivers is neither annual nor wholly seasonal, but is related to unpredictable rainfall patterns. Thus, floodplain inundation is usually temporally irregular or erratic (Nicholls 1991; Walker *et al.* 1995; Rogers and Ralph 2011). Depth and duration of inundation vary across floodplains with surface topography (Scown *et al.* 2015). The temporal variability in flooding along with spatial variability in topography across floodplains create habitat heterogeneity on floodplains (Welcomme 1979; Roberts *et al.* 2000), producing a spatial mosaic of habitat types (Thoms 2003). Plants on floodplains are

exposed to factors associated with flooding (e.g. soil anoxia) and drought (e.g. low soil water availability; Capon *et al.* 2009; Roberts and Marston 2011).

Variation in requirements for flooding and tolerances to the stresses of flooding and drought among plant species on floodplains (hereafter 'floodplain species' for brevity) structures their distribution and species assemblages (Blom *et al.* 1990; Walker *et al.* 1995; Naiman and Decamps 1997; Ward *et al.* 1999; Casanova 2015). In particular, trees and shrubs on floodplains (hereafter 'floodplain trees and shrubs') use stress avoidance or stress resistance mechanisms, such as water-conserving strategies and opportunistic growth, to persist through often unpredictable and episodic floods and droughts (Brock *et al.* 2003; Colloff and Baldwin 2010).

Along with persisting through floods and dry periods, some floodplain trees and shrubs require inundation and the drawdown of water for seed germination (Nicol and Ganf 2000; Nicol *et al.* 2003; Roberts *et al.* 2009). For example, germination of seeds of river red gum (*Eucalyptus camaldulensis*) and river cooba (*Acacia stenophylla*) occurs on wet sediments after inundation (Roberts and Marston 2011). Inundation also plays an important role in the dispersal of many floodplain species, through enabling dispersal of their seeds by water (hydrochory). Hydrochory allows floodplain species to exploit the temporally and spatially patchy nature of floodplains to colonise favourable sites (Howe and Smallwood 1982). Many floodplain species have buoyant fruits or seeds, which facilitates their transport by floodwaters (Sculthorpe 1967), including tangled lignum (*Duma florulenta*) (Chong and Walker 2005) and river red gum (*E. camaldulensis*) (Jensen *et al.* 2008).

Water resource development disrupts the natural flood regime of rivers and associated floodplains (Ward and Stanford 1995), changes the lateral connectivity of floodplain–river systems (Chapter 2) and reduces the frequency, extent and duration of floodplain inundation (Kingsford 2000b; Shilpakar and Thoms 2009). Flow peaks, and consequently durations of inundation, have been reduced in most rivers across the Murray–Darling Basin by river regulation and extraction of water for irrigation and other purposes (Driver *et al.* 2004a; Armstrong *et al.* 2009; Leblanc *et al.* 2012). Although the in-channel ecological effects of water resource development are often the focus of studies (Nilsson *et al.* 1991a; Bunn and Arthington 2002), floodplains are also affected (Lemly *et al.* 2000), and possibly to a greater extent than rivers (Kingsford 2000a).

Providing water as an environmental flow to improve or maintain the health of a river system is now commonly used in managing freshwater ecosystems (Driver *et al.* 2000; Murray-Darling Basin Authority (MDBA) 2011; Driver *et al.* 2013b). Environmental flows aim to improve or maintain biota and ecosystem processes (Lake 2000) by providing for the life stages of biota that depend on flooding. Understanding the relationships between germination and dispersal of floodplain species and hydrological regime, namely flooding, local rainfall and soil moisture, is necessary to establish environmental flow regimes to meet management goals for freshwater ecosystems.

Two common shrub species on the floodplains of the rivers of the Murray–Darling Basin are tangled lignum (*Duma florulenta*) and nitre goosefoot (*Chenopodium nitrariaceum*). Tangled lignum, from the Polygonaceae family, is a multistemmed perennial shrub that grows to 2.5 m in height (Cunningham *et al.* 1981) and contributes large amounts of carbon to floodplains, as well as providing habitat for nesting birds (Brandis *et al.* 2009; Kelleway *et al.* 2010; Roberts and Marston 2011). Tangled lignum grows on floodplains where flooding occurs every 3–10 years (Craig *et al.* 1991; Wilson 2009; Thomas *et al.* 2012) for ~3 months at a time (Newall *et al.* 2009). Tangled lignum is tolerant of dry periods and can survive without flooding for up to 10 years (Capon *et al.* 2009; Roberts and Marston 2011; Freestone *et al.* 2017). The seeds and vegetative propagules of tangled lignum are dispersed by water (Chong and Walker 2005). The hydrological requirements for germination of seeds of tangled lignum are unknown (see Roberts and Marston 2011; Casanova 2015). Capon *et al.* (2009) recommended that research be conducted to identify the conditions under which seed germination occurs in tangled lignum.

Nitre goosefoot, of the Chenopodiaceae family, is a spinescent shrub that grows up to 2 m high (PlantNET 2017b). Nitre goosefoot occurs at the edge of floodplains and other intermittently flooded areas (Briggs *et al.* 2000; W. Higgisson, S. Briggs and F. Dyer, pers. obs.) and can withstand inundation (Cunningham *et al.* 1981; Wassens *et al.* 2010). No studies have been undertaken on the relationships between seed germination or seed dispersal of nitre goosefoot and hydrological regime. The species is not considered in watering requirements of floodplain species of the Murray–Darling Basin (see Roberts and Marston 2011; Casanova 2015).

There are few floodplain shrub species in the semi-arid regions of the Murray–Darling Basin, and consequently tangled lignum and nitre goosefoot play an important role in the

functioning of these floodplain ecosystems. The conservation management of tangled lignum and nitre goosefoot requires understanding of relationships between germination and dispersal of the species and hydrological regime. The aims of the present study were to: (1) determine relationships between germination of tangled lignum and nitre goosefoot and hydrological regime; (2) determine the buoyancy of seeds of tangled lignum and nitre goosefoot, and hence their ability to disperse by hydrochory; and (3) provide recommendations for environmental flow requirements for the germination of tangled lignum and nitre goosefoot.

4.3   Methods

The seeds and soil were collected from two sites on the floodplain of the lower Lachlan River, in the lower Lachlan River Catchment (Fig. 4.1). The Lachlan River and its catchment form part of the Murray-Darling Basin. The Lachlan River Catchment covers an area of 84 700 km^2 (Hillman and Brierley 2002). The lower Lachlan River Catchment is a very low gradient alluvial plain (Kemp 2004) that experiences low and highly variable rainfall (370 mm annual rainfall at Hillston Airport; Bureau of Meteorology 2017). These geological and rainfall conditions result in large areas of irregularly flooded floodplains (Page 1994; Kingsford *et al.* 2004; Meredith and Beesley 2009). Flow regulation and water extraction mean that the Lachlan River now receives, on average, approximately half (56%) the daily flow under the current developed conditions compared with the average flow under undeveloped conditions (i.e. without water management infrastructure and water extraction; Driver *et al.* 2004b). The reduction in flow rates in the Lachlan River has reduced the average duration of floodplain inundation by ~65% (Driver *et al.* 2004b).

For the present study, seeds were collected from tangled lignum in the Lachlan State Conservation Area at Hunthawang, near the Lachlan River, north of Hillston, New South Wales (Fig. 4.1). Seeds were collected from nitre goosefoot near Whealbah Billabong, adjacent to the Lachlan River, south of Hillston, New South Wales (Fig. 4.1). Fully developed seeds were collected from plants (>50) of both tangled lignum and nitre goosefoot on 21 and 22 December 2016. Soil for the experiments was collected from near Whealbah Billabong, in the vicinity of where the nitre goosefoot seed was collected and where a large population of tangled lignum occurs, although not seeding at the time field work was conducted.
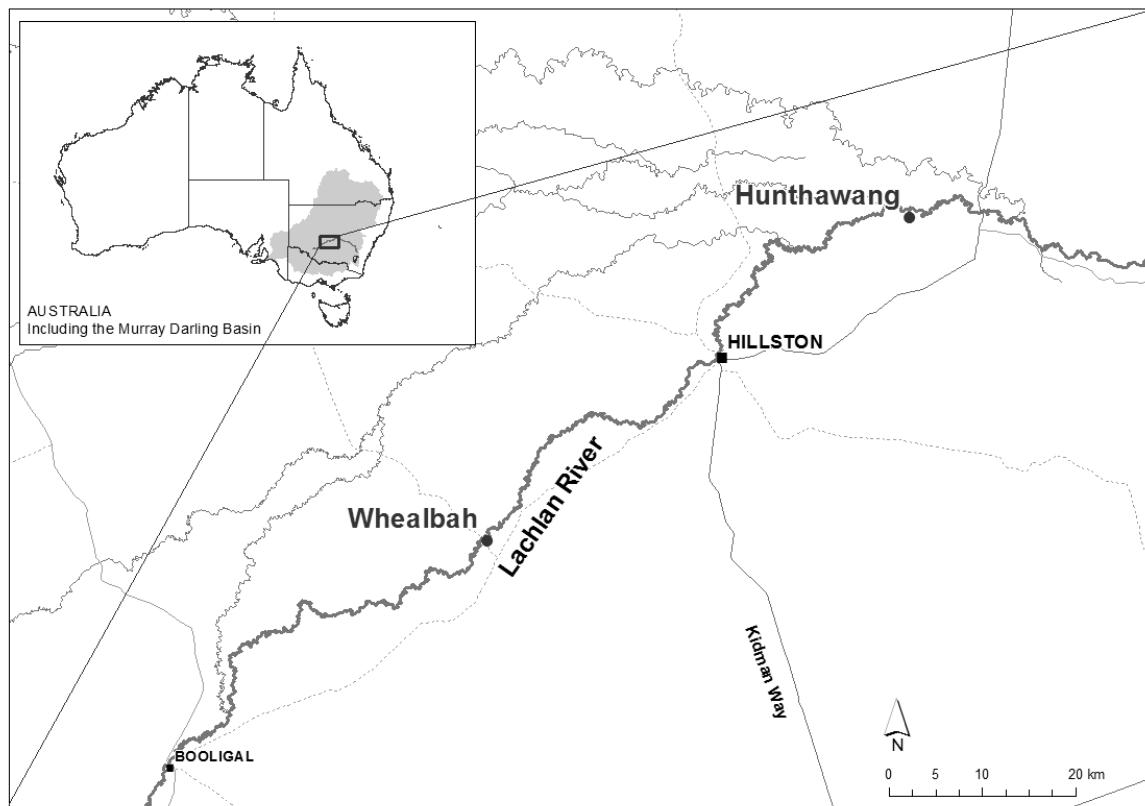
Figure 4.1 Locations where seeds of tangled lignum (Hunthawang) and nitre goosefoot (Whealbah) were collected on the floodplain of the lower Lachlan River, New South Wales, and location of the Murray-Darling Basin in south-eastern Australia.

Experimental methods

Two experiments were conducted in the University of Canberra glasshouse to investigate: (1) relationships between the germination of seeds of tangled lignum and nitre goosefoot and hydrological regime; and (2) the buoyancy of seeds of tangled lignum and nitre goosefoot, and hence their ability to disperse by hydrochory. The two experiments commenced on 5 January 2017. The seeds and soil were stored under dry conditions for 2 weeks between their collection and the commencement of the experiments.

The harvested seed material was cleaned at the Australian National Botanic Gardens, National Seed Bank (Canberra, ACT, Australia), using sieves and a seed aspirator to remove debris, before the experiments. The soil was sterilised by pasteurisation, by steaming it to a temperature of 64°C for 2 h at the Research School of Biology facility at the Australian National University (Canberra, ACT, Australia). The germination experiment was conducted in round plastic containers (740 mL) that were filled with sterilised soil to a depth of 50 mm.

Two hundred seeds of each species were placed in each container before the treatments were applied. Each treatment had four replicates. Containers were randomly assigned to a treatment. The containers were randomly repositioned in the glasshouse weekly throughout the experiment.

The experimental treatments for tangled lignum and nitre goosefoot were local rainfall, permanently soaked soil, inundated for 20 days, inundated for 40 days, inundated for 60 days and a control treatment. These treatments simulated local rainfall, soaked soil after floods recede and flood conditions on the floodplain of the Lachlan River. A further three experimental inundation treatments (inundated for 5, 10 and 15 days) were used for nitre goosefoot. This was because preliminary results indicated that the inundated 20 days treatment only resulted in the germination of a mean (±s.d.) 7.1 ± 1.5% of nitre goosefoot seeds, with only a small proportion (3.3 ± 1.0%) of the germinated seeds effective germinants (i.e. seeds that were alive on soil), and germination and effective germination were very low in the inundated for 40 and 60 days treatments. Nitre goosefoot occurs at the edge of floodplains, and is thus inundated for shorter periods than tangled lignum, which occurs in lower sections of floodplains than nitre goosefoot (Thomas *et al.* 2012). The additional treatments for nitre goosefoot commenced on 13 March 2017. Control treatments for both tangled lignum and nitre goosefoot were set up with soil and seed but no water applied.

The local rainfall treatment was watered using the same regime as rainfall for Hillston for the experimental period (January–March 2016) to mimic local rainfall. The rainfall data were obtained from the nearest weather station to the field collection sites (Hillston Airport; Bureau of Meteorology 2017). The 2016 rainfall regime was used for the rainfall treatment because it was close to typical rainfall for January–March for the Hillston area (i.e. rainfall for January–March 2016 = 94.6 mm *v.* long-term (120 years) average rainfall for Hillston January–March = 91.8 mm). The rainfall treatment received intermittent rainfall to mimic actual rainfall on Day 3 of the experiment (2.8 mm), Day 11 (1.0 mm), Day 12 (2.0 mm), Day 19 (1.0 mm), Day 20 (17.6 mm), Day 24 (12.4 mm), Day 25 (1.4 mm), Day 28 (20.1 mm), Day 57 (0.6 mm) and Day 58 (29.6 mm).

The containers with the soaked treatment were watered daily for 70 days to maintain full soil moisture, without inundation above the soil level, to mimic the conditions at the edge of a flooded area or moist soil after flood recession. Water was applied to the containers for the

soaked treatment until the soil was visibly wet at the surface but no water was above the surface of the soil. The soaked soil was maintained throughout the experiment.

The containers for the inundation treatments were filled with water to 25 mm above the soil surface for the duration of each inundation treatment. The water levels were maintained at 25 mm until approximately 2 days before the day that the water level was receded, and then receded manually on that day. Some evaporation occurred between when the addition of water was stopped and the manual drawdown of the water in the containers. No further water was added to the containers after drawdown. The inundation treatments were based on the position of each species on the floodplain (Cunningham *et al.* 1981; Newall *et al.* 2009; Casanova 2015), and the modelled average number of consecutive days during which commence to flow (resulting in floodplain inundation) was exceeded per year from 1898 to 2000 for Whealbah Billabong (where both species occur), under 'without development' (60 days inundation) and current (40 days inundation) flow scenarios (Chapter 2; Driver *et al.* 2004b).

The number of germinants and effective germinants in the replicates for each treatment were counted daily for 70 days. The total number of live germinants (hereafter 'germinants') was the number of seeds that germinated on the soil or floating in the water column and were alive on soil or in the water. The total number of live effective germinants (hereafter 'effective germinants') was the number of seeds that germinated on the soil or had germinated in the water column and then settled as live germinants on the soil. At the completion of the experiment (on Day 70), all ungerminated, visible seeds in each treatment were counted and then put on soaked soil for a further 20 days, after which all new germinants in each treatment were counted. The further 20 days of soaking investigated whether some seeds showed delayed germination.

The buoyancy experiment was conducted using the germination treatments of inundated for 40 days and inundated for 60 days, for both tangled lignum and nitre goosefoot. This provided eight replicates for the first 40 days of inundation and four replicates for the remaining 20 days of inundation. Each replicate contained 200 seeds. The experimental conditions were the same as for the inundation treatments of the germination experiment. All floating seeds, including germinated seeds, were counted daily until no floating seeds remained.

The mean (±s.d.) maximum daily temperature in the glasshouse was 37.4 ± 4.4°C over the period of the experiment, which is comparable to the mean January and February temperature at Hillston Airport for January and February 2016 (34.1°C). The long-term mean temperature for January and February at Hillston Airport is 33.2°C (Bureau of Meteorology 2017).

The number of total germinants and effective germinants was analysed using a Kruskal–Wallis non-parametric test (Conover and Iman 1981) in IBM SPSS Statistics 23 (IBM Corp., Armonk, NY, USA). *Post hoc* comparisons of treatment types were undertaken using Dunn–Bonferroni pairwise comparisons to identify significant differences between treatments. Bonferroni-adjusted significance values are used throughout the paper. The data were analysed as numbers, but are referred to in the text and presented in the figures as percentages for ease of comprehension. The results from the buoyancy experiment are presented as percentages; these data were not subjected to statistical analysis.

4.4   Results

4.4.1 Seed germination under different treatments

4.4.1.1 Tangled lignum

Treatment type had a significant effect on the number of germinants (χ^2_4 = 14.17, P = 0.007) and on the number of effective germinants of tangled lignum (χ^2_4 = 14.85, P = 0.005; Fig. 4.2). *Post hoc* pairwise comparisons showed that the number of germinants was significantly higher in the soaked treatment than in the rainfall treatment (P = 0.004), and that the number of effective germinants was significantly higher in the soaked treatment than in the rainfall treatment (P = 0.012) and in the inundated 60 days treatment (P = 0.019). The rainfall treatment had the lowest number of germinants and effective germinants (Fig. 4.2). The percentage of effective germinants of tangled lignum was inversely related to the length of inundation (Fig. 4.2). Germination of tangled lignum occurred following rainfall, soaking, inundation or recession of inundation (Fig. 4.3). No seed germination occurred in the control.
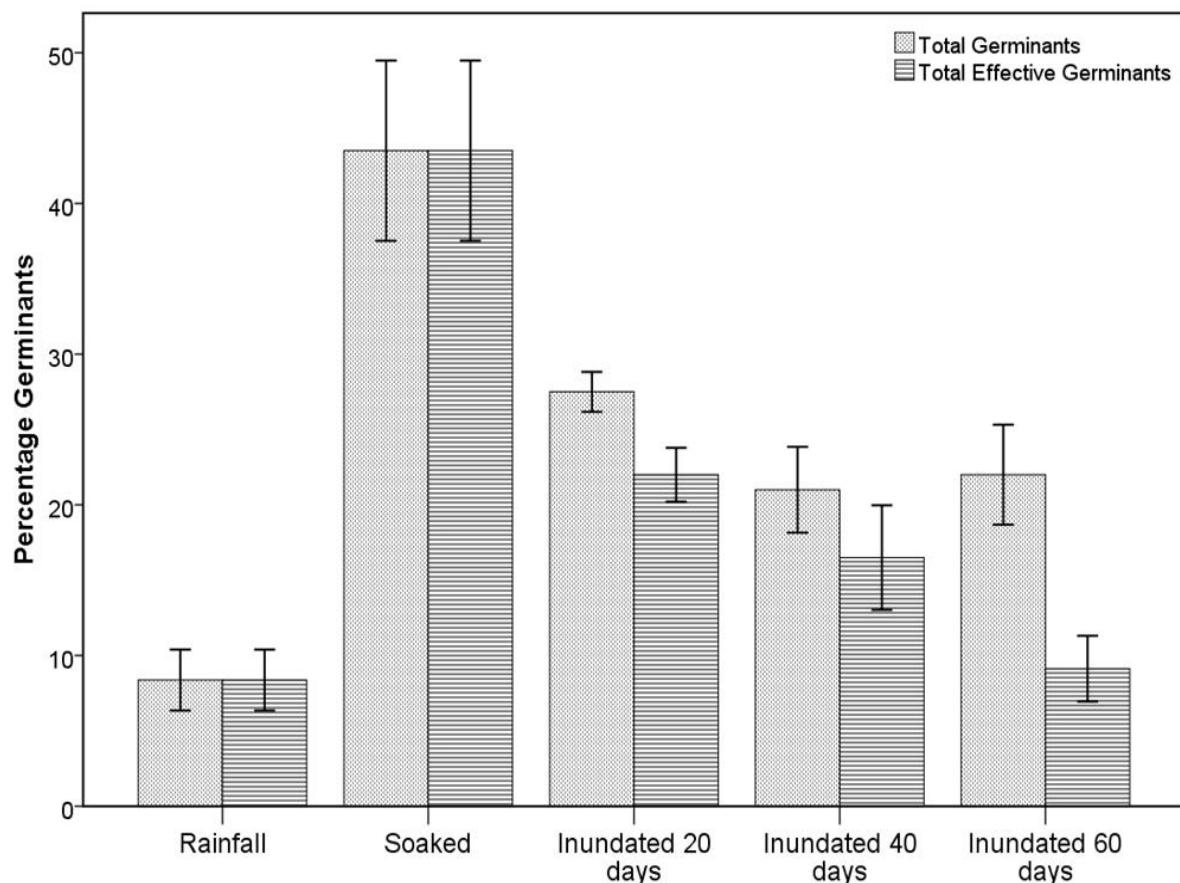
Figure 4.2 Mean (± SE) cumulative percentage of germinants and mean (± SE) cumulative percentage of effective germinants of tangled lignum in the five treatments: local rainfall; soaked soil; inundated 20 days; inundated 40 days; and inundated 60 days. The control treatment is not shown as no seed germination occurred in the control. Each treatment contained four replicates with 200 seeds each. Note that the scale on the Y axis differs from that on Figure 4. 4.

Germination of tangled lignum was greater in the soaked treatment than in the inundated 20 days treatment (although the difference did not reach statistical significance), but this result was confounded by the number of days that moisture was available in the two treatments. The inundated treatments did not receive further watering once the water was receded, and hence the soil in the inundated treatments, including the inundated 20 days treatment, dried out ~15 days after water recession. Moisture was available in the soaked treatment for 70 days. Germination in the inundated 20 days treatment ceased at Day 35, when the soil was dry, but germination in the soaked treatment continued up to Day 55. The peak percentage of germinants of tangled lignum in the inundated 20 days treatment (27.5 ± 1.3%) occurred on Day 35 (Fig. 4.3*c*). The percentage of germinants in the soaked treatment on Day 35 of the

80

experiment (28.3 ± 1.6; Fig. 4.3*b*) was similar to the number of germinants in the inundated 20 days treatment after 35 days.

The rainfall treatment resulted in two germination events, one at approximately Day 30 following three large rainfall events when 2.4% of seeds germinated, and the second at approximately Day 60 following the largest rainfall event (29.6 mm) when 5.5% of seeds germinated (Fig. 4.3*a*). The small rainfall events (<3 mm) did not result in any germination of tangled lignum, except a single germinate on Day 16. Germinants in the rainfall treatment died between the two main periods of rain; the soil in the containers dried out between the two main rainfall periods. In the soaked treatment, germination commenced on Day 4, increased rapidly from Day 16 to Day 26 and continued (at a slower rate) until Day 55 (Fig. 4.3*b*).

Germination in the inundation treatments commenced on Day 4. Germination in the inundated 20 days treatment accelerated at Day 10 and continued to accelerate after recession of water up to Day 23 (Fig. 4.3*c*). Germination in the inundated 20 days treatment peaked at Day 35 (15 days after recession of water). Germination in the inundated 40 days treatment diminished after 21 days inundation, and then accelerated up to Day 46 following recession of water at Day 40, with peak germination at Day 56 (16 days after recession of water; Fig. 4.3*d*). Germination in the inundated 60 days treatment diminished after 22 days inundation, then accelerated on Day 58 and continued to accelerate following recession of water at Day 60, up to Day 65 (Fig. 4.3*e*). Approximately half the germinants in the inundated 40 days and inundated 60 days treatments died after 22 days of inundation (Fig. 4.3*d*, *e*).
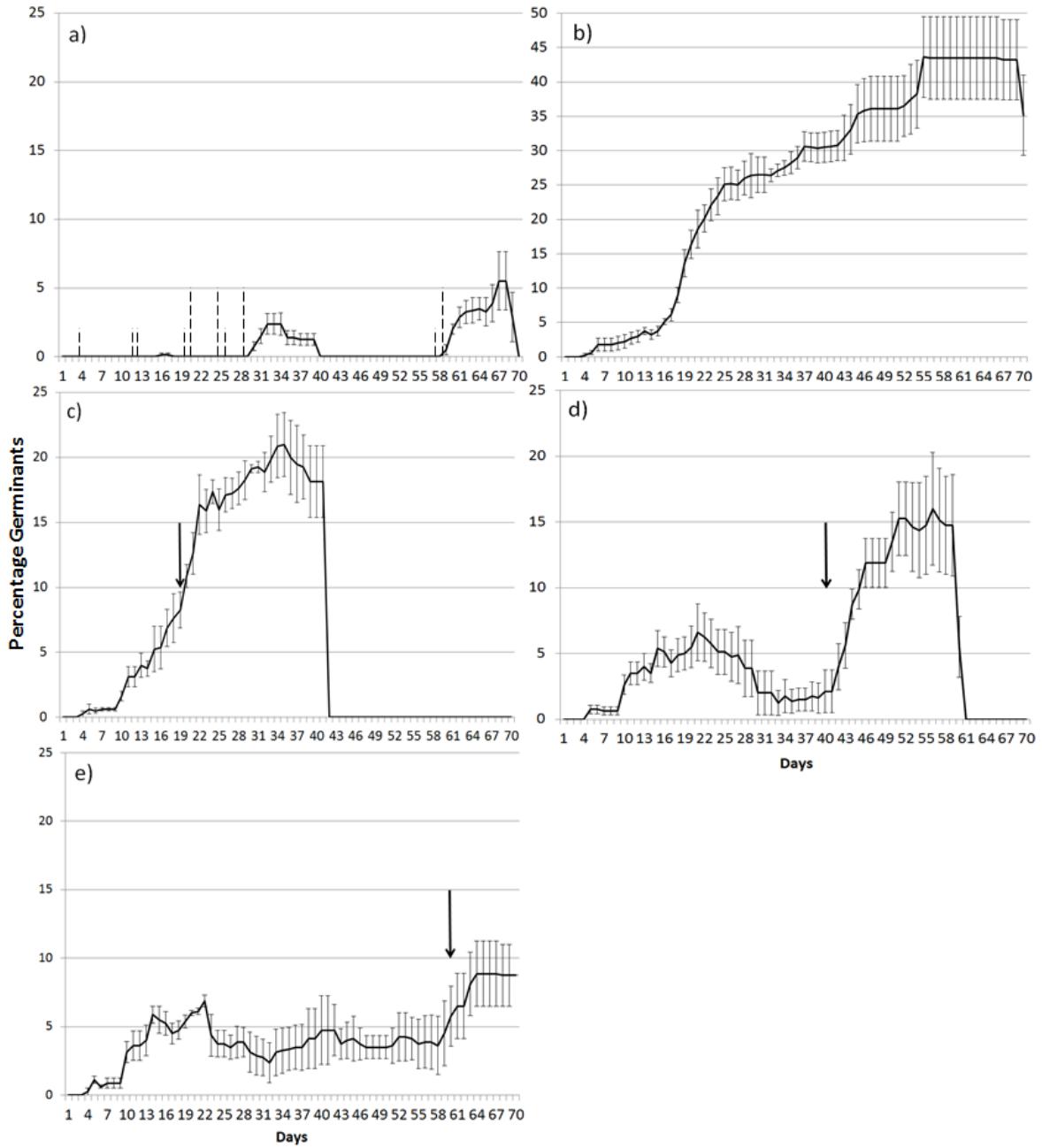
Figure 4.3 Mean (± SE) daily percentage of germinants of tangled lignum subjected to the five treatments (a) local rainfall; b) soaked soil; c) inundated 20 days; d) inundated 40 days; and e) inundated 60 days). The vertical lines on the local rainfall treatment show rainfall events; small lines represent small rainfall events (in order of occurrence: 2.8 mm, 1 mm, 2 mm, 1 mm, 1.4 mm, and 0.6 mm) and large lines represent large rainfall events (in order of occurrence: 17.6 mm, 12.4 mm, 20.1 mm, and 29.6 mm). The arrows on the figures for the inundated treatments show the date that water was manually receded. Each treatment contained four replicates with 200 seeds each. Note that the scale on the Y axes differs slightly from that on Figure 4.5.

A total of 191 ungerminated seeds remained after 70 days in the rainfall treatment (after the experiment), of which 106 seeds germinated during the subsequent 20 days on soaked soil. No seeds remained in the soaked treatment after the experiment; 44 seeds remained in the inundated 20 days treatment, of which 11 germinated during 20 days on soaked soil; and 42 and 57 seeds remained in the inundated 40 days and inundated 60 days treatments respectively, of which only a single seed from the inundated 40 days treatment germinated during 20 days on soaked soil.

4.4.1.2 Nitre goosefoot

Treatment type had a significant effect on the number of germinants ($\chi^2_7 = 22.69$, $P = 0.002$) and the number of effective germinants of nitre goosefoot ($\chi^2_7 = 28.10$, $P < 0.001$; Fig. 4.4). *Post hoc* pairwise comparisons showed that the number of germinants was significantly higher in the inundated 5 days treatment than in the inundated 40 days ($P = 0.018$) and inundated 60 days ($P = 0.014$) treatments. The number of effective germinants was significantly higher in the soaked treatment than in the inundated 40 days ($P = 0.035$) and inundated 60 days ($P = 0.009$) treatments. The number of effective germinants was significantly higher in the inundated 5 days treatment than in the inundated 40 days ($P = 0.011$) and inundated 60 days ($P = 0.003$) treatments. The percentage of effective germinants of nitre goosefoot dropped sharply from inundated 5 days to inundated 10 days, and then fell considerably from 10 days of inundation to 60 days of inundation (Fig. 4.4). Germination of nitre goosefoot in all treatments was triggered by wet soil following rainfall, soaking or recession of inundation (Fig. 4.5). No seed germination occurred in the control.
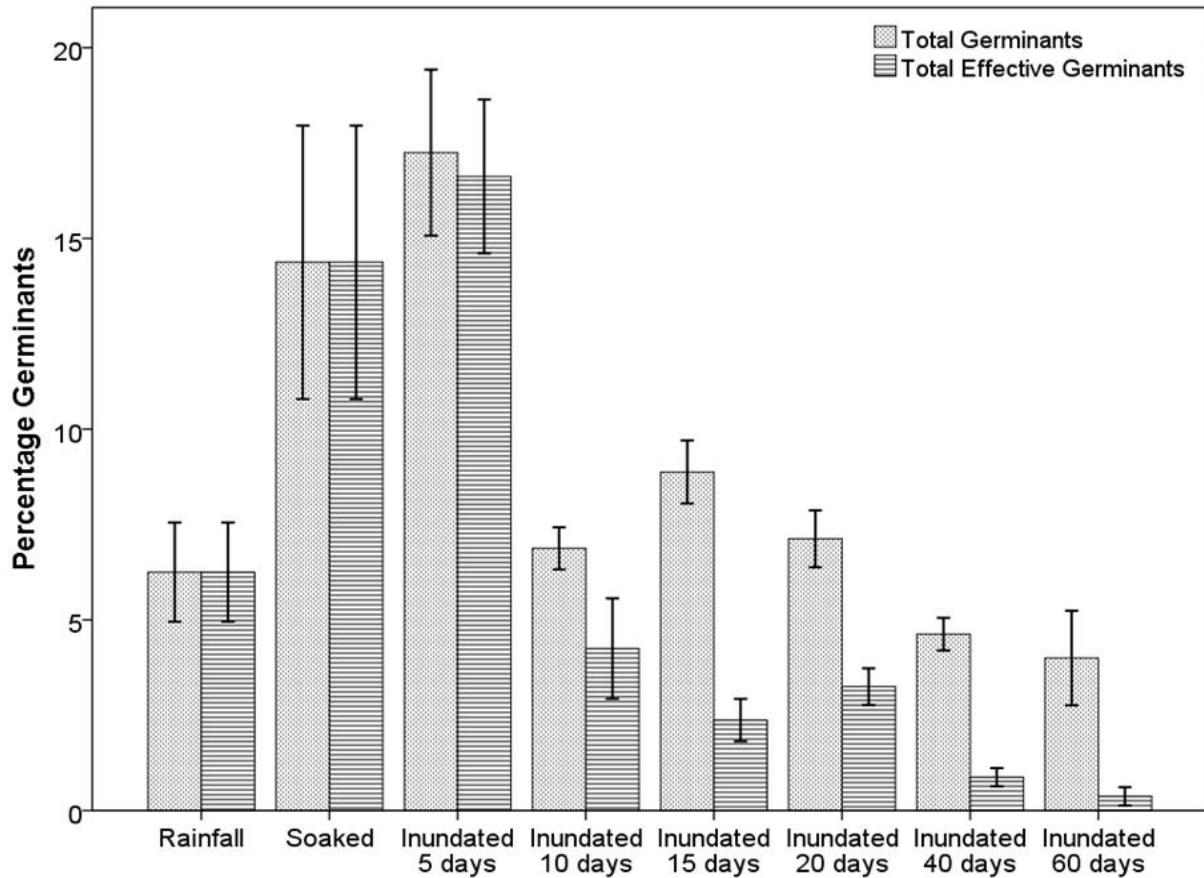
Figure 4.4 Mean (± SE) cumulative percentage of germinants and mean (± SE) cumulative percentage of effective germinants of nitre goosefoot in the eight treatments: local rainfall; soaked soil; inundated 5 days; inundated 10 days; inundated 15 days; inundated 20 days; inundated 40 days; and inundated 60 days. The control treatment is not shown as no seed germination occurred in the control. Each treatment contained four replicates with 200 seeds each. Note that the scale on the Y axis differs from that on Figure 4.2.

There were two main germination events in the rainfall treatment. The first of these occurred after Day 29, following the three large rainfall events in the previous 10 days. The second germination event was after Day 60 following 29 mm of rainfall on Day 58. The soil in the containers dried between the two main periods of rain and the germinants died. Germination of nitre goosefoot in the soaked and inundated treatments commenced on Day 3 (Fig. 4.5). In the soaked treatment, seed germination rose until a peak on Day 23, after which few seeds germinated (Fig. 4.5*b*). Germination in the inundated 5 days treatment occurred after inundation, continued to increase after the water level was receded on Day 5 and peaked on Day 12. No further germination occurred after that day. All nitre goosefoot germinants in the

inundated 5 days treatment died by Day 22 (Fig. 4.5*c*), just over 15 days after recession of inundation, when the soil was dry.

Germination in the inundated 10 days treatment peaked at Day 10, when the water level was receded. The percentage of germinants in the inundated 15 days and inundated 20 days treatments had a small peak at Day 8, then fell with ongoing inundation and then showed small increases after the water was receded (Fig. 4.5*e*, *f*). Most or all germinants of nitre goosefoot in the inundated 15 days and inundated 20 days treatments died after 10 days inundation (Fig. 4.5*e*, *f*). Very few seeds of nitre goosefoot germinated in the inundated 40 days and inundated 60 days treatments. All germinants in the inundated 40 days treatment died by Day 12, and all germinants died by Day 15 in the inundated 60 days treatment (data not shown). No visible seeds of nitre goosefoot remained in any treatment at the end of the experiment and soaking the soil in the treatments did not produce any germination.

Figure 4.5 Mean daily percentage (± SE) of germinants of nitre goosefoot subjected to the six treatments: a) local rainfall; b) soaked soil; c) inundated 5 days; d) inundated 10 days; e) inundated 15 days; and f) inundated 20 days. The vertical lines on the local rainfall treatment show rainfall events; small lines represent small rainfall events (in order of occurrence: 2.8 mm, 1 mm, 2 mm, 1 mm, 1.4 mm, and 0.6 mm) and large lines represent large rainfall events (in order of occurrence: 17.6 mm, 12.4 mm, 20.1 mm, and 29.6 mm). The arrows on the figures for the inundated treatments show the date that water was manually receded. Inundated 40 days and inundated 60 days treatments are not shown. Each treatment contained four replicates with 200 seeds each. Note that the scale on the Y axes differs slightly from that on Figure 4.3.

4.4.2 Seed buoyancy

The majority (98%) of seeds of tangled lignum in the inundated treatments floated for at least 5 days, after which the percentage of seeds remaining buoyant fell to 55% by Day 7 and to 10% by Day 30 of inundation (Fig. 4.6). All tangled lignum seeds had sunk by Day 49 (Fig. 4.6). The majority (84%) of seeds of nitre goosefoot floated for only 3 days (Fig. 4.6). Most of the seeds of nitre goosefoot had sunk by Day 7 of the experiment, when less than 10% of the seeds remained floating, and all nitre goosefoot seeds had sunk by Day 12 of inundation (Fig. 4.6).
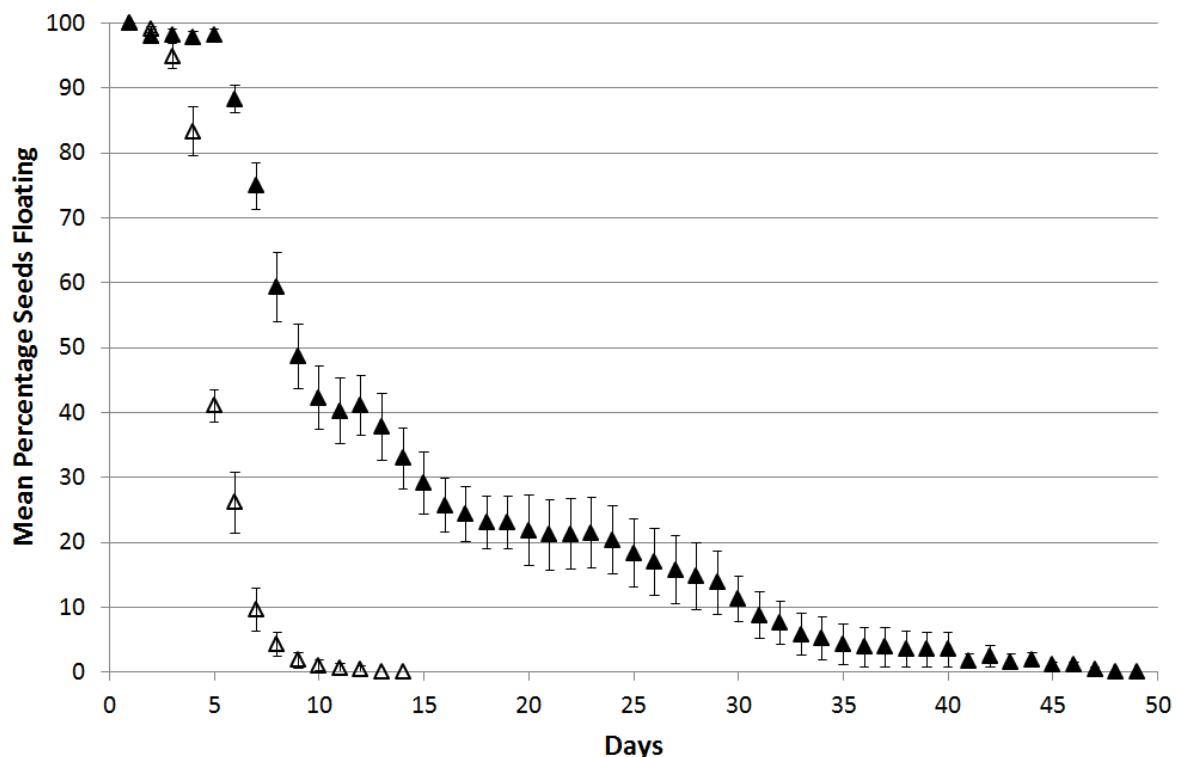
Figure 4.6 Mean (± SE) percentage of seeds of tangled lignum which were floating on each day of the experiment (black triangles), and the mean (± SE) percentage of seeds of nitre goosefoot which were floating on each day of the experiment (open triangles). There were eight replicates over the first 40 days of the experiment, and four replicates over the next 10 days. Each replicate contained 200 seeds.

4.5   Discussion

4.5.1 Seed germination

The present study showed that the highest germination and effective germination of tangled lignum occurred on soaked soil. Germination and effective germination were also high following recession of water in the inundated 20 days treatment, when the confounding effect of soil drying following recession of water is taken into account. Effective germination of tangled lignum seeds reduced with length of inundation. Tangled lignum had the lowest germination and effective germination in the rainfall treatment. Although tangled lignum did not germinate well on intermittent rainfall alone in the present study, this finding needs to be interpreted in the context of the experimental study. The species could possibly germinate during extended periods of high rainfall, if the soil remained continually wet for extended periods.

The results of the present study suggest that tangled lignum seeds require a certain period of contact with moisture to germinate. The contact with moisture can be with free water (of ~20 days duration) or with wet soil. The results of the present study suggest that seeds of tangled lignum germinate best in response to ~20 days of flooding followed by recession of water, and on wet soils such as occur following flood recession. Seed germination of tangled lignum has been reported to occur after inundation (Campbell 1973; Holland *et al.* 2013), and the results of the present study confirm this. Germinating on flood recession is a common characteristic of floodplain species, such as river red gum and black box (Pettit and Froend 2001; Casanova 2015). Germinating on wet soils following recession of floodwater provides tangled lignum seedlings with conditions that maximise growth and survival (Jensen 2008). Because floodplain inundation and the ensuing recession of floodwaters are intermittent, seed germination in tangled lignum is likely to be episodic, as in river red gum and black box (George *et al.* 2005).

The finding that 106 of the 191 ungerminated seeds from the rainfall treatment germinated on soaked soil over a 20-day period after the experiment finished suggests that some seeds of tangled lignum delay germination, and possibly exhibit dormancy (*sensu* Baskin and Baskin 1998). Chong and Walker (2005) concluded that seeds of tangled lignum do not exhibit dormancy. Further investigation is required to determine whether seeds of tangled lignum exhibit dormancy or not.

Although tangled lignum displayed most germination on soaked soil and with 20 days inundation, including after recession of water, some seeds germinated following recession of water after 40 days inundation and, to a lesser extent, after 60 days inundation, and some seeds germinated on soaked soil after the experiments concluded. Thus, some seeds of tangled lignum germinate on contact with free water, as would occur with flooding, whereas other seeds germinate on contact with moist soils, as would occur after floodwaters recede. Some germinated seeds (and seedlings; Capon *et al.* 2009) of tangled lignum are tolerant of extended flooding for a further period of time. Thus, tangled lignum is both flood respondent and flood tolerant. These traits of the species are suited to the temporally and spatially variable nature of flooding of Australian inland floodplains. Plasticity in reproductive phenology related to flooding has been shown in other floodplain species (Brock 2011). Tangled lignum also exhibits reproductive plasticity by having both sexual and asexual reproduction (Capon *et al.* 2009). The reproductive strategies of tangled lignum allow the species to reproduce in a landscape of highly variable flooding regimes.

Nitre goosefoot germinated best in response to 5 days of flooding, and on wet soils such as occur following flood recession. Although nitre goosefoot is not commonly considered a flood-dependent species, seed germination was much higher when seeds were inundated for 5 days or on soaked soils compared with intermittent rainfall. This indicates that nitre goosefoot germinates best where soil moisture levels are high following recession of short periods of flooding. Although nitre goosefoot did not germinate well on rainfall in the present study, this finding needs to be interpreted in the context of the experimental study. The species may germinate during prolonged periods of high rainfall that maintain soil moisture for 20 days or so.

Germination of nitre goosefoot seeds, particularly the percentage of effective germinants (i.e. seeds that germinated and were alive on soil), was inversely related to inundation period. Effective germination reduced considerably after ≥10 days inundation. Thus, nitre goosefoot is unlikely to occur in locations where prolonged flooding occurs. The results of the present experimental study are consistent with the position of nitre goosefoot in the landscape. Nitre goosefoot occurs on the edges of floodplains, lakes and other intermittently flooded areas (Briggs *et al*. 2000; Thomas *et al*. 2012, W. Higgisson, pers. obs.). Although flooding at the edges of floodplains and lakes is infrequent and usually of short duration, the results of the present study suggest that such short periods of inundation are vital for maintaining nitre goosefoot in the landscape.

Most of the nitre goosefoot seeds that germinated in the inundated treatments did so in the first 10 days following inundation (up to 22 days in the soaked treatment), very few seeds germinated in response to recession of water in the treatments of 10 days inundation and longer and no seeds germinated when they were put on soaked soil at the end of the experiment. These results suggest that nitre goosefoot seeds do not exhibit dormancy (*sensu* Baskin and Baskin 1998).

4.5.2 Seed buoyancy

Virtually all tangled lignum seeds floated for at least 5 days, with some remaining buoyant for more than 30 days. Chong and Walker (2005) found that seeds of tangled lignum remained buoyant for 5–24 days, and the seeds disperse by hydrochory. The results of the present study suggest that the seeds of tangled lignum have the ability to float, and hence move with water as it distributes down river channels and across floodplains, with the seeds dispersing by hydrochory. Hydrochory allows tangled lignum to colonise areas of floodplain that become inundated under a range of flood regimes, including with environmental flows. Other riparian species also show hydrochory (Pettit and Froend 2001).

Most seeds of nitre goosefoot remained buoyant for only 4 days, and almost all seeds had sunk within 1 week. Thus, hydrochory is unlikely to be an important dispersal mechanism for seeds of nitre goosefoot (see Nilsson *et al.* 1991b). It is possible that the species uses other means of dispersal, such as wind or epizoochory (dispersal on the outside of animals).

4.5.3 Implications

Water resource development has changed the hydrological regimes of floodplain ecosystems in the Murray–Darling Basin (Kingsford 2000a; Driver *et al.* 2004a). Changes in flood regimes can cause plant species and vegetation communities to contract or expand on floodplains according to their inundation requirements (Shilpakar and Thoms 2009; Thomas *et al.* 2012; Roberts *et al.* 2016).

The inundation requirements for the germination of both tangled lignum and nitre goosefoot should be considered in the management of environmental flows. The inundation requirements for germination of tangled lignum are not considered in managing

environmental flows (but see Roberts and Marston 2011). Tangled lignum requires 17–20 days contact with moisture for germination of more than a few seeds. Duration of inundation varies with position on floodplains. Thus, inundation periods of longer than 17–20 days across the floodplain will usually be required to ensure germination of tangled lignum. If inundation periods are too short for most seeds of tangled lignum to germinate, then the species may colonise lower areas of floodplains where inundation periods are longer, and retreat from higher areas of floodplains where inundation periods are shorter.

The inundation requirements of nitre goosefoot are not considered in current management of environmental flows. The results of the present study demonstrate that nitre goosefoot is a flood-dependent species. It germinates best on short periods of inundation and on wet soil after recession of short periods of flooding. Although nitre goosefoot requires short periods of inundation to germinate, its position at the edge of floodplains means that inundation of the floodplain needs to be of sufficient duration for water to reach the higher areas, usually edges, of floodplains where nitre goosefoot occurs. Nitre goosefoot may expand into lower areas of floodplains, and retreat from the edges of floodplains, if the edges of floodplains no longer flood.

As well as inundation requirements for the germination of seeds of tangled lignum and nitre goosefoot, environmental flows need to consider inundation (and drying) requirements for the survival of the seedlings of these species. Capon *et al*. (2009) found that lignum seedlings exhibited considerable tolerance of flooding and drying, although the biomass of seedlings was reduced after 60 days flooding. Survival of nitre goosefoot seedlings under different conditions of flooding and drying has not been investigated.

The present study used an experimental approach to investigate germination requirements of the floodplain species tangled lignum and nitre goosefoot in relation to hydrological regimes. Further work should be undertaken to test the findings of the present study in the field, and to investigate the responses of the study species to altered flooding regimes on floodplains. The study did not specifically investigate the survival of seedlings in relation to hydrological regime. I recommend that the survival of seedlings of tangled lignum under field conditions be investigated (noting that Capon *et al*. (2009) investigated the survival of lignum seedlings under experimental conditions), and that the survival of nitre goosefoot seedlings under experimental and field hydrological regimes be investigated. Further investigation of whether seeds of tangled lignum exhibit dormancy is also warranted.

4.6 Addendum

While the manuscript presented here was published prior to the examination of the thesis, the examiners raised several pertinent questions that are addressed below:

1. Why was no overall viability test undertaken as a comparison?

This chapter focused on seed germination and dispersal of tangled lignum and nitre goosefoot in relation to duration of inundation and as such overall seed viability was not included as part of this study.

2. During the inundation experiment - in processing was there any removal of empty seed, winnowing?

The cleaning and processing of the seeds occurred using sieves and a seed aspirator (winnowing) which removes material based on weight and size, this process removes empty seeds.

3. *No visible seeds of nitre goosefoot remained in any treatment at the end of the experiment and soaking the soil in the 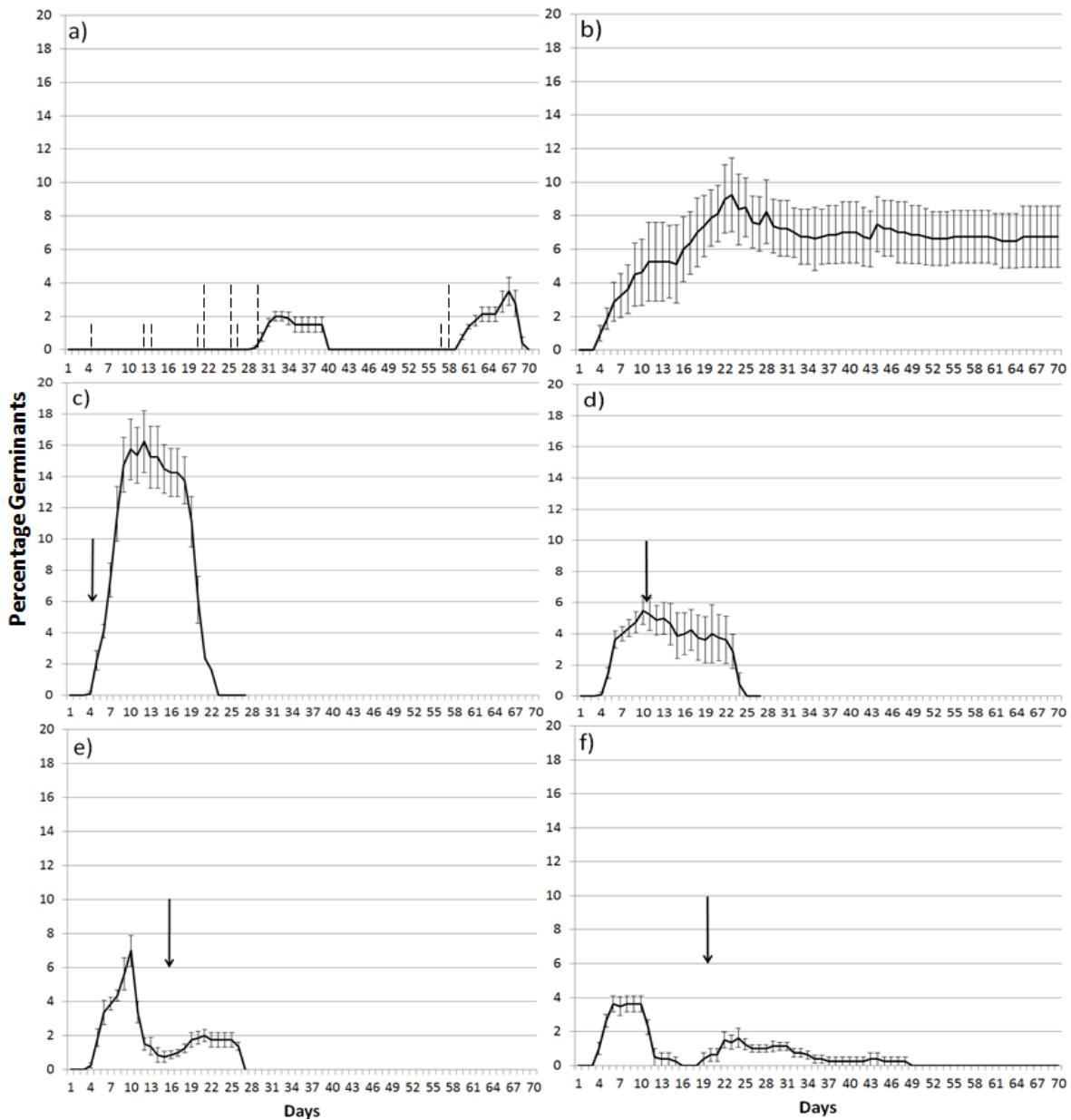treatments did not produce any germination.* How does this tally with the cumulative germination % which at max is only about 17% - what happened to the rest of the seed?

The fact that no seeds of nitre goosefoot were observed during inspection of the containers at the end of the experiment and the fact that no seeds germinated following re-wetting indicates that the seeds disintegrated during the experiment and became unviable.

4. It would be easier to think about seed dormancy in tangled lignum if there was some basic information about the nature of the seeds, seed coats etc, to understand whether and what sort of dormancy is likely.

No information was taken on the nature of the seeds or seed coat during or prior to the experiment. This information is important in understanding whether and what sort of dormancy tangled lignum may exhibit.

5. *It is possible that the species uses other means of dispersal, such as wind or epizoochory (dispersal on the outside of animals).* As there is no description of the seed it is impossible to judge whether this is a reasonable assertion or not. Critical to

this discussion is the question as to whether it is disappearing from the landscape or not? Is it getting by ok and doesn't need active management?

The focus of this study was the hydrological requirements of tangled lignum and nitre goosefoot for germination and dispersal. While the results of this chapter demonstrate that germination rates are related to inundation, and that changes to floodplain inundation may result in changes in the distribution and abundance of the species. Understanding if these species are disappearing from the landscape and requiring active management was out of the scope of this study. To understand the distribution and abundance of these species requires field-based vegetation surveys.

DECLARATION OF CO-AUTHORED PUBLICATION CHAPTER

For use in theses which include publications. This declaration must be completed for each co-authored publication and to be placed at the start of the thesis chapter in which the publication appears.

Declaration for Thesis Chapter 5

Declaration by candidate

In the case of Chapter 5 the nature and extent of my contribution to the work was the following:

Nature of Contribution	Extent of contributions
I designed the study, conducted all field work and experimental work, analysed the data and led the writing of the manuscript.	90%

The following co-authors contributed to the work:

Name	Nature of Contribution	Contributor is also a student at UC Y/N
Fiona Dyer	Study design and manuscript writing	N
Sue Briggs	Study design and manuscript writing	N

Candidate's Signature: Date: 01/02/2019

Declaration by co-authors

The undersigned hereby certify that:

1) the above declaration correctly reflects the nature and extent of the candidate's contribution to this work, and the nature of the contribution of each of the co-authors.
2) they meet the criteria for authorship in that they have participated in the conception, execution, or interpretation, of at least that part of the publication in their field of expertise;
3) they take public responsibility for their part of the publication, except for the responsible author who accepts overall responsibility for the publication;
4) there are no other authors of the publication according to these criteria;
5) potential conflicts of interest have been disclosed to (a) granting bodies, (b) the editor or publisher of journals or other publications, and (c) the head of the responsible academic unit; and
6) the original data are stored at the following location(s) and will be held for at least five years from the date indicated below:

Location(s):	Institute for Applied Ecology, University of Canberra, Canberra, Australia

Signatures	Date
Josie Dye	24/01/2019
S.V. BRIGGS	24/01/2019

5. Responses of nitre goosefoot (*Chenopodium nitrariaceum*) to simulated rainfall and depth and duration of experimental flooding

Background: Nitre goosefoot is not currently recognised as a flood dependent species, and the tolerances and requirements to flooding have not been established. While chapter 4 showed the hydrological requirements and limits for seed germination in nitre goosefoot, little is known about growth, survival and reproduction of nitre goosefoot and the species requirements and tolerances for watering. As such, chapter 5 explores the tolerance and requirements of nitre goosefoot to depth and duration of flooding. This chapter contains a manuscript that has been published in the journal of **Marine and Freshwater Research** as:

Higgisson, W., Briggs, S., and Dyer, F. (2018) Responses of nitre goosefoot (*Chenopodium nitrariaceum*) to simulated rainfall and depth and duration of experimental flooding

5.1   Abstract

Nitre goosefoot (*Chenopodium nitrariaceum*) is a woody shrub that occurs at the edges of floodplains and other intermittently flooded areas across the Murray–Darling Basin. No studies have been conducted on the hydrological requirements of nitre goosefoot, and the species is not considered in watering requirements of floodplain species of the Murray–Darling Basin. This study investigated the effects of simulated rainfall and depth and duration of experimental flooding on mortality, leaf production, biomass and seed production of nitre goosefoot. Nitre goosefoot plants were grown from seeds collected near Hillston, New South Wales, Australia. The plants were subjected to the following 14 hydrological treatments: dry (no water applied), rainfall (simulating rainfall conditions at Hillston) and 12 combinations of three water depths (10 cm, 50 cm, 75 cm) with four durations of inundation (5 days, 10 days, 20 days, 40 days). The study found that nitre goosefoot plants survived flooding, providing plants were not totally submerged, leaf production increased during flooding and after drawdown, and leaf production, biomass and seeding were highest under shallow flooding for approximately 1 month. The results of the study allow the hydrological requirements of nitre goosefoot to be considered in environmental watering programs.

5.2   Introduction

Perennial plants on floodplains are exposed to effects of flooding (e.g. soil anoxia) and of drying (e.g. low soil water; Capon *et al.* 2009; Roberts and Marston 2011). Flooding can cause an energy deficit because of a shortage of oxygen in the soil, which is stressful or lethal to plants (Armstrong 1979; Voesenek *et al.* 2006). Plants regularly exposed to flooding have a range of morphological and physiological traits (Mommer *et al.* 2006), including elongation of shoots that maintain contact with the atmosphere under flooded conditions, transport of oxygen from leaves to roots, and adventitious roots (Kozlowski 1984; Blom *et al.* 1990; Armstrong *et al.* 1994; Kozlowski 1997). The capacity of these traits to mitigate the effects of flooding depends on the depth and duration of flooding (Laan and Blom 1990; Voesenek *et al.* 2006) and the age of the plant (Blom *et al.* 1990).

Perennial plants on floodplains must persist through dry conditions, as well as flooded conditions. Adaptations of plants to dry conditions include shedding leaves, reducing leaf production and decreasing stomatal conductance to reduce water loss, and increasing

investment in production of roots to increase water uptake (Chaves *et al.* 2003). Variations in tolerances to the stresses of flooding and drying among floodplain plant species influence their distribution and shape community assemblages on floodplains (Blom *et al.* 1990; Walker *et al.* 1995; Naiman and Decamps 1997; Ward *et al.* 1999). Woody perennial plants on the floodplains of the Murray-Darling Basin, such as river red gum (*Eucalyptus camaldulensis*), black box (*Eucalyptus largiflorens*), tangled lignum (*Duma florulenta*) and river cooba (*Acacia stenophylla*), require flooding for survival, growth and reproduction (Robertson *et al.* 2001; George 2004; Capon *et al.* 2009; Roberts and Marston 2011; Doody *et al.* 2014; Catelotti *et al.* 2015). Woody perennial plants on the floodplains of the Murray-Darling Basin do not occur on permanently flooded soils, and, thus, also require dry periods for long-term survival (Roberts and Marston 2011).

Water resource development, i.e. river regulation and extraction of water for irrigation and other purposes, has changed the flow patterns of many rivers in the Murray-Darling Basin (Kingsford 2000a). Together with engineering structures on floodplains, these changes have altered the natural flood regimes of floodplains by changing the lateral connectivity of floodplain-river systems (Thoms 2003), and by reducing the frequency, extent and duration of floodplain inundation (Kingsford 2000a; Driver *et al.* 2004b; Armstrong *et al.* 2009; Shilpakar and Thoms 2009; Leblanc *et al.* 2012). Flow regulation and water extraction mean that the Lachlan River now receives approximately half (56%) the average daily flow under the current developed conditions, compared with the average daily flow it would have received under undeveloped conditions (i.e. without water-management infrastructure and water extraction; Driver *et al.* 2004). The reduction in flows in the Lachlan River has reduced the average duration of floodplain inundation by ~65% (Driver *et al.* 2004b).

Changes to floodplain inundation, particularly changes to depth and duration of flooding, affect the distribution and abundance of plants on floodplains (Voesenek *et al.* 2006; Capon *et al.* 2012). Environmental flows to improve the health of river and floodplain ecosystems, including providing for life stages of biota that depend on periodic flooding, are commonly used in Australia (Driver *et al.* 2000; Murray-Darling Basin Authority (MDBA) 2011; Driver *et al.* 2013a). Understanding the relationships between flooding and mortality, growth, and reproduction of species on floodplains is necessary to establish environmental flows to meet management goals.

Nitre goosefoot (*Chenopodium nitrariaceum*) is a spinescent shrub up to 2.5 m high in the Chenopodiaceae family (Cunningham *et al.* 1981; PlantNET 2017a). It occurs across much of inland Australia (AVH 2016), at the edge of floodplains and other intermittently and temporarily flooded areas (Seddon and Briggs 1998; Briggs *et al.* 2000), and sometimes in areas that rarely flood (Keith 2004). Nitre goosefoot is part of several vegetation communities in inland New South Wales, often with or grading into tangled lignum, black box and river cooba on wetter areas on floodplains, and terrestrial chenopods in drier areas at the edges of floodplains (Keith 2004; Benson *et al.* 2006; McGinness *et al.* 2013). Only a few species of shrubs, especially large shrubs (i.e. >2 m tall), occur on the floodplains of the Murray-Darling Basin (Cunningham *et al.* 1981; Keith 2004; Benson *et al.* 2006). Habitat structure in these floodplain communities is enhanced by shrubs, which are influenced by flood regime (McGinness *et al.* 2013). Habitat structure is generally provided by tangled lignum in wetter sites and nitre goosefoot in drier sites (McGinness *et al.* 2013). Vegetation structure on floodplains (such as provided by nitre goosefoot and tangled lignum) provides habitat for terrestrial fauna, particularly for terrestrial birds (McGinness *et al.* 2010).

Apart from requirements and tolerances for seed germination (Chapter 4), little is known about the requirements and tolerances of nitre goosefoot for watering to inform environmental flows. Casanova (2011) classified wetland species into functional groups to enable prediction of their responses to watering events. Nitre goosefoot was not included in Casanova's (2011) paper. Campbell *et al*. (2014) used the approach of Casanova (2011) to classify nitre goosefoot into the functional group Tda, i.e. terrestrial plants that grow in damp places but cannot tolerate flooding in the vegetative state. McGinness *et al*. (2013) noted that nitre goosefoot is found in drier sites than is tangled lignum, is less tolerant of long-duration flooding, suffers under deep flooding and does not appear to require frequent wetting to survive and reproduce, although little is known of precise limits. The species has not been considered in watering requirements for floodplain species of the Murray-Darling Basin (see Roberts and Marston 2011; Casanova 2015).

Here, I use an experimental approach to investigate mortality, growth and reproduction of nitre goosefoot plants under a range of hydrological conditions. The aims of the present study were to: (1) determine relationships between hydrological regime and mortality, biomass, leaf production, and seed set of nitre goosefoot plants, and (2) provide recommendations for watering strategies to maintain populations of nitre goosefoot. I use the term hydrological regime to mean simulated, experimental rainfall and experimental flooding.

5.3   Materials and methods

Fully developed seeds were collected from >50 plants of nitre goosefoot on 22 December 2016, near Whealbah Billabong, adjacent to the Lachlan River, near Hillston, New South Wales, Australia (Fig. 5.1). The sites from which seeds and soil were collected are on the floodplain of the lower Lachlan River, in the lower Lachlan River Catchment, in the Murray-Darling Basin (Fig. 5.1). The soil for the experiment was collected from near where the nitre goosefoot seed was collected (Fig. 5.1). Soils in the study area are vertosols (Isbell 2016). The annual rainfall at Hillston Airport is 370 mm (Bureau of Meteorology 2017). The average mean maximum daily temperature at Hillston Airport ranges from 15°C in July to 33.5°C in January (Bureau of Meteorology 2017).
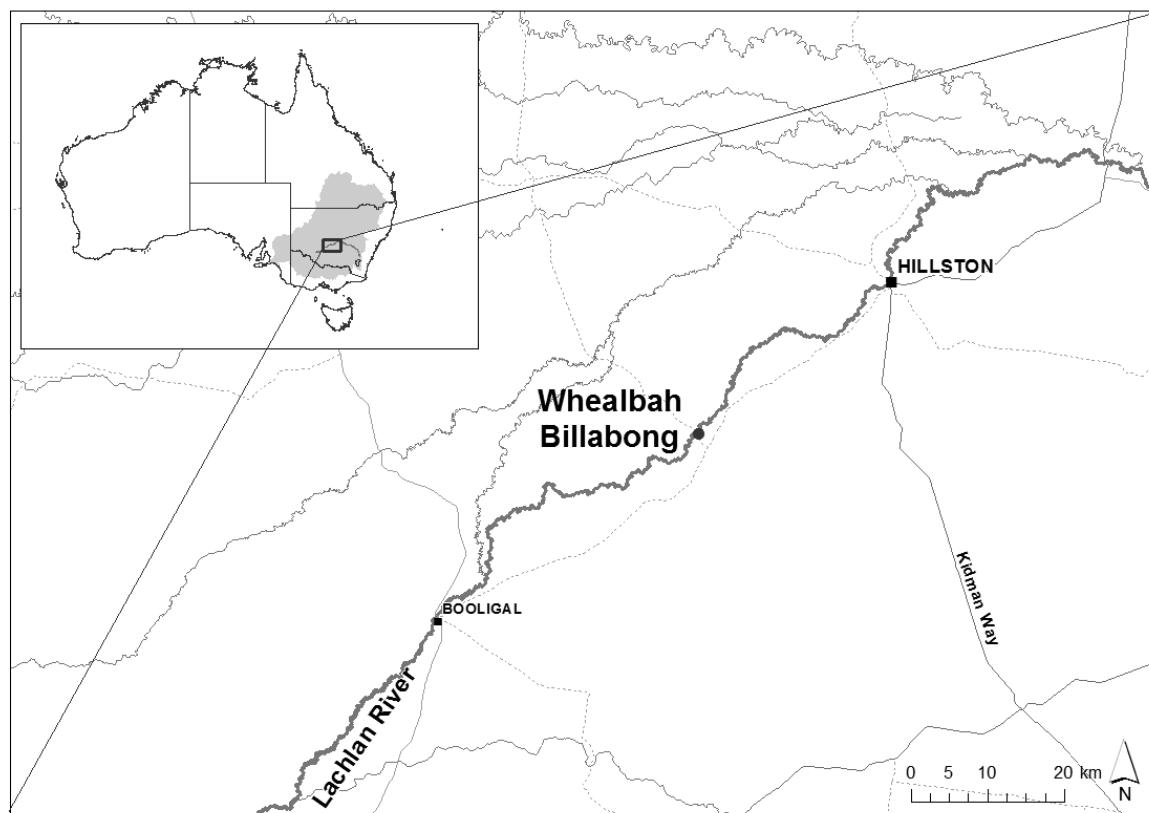
Figure 5.1 Location of site (Whealbah Billabong) where seeds of nitre goosefoot and soil were collected on the floodplain of the lower Lachlan River, New South Wales, and location of the Murray-Darling Basin in south-eastern Australia.

The harvested seed material was cleaned at the National Seed Bank, Australian National Botanic Gardens in Canberra, using sieves and a seed aspirator to remove debris. The soil was then sterilised by pasteurisation, by steaming it to a temperature of 64°C for 2 h at the Research School of Biology facility at the Australian National University, Canberra.

On 6 February 2017, seeds of nitre goosefoot were put on soil in an open tray and watered regularly to allow germination. On 27 March 2017, 120 germinated plants were selected randomly and transplanted individually into PVC pipes (30-cm length, 9-cm diameter) that were filled with soil, to grow the plants. The lower end of each pipe was covered with gauze that held the soil in the pipe, while allowing uptake of water. The pipes were placed on a bench in the glasshouse and watered approximately once a week until the experiment commenced. Plants that died during the first 10 days after transplanting were replaced.

The experiment commenced on 7 October 2017, to coincide with the typical timing of floodplain inundation in the Lachlan River under natural conditions (September to November; Chapter 2; Driver *et al.* 2000; SEWPaC 2011), when the nitre goosefoot plants were 8 months old. The soil in the pipes was moist at the start of the experiment. The experimental treatments (Table 5.1) were applied for 50 days. Pipes for the dry and rainfall treatments were placed on open-mesh crates on the concrete floor of the glasshouse. Pipes for the inundation treatments were placed on shelves in six barrels (100 cm deep), with the shelves positioned to provide the three experimental depths. The pipes, each containing one plant, were randomly assigned to treatments.

Table 5.1 Treatments in the experiment and the abbreviation for the inundated treatments, as referred to in the text and figures. NA, not applicable.

Treatment	No. days inundated	Abbreviation
Dry	NA	Dry
Rainfall	NA	Rainfall
Inundated 10 cm	5	10 cm - 5 days
	10	10 cm - 10 days
	20	10 cm - 20 days
	40	10 cm - 40 days
Inundated 50 cm	5	50 cm - 5 days
	10	50 cm - 10 days
	20	50 cm - 20 days
	40	50 cm - 40 days
Inundated 75 cm	5	75 cm - 5 days
	10	75 cm - 10 days
	20	75 cm - 20 days
	40	75 cm - 40 days

No water was applied to the plants in the dry treatment after the experiment commenced. The rainfall treatment mimicked the recorded rainfall at Hillston (Airport) for October and November 2015, which was 93.4 mm. This was higher than the long-term average (64.6 mm) at Hillston for this period, but is not uncommon, because rainfall for October to November equals or exceeds 94 mm in 4 out of 10 years (Bureau of Meteorology 2017). The rainfall treatment, thus, mimicked high, but not extremely high, spring rainfall to avoid any confounding effects of low rainfall on growth of the plants. The plants in the rainfall treatment were watered on Day 11 of the experiment (0.2 mm), Day 12 (0.4 mm), Day 21 (0.4 mm), Day 22 (8.2 mm), Day 26 (0.2 mm), Day 31 (16.6 mm), Day 32 (23.2 mm), Day 33 (21.6 mm), Day 35 (0.6 mm), Day 36 (12.2 mm), Day 37 (0.2 mm), Day 43 (4.2) and Day 44 (5.4 mm). The amount of water allocated to each plant was calculated by multiplying the daily rainfall amount (mm) by the surface area of the pipe (cm^2), giving the amount of water per plant, per day.

The inundation treatments were based on the position of nitre goosefoot at the edge of floodplains (Briggs *et al.* 2000; Newall *et al.* 2009), seed germination of nitre goosefoot in relation to flooding (chapter 4), and the approximate duration of flooding on the floodplain of the lower Lachlan River where nitre goosefoot occurs (chapter 2). The inundation treatments mimicked inundation regimes that are likely to be experienced by nitre goosefoot. Water depths to which the plants were experimentally exposed were measured from the surface of the soil to the water surface. The plants in the inundation treatments were inundated at the water depth for their treatment for the number of days assigned for that treatment (Table 5.1). Plants were watered under rainfall conditions after they were removed from inundation conditions, to simulate natural conditions after drawdown.

The dry and rainfall treatments had 12 individual replicates, and the inundation treatments had eight replicates. All replicates had one plant per replicate. The pipes containing the plants in the dry and rainfall treatments were randomly re-assigned to a position on a crate, and the pipes containing the plants in the inundation treatments were randomly re-assigned a position in barrels on Days 5, 10, 20, 30 and 40 of the experiment. The mean (±s.d.) maximum daily temperature in the glasshouse for the experimental period was 35.7°C ± 4.3, which is slightly higher than the mean maximum daily temperature at Hillston for the experimental period (i.e. 30.9°C in November 2017; Bureau of Meteorology 2017).

Plant heights were measured at the commencement of the experiment on Day 0. Mortality, number of live leaves per plant and presence or absence of seeds on each plant were recorded on Day 0, Day 5, Day 10, Day 20, Day 30, Day 40 and Day 50. All plants were harvested after the measurements were taken on Day 50. Aboveground and belowground components of the harvested plants were separated, cleaned of soil, dried to a constant weight at 70°C for 3 days, and weighed to obtain dry weights of aboveground and belowground biomass per plant.

Mortality is reported as numbers per treatment, without statistical analysis because of low numbers. Net production of live leaves per plant over the experiment (i.e. the number of live leaves on Day 50 of the experiment minus the number of live leaves on Day 0 of the experiment) was analysed using a one-way ANOVA in IBM SPSS Statistics 23, Armonk, NY: IBM Corp.. Number of leaves and net production of leaves refer to the number of live leaves. The data met the assumptions of ANOVA (Field 2013). Post hoc comparisons of treatments were undertaken for leaf production by using the Tukey HSD test. Total biomass,

aboveground biomass and belowground biomass were analysed with analysis of covariance (ANCOVA) in IBM SPSS Statistics 23, Armonk, NY: IBM Corp.. Height of plants on Day 0 was used as the covariate to adjust the biomass data for any difference in biomass between plants on Day 0. The adjusted biomass data met the assumptions of ANCOVA (Field 2013). *Post hoc* comparisons of treatment were undertaken for the biomass data by using the Tukey HSD test. Statistical analysis of biomass was performed only on plants that were alive when harvested (on Day 50). All biomass data refer to dry biomass and adjusted means. The numbers of plants that set seeds are reported as numbers without statistical analysis because of low numbers.

5.4   Results

5.4.1 Mortality

All plants in the dry treatment died by Day 40 of the experiment. One plant in the rainfall treatment died between Day 20 and Day 30 of the experiment. Three plants in the 75 cm–40 days treatment died between Day 30 and Day 40. No other plants died throughout the experiment. The three plants which died in the 75 cm–40 days treatment were all completely submerged throughout the experiment, because they were less than 75 cm high. Seven plants in the 75 cm–5 days treatment, four plants in the 75 cm–10 days, and five plants in the 75 cm–20 days treatment were also fully submerged. None of these plants died. Four plants in the 75 cm–40 days treatment were fully submerged, including the three plants that died.

5.4.2 Leaf production

Plants in the dry treatment had significantly lower net production of leaves than did plants in all other treatments ($F_{13,106} = 7.39$, $P = 0.001$). Plants in the rainfall treatment produced significantly fewer leaves than did plants in the 10 cm–40 days treatment ($F_{13,106} = 7.39$, $P = 0.024$).

Plants in the 10 cm–40 days (highest net production of leaves), 50 cm-40 days and 75 cm–5 days treatments produced more leaves than did plants in the other treatments (Table 5.2). Plants in the rainfall and 75 cm–40 days treatments produced fewer leaves than did plants in

the other treatments (Table 5.2). Net production of leaves by plants in the 10 cm-40 days and 50 cm-40 days treatments (especially 10-cm depth) was higher than in plants in treatments with shorter periods of inundation (Table 5.2). In contrast, net leaf production in the 75 cm-40 days treatment was lower than in the treatments with shorter periods of inundation (Table 5.2). Plants that were inundated for 5 days had the highest production of leaves in the 75-cm-depth treatments (Table 5.2). The submerged branches on plants at 50- and 75-cm depths, which were inundated for 20 days or longer, became leafless after 20–30 days of inundation.

Table 5.2 Number of plants that died, net leaf production (mean ± s.e.m.) per plant, ratio of belowground to aboveground dry biomass of live plants on day 50 of the experiment, and cumulative number (%) of plants that produced seed, during the experiment, by treatment. n = 12 for dry and rainfall treatments; n = 8 for inundation treatments. NA, not applicable.; NS, not shown, see text for details.

Treatment	No. days inundated	No. plants that died	Net leaf production ± s.e.m.	Ratio of belowground to aboveground biomass	No. plants that produced seeds (%)
Dry	NA	12	-438 ± 19	NS	1 (8.3)
Rainfall	NA	1	40 ± 50	0.31	1 (8.3)
Inundated 10 cm	5	0	268 ± 56	0.34	2 (25.0)
	10	0	207 ± 47	0.30	1 (12.5)
	20	0	226 ± 92	0.31	4 (50.0)
	40	0	446 ± 75	0.35	5 (62.5)
Inundated 50 cm	5	0	305 ± 52	0.30	2 (25.0)
	10	0	290 ± 66	0.29	2 (25.0)
	20	0	182 ± 96	0.34	4 (50.0)
	40	0	357 ± 48	0.33	3 (37.5)
Inundated 75 cm	5	0	346 ± 73	0.34	1 (12.5)
	10	0	274 ± 93	0.31	2 (25.0)
	20	0	189 ± 90	0.33	3 (37.5)
	40	3	100 ± 129	0.27	3 (37.5)

The mean ± s.e.m. number of leaves per plant in the dry treatment fell from 436 ± 29 to 150 ± 15 by Day 30, and to zero by Day 40. The temporal patterns of leaf production in the treatments that received rainfall or were inundated followed several trends (Fig. 5.2–5.4). The mean number of leaves in the rainfall treatment stayed fairly constant during the experiment, with some small rises and falls that were not obviously related to rainfall. Leaf production in all inundated treatments rose in response to initial flooding and went up during the 10 days (and sometimes longer periods) following recession of water (Fig. 5.2-5.4). The number of leaves per plant generally fell 10-20 days after water was receded (Fig. 5.2-5.4).

The mean number of leaves on nitre goosefoot plants in the 10-cm and 50-cm water-depth treatments that were inundated for 20 or fewer days usually peaked at 20 or 30 days (if inundated for 20 days), and then declined. The number of leaves on plants in the 10- and 50-cm water-depth treatments that were inundated for 40 days went up during the experiment, with the largest rises following drawdown (Fig. 5.2, 5.3). The number of leaves on plants in the 75-cm-depth treatments that were inundated for 5 days and for 10 days peaked at 30 days, and then declined. The number of leaves on plants in the 75 cm-20 days treatment peaked at 40 days (with a lower peak at 10 days; Fig. 5.4). The number of leaves on plants in the 75 cm-40 days treatment peaked at 10 days, and then declined, with a small rise after Day 40 (Fig. 5.4). Excluding the three dead plants, the mean ± s.e.m. numbers of leaves at Days 40 and 50 in the 75 cm-40 days treatment were 431 ± 137 and 675 ± 115 respectively.
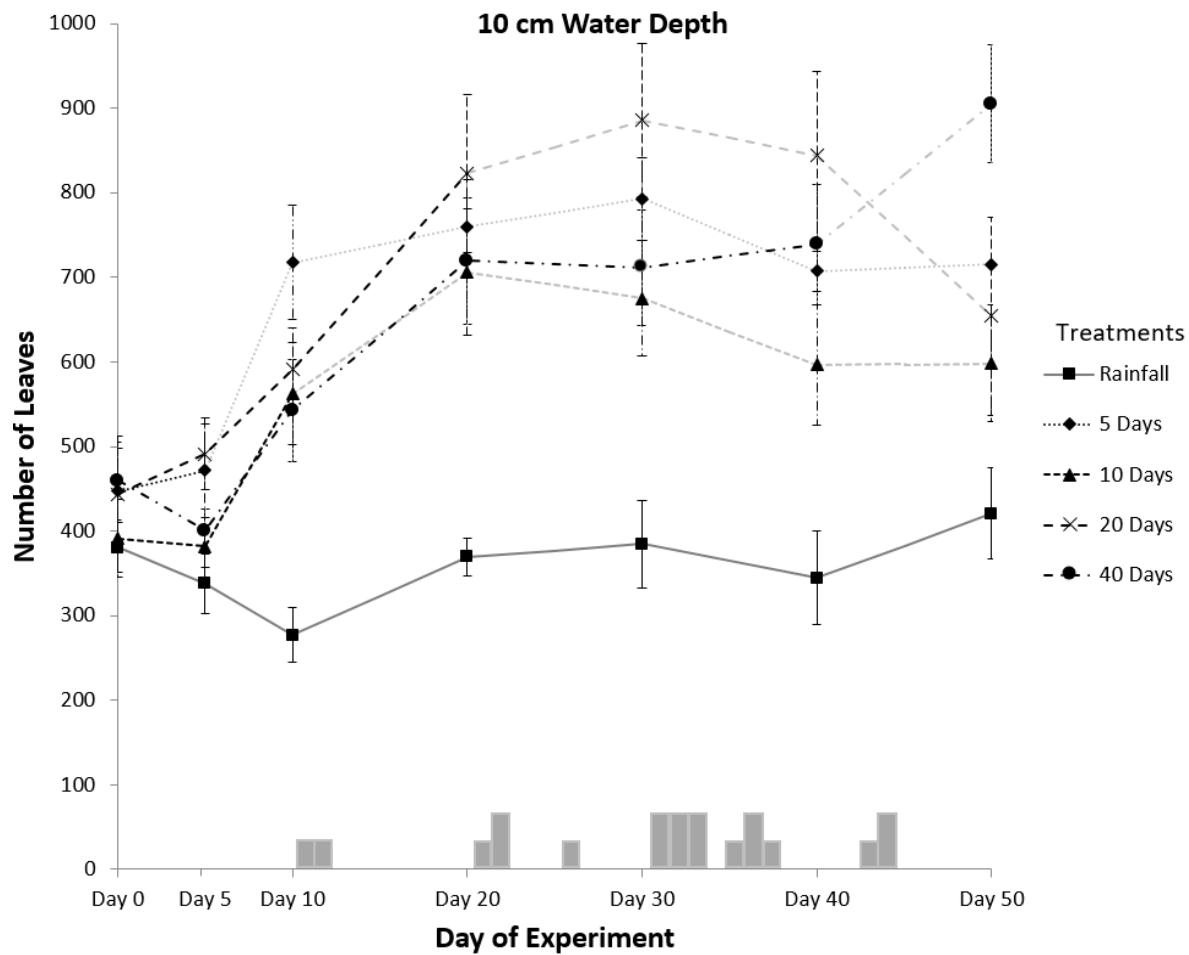
Figure 5.2 Mean ± s.e.m. number of live leaves per nitre goosefoot plant over time in the rainfall and inundated 10 cm treatments. The inundation treatments each contained eight plants and the rainfall treatment contained 12 plants. Black lines show inundated period, and grey lines show non-inundated period. Small grey bars represent small rainfall events (<5 mm), and large grey bars represent larger rainfall events (>5 mm). No rainfall was applied to plants during the inundated periods.
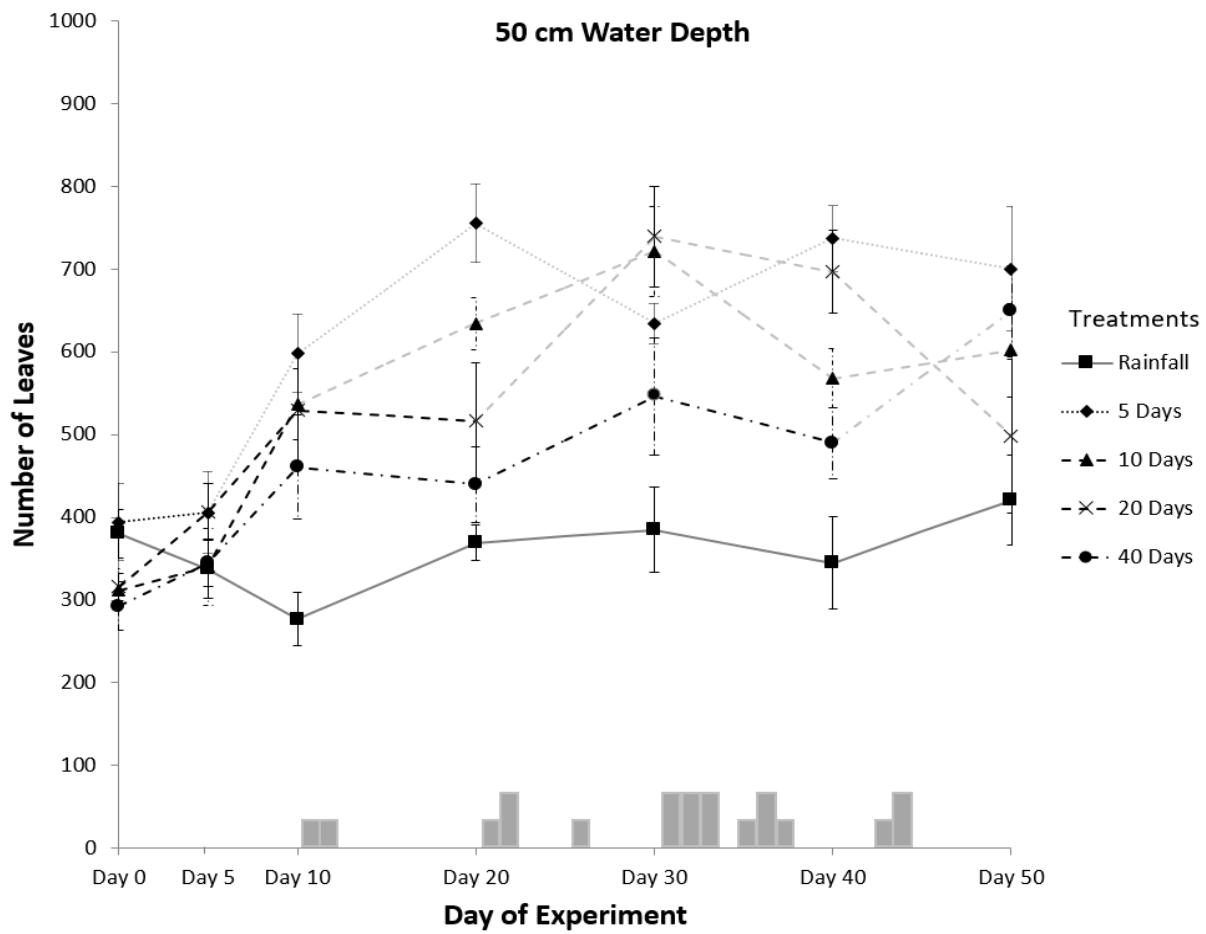
Figure 5.3 Mean ± s.e.m. number of live leaves per nitre goosefoot plant over time in the rainfall and inundated 50 cm treatments. The inundation treatments each contained eight plants and the rainfall treatment contained 12 plants. Black lines show inundated period, and grey lines show non-inundated period. Small grey bars represent small rainfall events (<5 mm), and large grey bars represent larger rainfall events (>5 mm). No rainfall was applied to plants during the inundated periods.
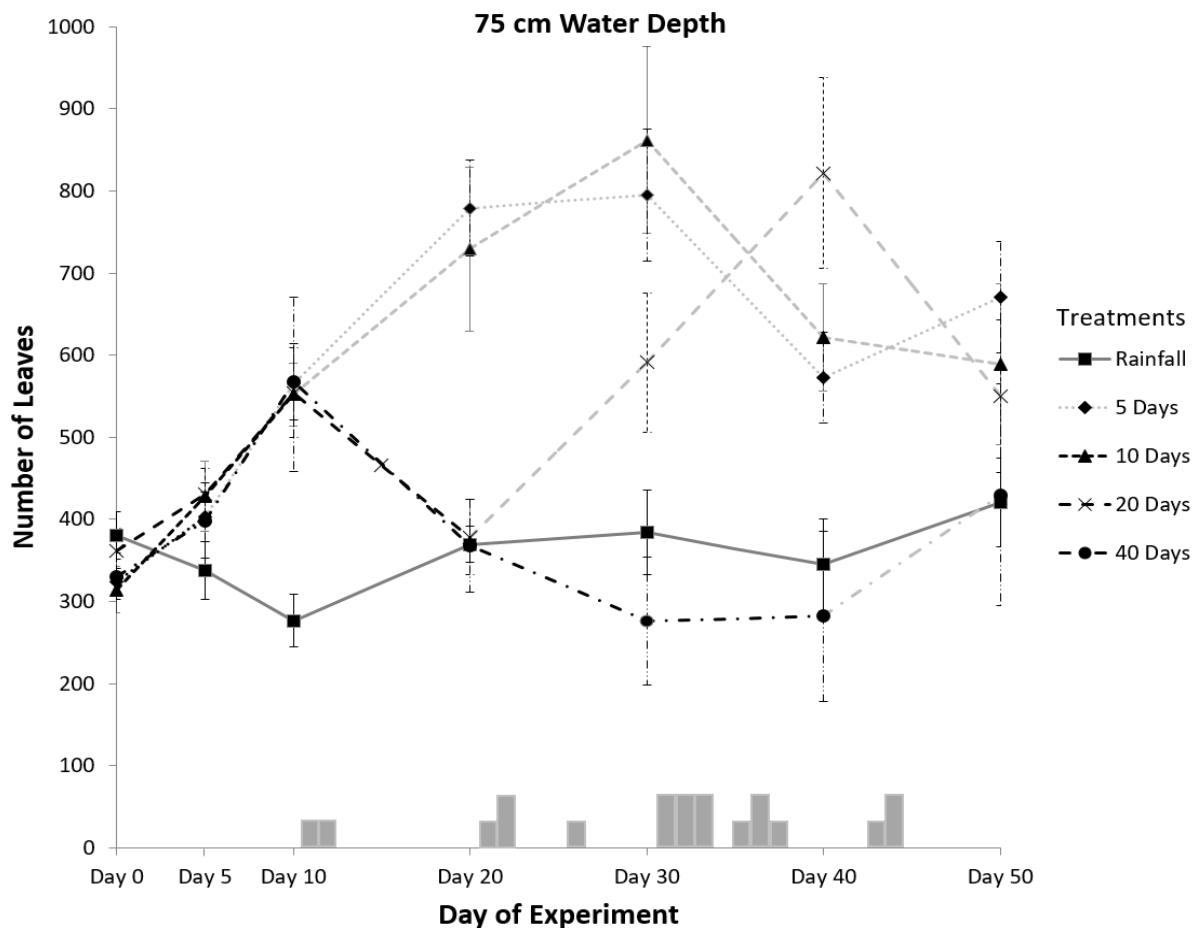
Figure 5.4 Mean ± s.e.m. number of live leaves per nitre goosefoot plant over time in the rainfall and inundated 75 cm treatments. The inundation treatments each contained eight plants and the rainfall treatment contained 12 plants. Black lines show inundated period, and grey lines show non-inundated period. Small grey bars represent small rainfall events (<5 mm), and large grey bars represent larger rainfall events (>5 mm). No rainfall was applied to plants during the inundated periods.

5.4.3 Biomass

Treatment had a significant effect on total biomass ($F_{12,91} = 4.448$, $P = 0.001$). *Post hoc* testing (Tukey's HSD) showed that plants in the 10 cm-20 days treatment had a significantly higher total biomass on Day 50 than did plants in the rainfall treatment ($P = 0.01$), in the 10 cm-10 days treatment ($P = 0.008$), and in the 75 cm-5 days ($P = 0.002$), 75 cm-20 days ($P = 0.001$) and 75 cm-40 days ($P = 0.002$) treatments (Fig. 5.5). Plants in the 10 cm-40 days treatment had a significantly higher total biomass on Day 50 than did plants that were inundated at 75 cm for 5 days ($P = 0.02$), 20 days ($P = 0.011$) and 40 days ($P = 0.014$; Fig. 5.5).

111

Treatment had a significant effect on aboveground biomass ($F_{12,91}$ = 3.767, P = 0.001). Plants in the 10 cm-20 days treatment had a significantly higher aboveground biomass on Day 50 than did plants in the rainfall treatment (P = 0.018), in the 10 cm-10 days (P = 0.020), and 75 cm-5 days (P = 0.002), 75 cm-20 days (P = 0.002) and 75 cm-40 days (P = 0.010) treatments (Fig. 5.5). Plants in the 10 cm-40 days treatment had significantly higher aboveground biomass than did plants in the 75 cm-20 days treatment (P = 0.042; Fig. 5.5).

Treatment had a significant effect on belowground biomass ($F_{12,91}$ = 2.351, P = 0.011). Despite this, no treatments were significantly different at P = 0.05. Plants in the 10 cm-40 days treatment had a higher belowground biomass on Day 50 than did plants in the 75 cm-40 days treatment at P = 0.056 (Fig. 5.5). The plants in the dry treatment (ratio of belowground to aboveground biomass, 0.36), and in the 10 cm-5 days, 10 cm-40 days, in the 50 cm-20 days, and in the 75 cm-5 days treatments had slightly higher ratios of belowground to aboveground biomass than did plants in the other treatments (Table 5.2, Fig. 5.5). The plants in the 75 cm-40 days treatment had the lowest ratio of belowground biomass to aboveground biomass (Table 5.2, Fig. 5.5).

All plants in the dry treatment died during the experiment and were not included in the analyses of dried biomass of live plants. Plants in the dry treatment had mean ± s.e.m. aboveground and belowground biomasses of 4.32 ± 0.33 and 1.54 ± 0.16 g respectively, measured at Day 50 of the experiment. One plant in the rainfall treatment died during the experiment and was excluded from the analyses of biomass. Including this dead plant, the mean ± s.e.m. aboveground and belowground biomasses in the rainfall treatment were 5.21 ± 0.33 and 1.61 ± 0.16 respectively. Three plants in the 75 cm-40 days treatment died during the experiment and were excluded from the analyses of biomass. Including these dead plants, the mean ± s.e.m. aboveground and belowground biomasses for this treatment were 4.84 ± 0.40 and 1.26 ± 0.20 respectively.
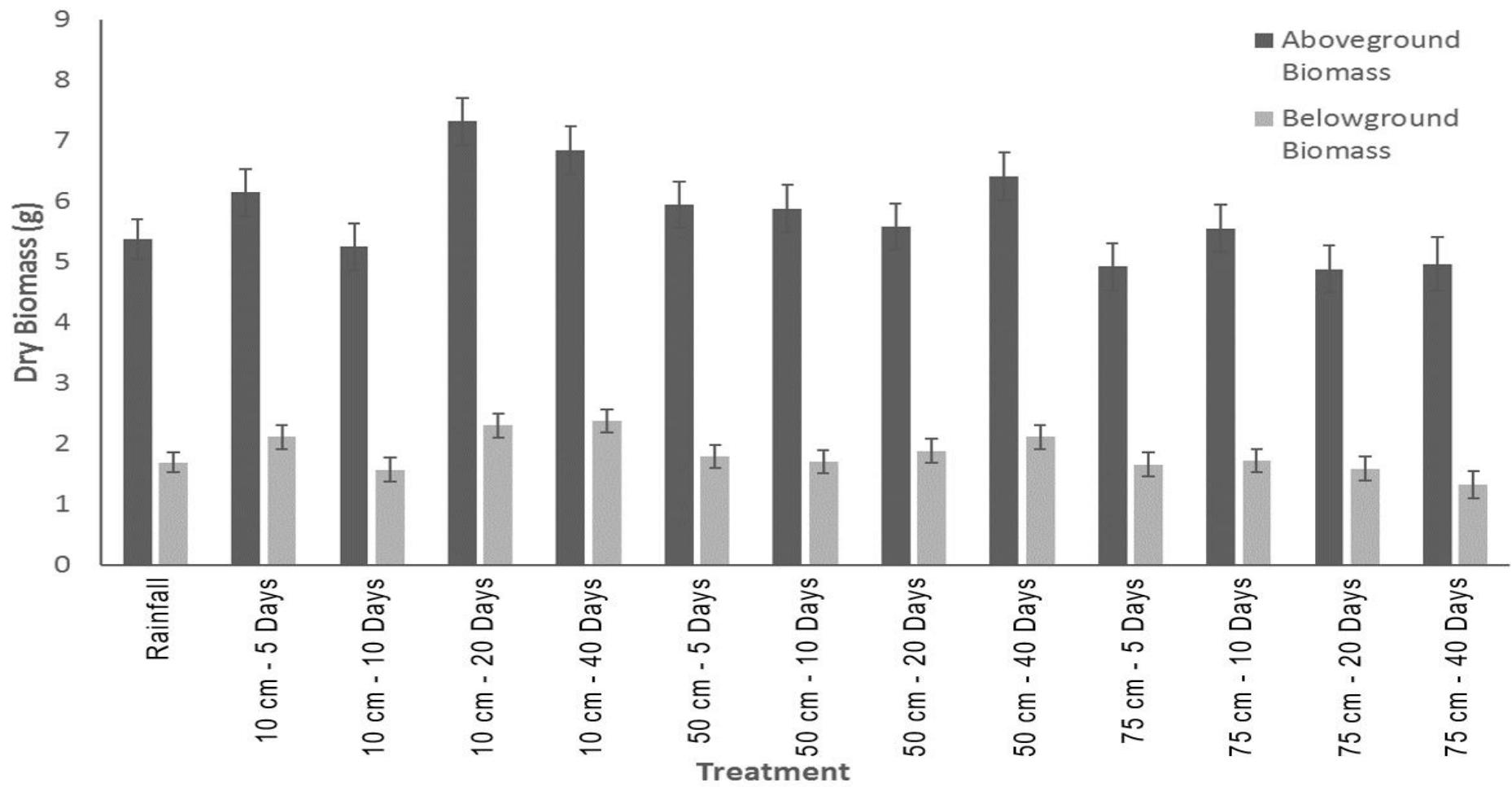
Figure 5.5 Mean ± s.e.m. aboveground dry biomass (g) and mean ± s.e.m. belowground dry biomass (g) per plant of nitre goosefoot plants on day 50 of the experiment in the rainfall and inundation treatments (see Table 5.1 for explanation of abbreviations). Only plants which were alive on day 50 of the experiment are included in the figure.

113

5.4.4 Seeding

In total, 34 plants set seed across all treatments. Seed setting was not synchronous but occurred at various times over the experimental period. The treatment with the highest number of seeding plants was the 10 cm-40 days treatment, with five of eight plants setting seed (Table 5.2). These plants set seed by Day 10, Day 20 (three plants) and Day 50. Four of the eight plants in the 10 cm-20 days and 50 cm-20 days treatment set seed (Table 5.2). These plants set seed by Days 5, 20, 30 and 40 in the 10 cm-20 days treatment and by Days 10, 20 (two plants) and Day 30 in the 50 cm-20 days treatment. The two or three plants in the other treatments in which more than one plant set seed (Table 5.2), did so by Day 30 of the experiment.

One plant in the dry, rainfall, the 10 cm-10 days, and the 75 cm-5 days treatments set seed during the experiment (Table 5.2). The plant in the dry treatment seeded in the first 10 days of the experiment. The plants in the rainfall and the 10 cm-10 days treatments both seeded between Day 40 and Day 50 of the experiment. The plant in the 75 cm-5 days treatment seeded between Day 20 and Day 30 of the experiment.

5.5   Discussion

5.5.1 Mortality

Most (97%) nitre goosefoot plants survived the rainfall and inundation treatments. One plant in the rainfall treatment, and three of the four plants that were flooded for 40 days at 75-cm depth and were fully submerged, died during the experiment. The survival of the inundated nitre goosefoot plants that were partially submerged for up to 40 days, or that were fully submerged for 20 or fewer days, suggests that nitre goosefoot can survive under flooded conditions, and, thus, can grow under hypoxic conditions for at least 40 days, providing the period of submersion is short or some of the plant remains out of water. Other studies have found that plants can survive partial submergence better than full submergence, and mortality increases with the duration of inundation (Auchincloss *et al.* 2012; Striker 2012; Greet 2015). Although plant responses to flooding are complex (Kozlowski 1997), nitre goosefoot plants that are not fully submerged might survive hypoxic conditions by transporting oxygen from

aerial tissues to the roots, which is a strategy used by other flood-tolerant species (Blom *et al.* 1990).

Taller plants cope with deeper periods of flooding better than do shorter plants, because taller plants are less likely to be fully submerged. Juvenile (<24 month old) river red gums experienced increased stress when totally submerged, compared with plants that were only partially submerged (Roberts and Marston 2011). Higher mortality rates have been observed in small river cooba recruits than in larger recruits following a flood event (Capon *et al.* 2012). The nitre goosefoot plants in the present experiment were ~8 months of age and had a mean ± s.d. height of 67.8 cm ± 15.2 at the commencement of the experiment. Nitre goosefoot plants grow up to 2.5 m tall (Cunningham *et al.* 1981). Because the only inundated plants that died were completely submerged for 40 days, it is possible that tolerance to extended flooding or to deeper water would increase as plants grow taller. Older and, therefore, taller plants would be partially, rather than fully, submerged, by deeper floods.

5.5.2 Leaf production

Nitre goosefoot plants generally increased leaf production in response to inundation, and again following recession of water. Increasing leaf production in response to flooding is a common characteristic of floodplain species such as tangled lignum and river red gum (Capon *et al.* 2009; Roberts and Marston 2011; Parsons and Thoms 2013). Nitre goosefoot shed leaves after 10 days following flood recession, as the soil dried. Shedding leaves has been observed in tangled lignum on drying floodplains (Roberts *et al.* 2016).

The present study showed that nitre goosefoot produced most leaves with shallow flooding for 40 days or so. The species also had a high production of leaves with deeper flooding for a few days. Lower numbers of leaves were produced under rainfall, shallow flooding for short periods, and long, deep inundation. These results suggest that leaf production in nitre goosefoot responds positively to shallow flooding for a month or so and to a few days of deep flooding, but not to deep, long floods. Capon *et al*. (2009) found that allocation of resources by tangled lignum plants to leaves was lower in more deeply flooded plants. All branches on plants in our study that were submerged for 20 days or more became leafless by 20–30 days. Leaf shedding and the suppression of new leaves has been observed in other woody species in response to submergence (Kozlowski 1997).

5.5.3 Biomass

The present study found that total biomass, aboveground biomass and belowground biomass of nitre goosefoot plants at the end of the study were generally higher with shallow flooding for 20-40 days than with shorter or deeper flooding, or with rainfall alone. Higher growth during flooding is characteristic of flood-dependent species such as tangled lignum (Roberts and Marston 2011). The low ratio of belowground biomass to aboveground biomass in plants that were inundated at 75-cm depth for 40 days is consistent with the results of studies that showed that the ratio of root to shoot biomass decreased with the length of flooding period in river red gum (Argus *et al.* 2015), and in other plant species (Megonigal and Day 1992 and references therein; Sauter 2013). The ratios of belowground to aboveground biomass in nitre goosefoot plants that were flooded at shallow depths for 40 days were not lower than were the ratios in plants that received shallow flooding for shorter periods of time. This was possibly because roots of plants in the shallower treatments could obtain more oxygen from their greater area of non-submerged material during the experiment, than the area of non-submerged material in the deeper, 75-cm-flood treatments (see Sauter 2013).

5.5.4 Seeding

Nitre goosefoot plants that were inundated for longer were more likely to set seed than were plants that were inundated for shorter periods of time or that received rainfall or no water. Seed set occurred during inundation and after drawdown. The duration of inundation was more important than was the depth of inundation for seeding (as long as plants were not completely submerged), because three or more plants in the treatments that were inundated 20 days or longer set seed at all three depths. Setting seed during flooding or just after flood recession allows mature seeds to fall on the receding flood waters or on wet or damp soil, which maximises seed germination (chapter 4) and seedling establishment. This is a common strategy in plants on the floodplains of the Murray-Darling Basin (Casanova 2015).

5.6 Implications and recommendations

Flooding is a fundamental driver of productivity in semi-arid floodplains (Parsons and Thoms 2013) and relationships between flooding and nitre goosefoot productivity is no exception. The present study showed that leaf production, biomass and seed set in nitre goosefoot were highest under shallow flooding for 20-40 days, depending on the attribute. Leaf production was also high with short, deep floods. Leaf production increased after flooding and after recession of water. Although leaf production, biomass and seed set were low under rainfall, these findings need to be interpreted in the context of the experimental study. Biomass, net leaf production, and seed set could be high during extended periods of very high rainfall if the soil remained continually wet.

Nitre goosefoot occurs with or near other flood-dependent species, particularly black box and tangled lignum (Cunningham *et al.* 1981). These species typically occur in periodically flooded areas (Capon *et al.* 2016; Roberts *et al.* 2016) on floodplains and in local depressions, mostly in semi-arid regions. Black box and tangled lignum exhibit characteristics (such as increased growth and seed production) in response to flooding (George 2004; Capon *et al.* 2016) similar to those that nitre goosefoot did in the present study.

The present study showed that nitre goosefoot both responds positively to flooding and tolerates flooding. Growth and seed set of nitre goosefoot plants were high with extended, shallow flooding and with short periods of deeper flooding (depending on attribute), and the plants survived flooding for 40 days, providing they were not totally submerged. Nitre goosefoot survived, grew, and one plant set seed, under rainfall conditions. The requirement of the species for a month or so of shallow flooding for growth and reproduction, the survival and some growth of the species under rainfall conditions (without flooding), the species' intolerance of total submergence for extended periods, and the highest germination of seeds with a few days flooding and on moist soils (see thesis chapter four), allows nitre goosefoot to occur on the higher parts of intermittently and temporarily inundated areas, such as on the edges of floodplains and wetlands.

Plants on floodplains can be grouped on the basis of shared traits (Casanova 2011), so as to allow interpretation of responses to flooding for allocation of environmental flows (Merritt *et al.* 2010b; Campbell *et al.* 2014). Campbell *et al.* (2014) placed nitre goosefoot within the terrestrial damp (Tda) water-plant functional group (defined by Casanova 2011), i.e.

terrestrial plants that grow in damp places but cannot tolerate flooding in the vegetative state. The present study has shown that nitre goosefoot does not have the characteristics of Tda plants, because it tolerates flooding, and it responds positively to flooding, providing the floods are not too deep or too long. Consistent with the findings of the present study, Cunningham *et al*. (1981) described the habitat of nitre goosefoot as depressions, river flats, floodplains and other low-lying areas subject to occasional inundation (but see Keith (2004) and McGinness (2013) who considered that nitre goosefoot occurs in areas that rarely flood).

The flood tolerance of nitre goosefoot suggest that the species is aligned more closely with the water-plant functional group of amphibious, fluctuation tolerator-emergent (ATe) or with amphibious fluctuation tolerator-woody (ATw) than with the Tda group (Casanova 2011). Plants in the ATe group are emergent monocots and dicots that survive in saturated soil or shallow water but require most of their photosynthetic parts to remain above the water (emergent; Casanova 2011). They tolerate fluctuations in the depth of water, as well as water presence. They need water to be present for ~8-10 months of the year, and the dry time to be in the cooler times of the year. Plants in the functional group ATw are woody perennial species that hold their seeds on their branches and require water to be present in the root zone all year round, but will germinate in shallow water or on a drying profile. If they grow on floodplains, they require flooding and restoration of the groundwater levels on a regular basis.

Nitre goosefoot has some of the characteristics of both ATe and ATw species. This study has shown that the species survives in saturated soil or shallow water and requires most of its photosynthetic parts to remain above the water. Nitre goosefoot does not require water to be present for 8-10 months of the year because the species occurs on the edges of floodplains (Seddon and Briggs 1998; Briggs *et al.* 2000), which flood less frequently and for shorter durations than the lower, more central parts of floodplains. Nitre goosefoot will germinate in shallow water or, on a drying profile (chapter 4), the species holds its seeds on its branches for at least a few months (W. Higgisson, pers. obs.), and the present study has shown that it requires flooding for optimum growth and seed set. The characteristics of the root zone of nitre goosefoot are unknown. Further investigation of the ecology of nitre goosefoot is required to determine the water-plant functional group (Casanova 2011) that most closely aligns with the characteristics of nitre goosefoot.

Only a few species of large shrubs occur on floodplains of the Murray-Darling Basin (Cunningham *et al.* 1981; Keith 2004; Benson *et al.* 2006). Habitat structure on these floodplains is generally provided by tangled lignum at wetter sites and nitre goosefoot at drier sites (McGinness *et al.* 2013). Thus, nitre goosefoot is an important component of the floodplains of the Murray-Darling Basin. The current study showed that a month or so of shallow flooding provided best conditions for growth and seed set of nitre goosefoot. Chapter 4 found that seeds of nitre goosefoot germinated best when subjected to 5 days of flooding, and on wet soils such as occur following flood recession. On the basis of the results of the present study and chapter 4, nitre goosefoot appears to have an ecological strategy of optimal growth and seed set under short, shallow floods, and optimal germination of seeds during short floods and following flood recession. Together, these results provide the information to factor the hydrological requirements of nitre goosefoot into environmental flow regimes.

5.7   Addendum

While the manuscript presented here was published prior to the examination of the thesis, the examiners raised several pertinent questions that are addressed below:

1. I would suggest that a 5°C difference is considerably higher. Could this have influenced mortality more than would have been the case naturally?

Whilst the average maximum daily temperature in the glasshouse during the experiment (35°C) was approximately 5°C greater than the temperature in Hillston, NSW during the experimental period. The glasshouse temperature was comparable to the average temperature in Hillston in January (34°C), therefore it is likely that nitre goosefoot would regularly experience temperatures around or in excess of 35°C. It is also hard to predict the effect of the higher temperature on mortality as very few plants died during the experiment. A total of four plants died during the experiment not including plants in the control treatment. Three of the four plants which died were inundated to a depth of 75 cm for 40 days and were completely inundated. One plant died within the rainfall treatment. This chapter was interested in the effect of depth and duration of flooding and not the influence of temperature.

2. Seed set discussion – there is no context to place this in terms of what is known or not known about the reproductive biology of this species. What is the point of looking at this as a variable? At what age/size do plants typically flower/set seed. At what time of the year do they normally flower? Did any plants flower, but not set seed e.g. was seed set constrained by anything other than the treatments (such as self/cross pollination)?

This study was interested in the influence of flooding depth and duration on a range of life history stages in nitre goosefoot including whether flooding promotes seed set. There is very little known regarding the reproductive biology of nitre goosefoot, including at what age, size and time of year does the species typically flowers or set seed, or if pollination is constrained by factors such as self/cross pollination.

DECLARATION OF CO-AUTHORED PUBLICATION CHAPTER

For use in theses which include publications. This declaration must be completed for each co-authored publication and to be placed at the start of the thesis chapter in which the publication appears.

Declaration for Thesis Chapter 6

Declaration by candidate

In the case of Chapter 6 the nature and extent of my contribution to the work was the following:

Nature of Contribution	Extent of contributions
I conducted all field and laboratory work, analysed the data and led the writing of the manuscript.	80%

The following co-authors contributed to the work:

Name	Nature of Contribution	Contributor is also a student at UC Y/N
Fiona Dyer	Study design and manuscript writing	N
Dianne Gleeson	Study design and manuscript writing	N
Linda Broadhurst	Study design and manuscript writing	N

Candidate's Signature: Date: 01/02/2019

Declaration by co-authors

The undersigned hereby certify that:

1) the above declaration correctly reflects the nature and extent of the candidate's contribution to this work, and the nature of the contribution of each of the co-authors.
2) they meet the criteria for authorship in that they have participated in the conception, execution, or interpretation, of at least that part of the publication in their field of expertise;
3) they take public responsibility for their part of the publication, except for the responsible author who accepts overall responsibility for the publication;
4) there are no other authors of the publication according to these criteria;
5) potential conflicts of interest have been disclosed to (a) granting bodies, (b) the editor or publisher of journals or other publications, and (c) the head of the responsible academic unit; and
6) the original data are stored at the following location(s) and will be held for at least five years from the date indicated below:

Location(s):	Institute for Applied Ecology, University of Canberra, Canberra, Australia

Signatures	Date
	06/07/2019
	27/07/2019
	06/07/2019

122

6. Genetic diversity and gene flow patterns in tangled lignum (*Duma florulenta*) and river cooba (*Acacia stenophylla*) across a large inland floodplain

Background: Little is currently known about the effect of hydrochory on the genetic structure of plants which occur on floodplains and the role of river-floodplain connection in the dispersal of floodplain plants and connection between habitat patches. Determining a species' genetic structure and how this is maintained across a landscape is an important step in helping to set conservation priorities. Therefore, chapter 6 explores and compares the genetic variability and possible pathways of gene flow of tangled lignum and river cooba. This chapter contains a manuscript in preparation for publication.

6.1   Abstract

Gene flow is a key evolutionary driver of spatial genetic structure. Spatial patterns in genetic structure reflect demographic processes and dispersal mechanisms. Understanding how genetic structure is maintained across a landscape can assist in setting conservation priorities. In Australia, floodplains naturally experience a highly variable flooding regime, which structures the vegetation communities. Temporal variability in flooding, and spatial variability in topography, means communities on floodplains are often spatially and temporally discrete. Flooding plays an important role, connecting communities on floodplains, through enabling dispersal via hydrochory. Water resource development has changed the lateral-connectivity of floodplain-river systems. One possible consequence of these changes is the reduced physical and subsequent genetic connections. This study aimed to identify and compare the population structure and dispersal patterns of tangled lignum (*Duma florulenta*) and river cooba (*Acacia stenophylla*), across a large inland floodplain using a landscape genetics approach. Both species are widespread throughout flood prone areas of arid and semi-arid Australia. Leaves were collected from 144 lignum plants across 10 sites and 84 river cooba plants across six sites, on the floodplain of the lower and mid Lachlan Catchment, NSW. DNA was extracted and genotyped using DArTseq platforms (double digest RADseq). Genetic diversity was compared with floodplain-river connection frequency, and genetic distance (F_{ST}) was compared with river distance, geographic distance and floodplain-river connection frequency between sites. Genetic connectivity increased with increasing floodplain-river connection frequency in tangled lignum but not in river cooba. In tangled lignum, sites that experience more frequent flooding had higher levels of genetic diversity and were more genetically homogenous. There was also an isolation by distance effect where increasing geographic distance correlated with increasing genetic differentiation in lignum, but not in river cooba. Waterbirds may play an important role in the dispersal of tangled lignum, as water dispersal alone does not explain connectivity between patches. These results demonstrate how genetic patterns can highlight influential mechanisms over species distribution and persistence on floodplains.

6.2 Introduction

World-wide, the patterns of river flow have been, and continue to be, affected by water resource developments such as dams and water abstraction for irrigated agriculture and other purposes (Nilsson *et al.* 2005; Haddeland *et al.* 2014; Grill *et al.* 2015). These changes to river flow disrupt the natural flood regime of rivers and associated floodplains, changing the lateral connectivity of floodplain-river systems (Ward and Stanford 1995; Thoms 2003; Chapter 2). One possible consequence of altered river flow patterns is physical disruption to established patterns of gene flow in organisms such as fish (Gouskov *et al.* 2016) and plants. Consequently, impacts on riverine species can include demographic factors such as smaller population sizes and range reductions, as well as genetic factors such as reduced genetic diversity, inability to adapt to environmental changes and inbreeding depression (Pavlova *et al.* 2017).

Genetic diversity within individuals and populations favours species persistence by helping to maintain fitness and adaptation to a changing environment (Falconer *et al.* 1996; Mandák *et al.* 2006; Barrett and Schluter 2008). Gene flow is a key driver of spatial genetic structure within a species (Ellstrand 2014) influencing genetic diversity within and among populations as well as genetic differentiation between populations (Slatkin 1977; Young *et al.* 2000). Gene flow via migration between patches maintains genetic variation while retarding genetic divergence (Meffe and Vrijenhoek 1988). Many factors influence gene flow including geographic distance (Wright 1943; Ellstrand 2014), landscape features such as river networks (Knaapen *et al.* 1992; Yan *et al.* 2016), and reproduction and dispersal rates (Iwaizumi *et al.* 2013). These factors may facilitate or inhibit gene flow between populations, influencing the amount of genetic differentiation that may occur across a species geographic range (Kawecki and Ebert 2004; Dillon *et al.* 2014).

Globally, habitat fragmentation has led to a decrease in genetic diversity in many plant species and increased the likelihood of inbreeding (Young *et al.* 1996; Aguilar *et al.* 2008; Frankham *et al.* 2017). This can lead to inbreeding depression and reduce fitness, increasing the risk of extinction (Templeton *et al.* 1990; Frankham 2005).

Since population genetic structure reflects demographic processes such as migration rates, population arrangement (for example continuous or discrete), and dispersal mechanisms and limitations, determining a species' genetic structure and how this is maintained across a

landscape is an important step in helping to set conservation priorities (Stow *et al.* 2001; Dudaniec *et al.* 2013). Genetic approaches can identify spatial genetic variation and how this is associated with landscape and ecological features (for example see Wei *et al.* 2013; Dillon *et al.* 2015; Yan *et al.* 2016; Coleman *et al.* 2018), and recent advances in genomic technologies have increased the applicability and use of genetics in conservation biology (Hoffmann *et al.* 2015; Garner *et al.* 2016; Pavlova *et al.* 2017).

In arid and semi-arid environments, floodplain inundation is often temporally irregular (Nicholls 1991; Walker *et al.* 1995; Rogers and Ralph 2011) and varies spatially across floodplains, with surface topography influencing the depth and duration of inundation (Scown *et al.* 2015). This temporal variability in flooding along with spatial variability caused by topographic variability across floodplains, creates a heterogeneous mosaic of habitat types (Welcomme 1979; Roberts *et al.* 2000; Thoms 2003), with floodplain species and communities often being spatially and temporally discrete.

Many plants which occur along rivers and on floodplains have buoyant fruits or seed which favours their dispersal by water (hydrochory) (Sculthorpe 1967). The dendritic nature, and unidirectional flow of rivers means hydrochory connects habitat patches linearly as propagules move downstream (Nilsson *et al.* 2010). On floodplains, inundation enables the dispersal of seeds (Chong and Walker 2005; Roberts and Marston 2011), including through connection between otherwise disconnected habitat patches (Nilsson *et al.* 2010). Waterbirds also play an important role in the dispersal of aquatic plant propagules (Green *et al.* 2008). Waterbirds transport propagules both internally and externally among habitat patches (Green *et al.* 2008). In arid and semi-arid Australia, waterbirds are nomadic, moving among temporary wetlands for breeding and feeding (Kingsford *et al.* 1999) and often covering vast distances along commonly used flyway paths (McGinness 2017).

Currently, little is known about population structure and gene flow in plants which occur on floodplains or wetlands (except see Kudoh and Whigham 1997; Schleuning *et al.* 2011) and whether these patterns reflect landscape features such as river architecture, abiotic variables including floodplain inundation, and biotic influences such as bird movements. Kudoh and Whigham (1997) propposed that there are three possible models of pathways of gene flow in a hydrochorous wetland species. that is, 1) a two-dimensional stepping stone distance model, where gene flow depends on the Euclidian distance between sites, 2) a one-dimensional stepping stone distance model, where gene flow depends on the distance measured along the

river network, and 3) the stream accessibilty model, where gene flow depends on distance to the river channel and flooding frequency for populations not adjacent to the river.

The stream hierarchy model (Meffe and Vrijenhoek 1988), proposed for fish species which occur in desert streams, suggests that genetic divergence is a function of geographic connectedness of habitats, and that populations located along different river distributaries and more so in different drainage catchments will have a higher genetic divergence than populations occurring along the same river or within the same drainage catchment . This has been observed in a freshwater shrimp (Carini and Hughes 2004) and two freshwater fishes (Hughes and Hillyer 2006) in western Queensland. These pathways of dispersal, influence a species vulnerability to the effects of water resource development and informs the way in which we manage them.

This study aimed to identify and compare the population structure and dispersal patterns of two ecologically important plant species with varying distribution on rivers and floodplains: tangled lignum (*Duma florulenta*) and river cooba (*Acacia stenophylla*) across a large inland floodplain, using a landscape genetics approach. River cooba commonly occurs along rivers and creek banks (Cunningham et al. 1981) whilst tangled lignum typically occurs away from the main river channel on floodplains (Roberts et al. 2016). Tangled lignum is assumed to disperse by water owing to its seeds ability to float for >30 days (Chapter 4). The riverine distribution of river cooba also suggests its propagules may disperse by water. I hypothesized that species which occur along rivers such as river cooba, will display free flowing gene-flow related to distance along rivers while species such as tangled lignum which occur away from the main river channel on floodplains, will be more dependent on river-floodplain connection for gene-flow between patches. I addressed the following questions: i) how much genetic diversity exists within the study region for both species, ii) does population genetic structure exist in either species? iii) is there evidence of gene flow within either species? and, iv) does gene flow relate to landscape features such as river networks, frequency of floodplain-river connection by flooding, or biological features such as bird migration?

6.3 Methods

6.3.1 Study species, sampling strategy and study sites

Two common and ecologically important plant species distributed on floodplains and along rivers of the Murray-Darling Basin are tangled lignum (Polygonaceae) and river cooba (Fabaceae) (Cunningham *et al.* 1981). Both species are widespread through arid and semi-arid Australia (AVH 2016) and provide important habitat for colonially nesting waterbirds during floodplain inundation (Brandis *et al.* 2009; Kelleway *et al.* 2010; Roberts and Marston 2011). Tangled lignum is a multi-stemmed, perennial shrub to 2.5 m high (Cunningham *et al.* 1981). Tangled lignum grows on floodplains where flooding occurs every 3-10 years (Craig *et al.* 1991; Wilson 2009; Thomas *et al.* 2012; Freestone *et al.* 2017). Tangled lignum is dioecious (Chong and Walker 2005). Tangled lignum can reproduce sexually via seed and asexually by lateral growth of rhizomes and stolons as well as through root development along branches which can disconnect to form new individuals (Craig *et al.* 1991; Roberts and Marston 2011). The seeds of tangled lignum are assumed to disperse by water, because they can float, and seed germination occurs following the recession of floodwaters (Chapter 4).

River cooba is a medium tree to 13 m (PlantNET 2017a) which occurs along the margins of rivers and on higher elevated areas of floodplains which receive flooding every three to five years (Cunningham *et al.* 1981; Doody and Overton 2009). Seed germination has been observed following the recession of floodwaters (Cunningham *et al.* 1981). Little is known on the effect of flooding on river cooba, including the dispersal and establishment of seeds (see Johns *et al.* 2009; Roberts *et al.* 2016).

During 2016-2017 a total of 144 tangled lignum plants were sampled across 10 sites and 84 river cooba plants were sampled across six sites in the lower and mid Lachlan River Catchment, and lower Murrumbidgee River Catchment (Figure 6.1 and Table 6.1). Remnant study sites for both species were chosen based on field surveys in April and May 2016 in consultation with water managers to ensure (as best as possible) that study sites were evenly distributed along the main channel as well as anabranches and distributaries of the Lachlan River. For tangled lignum, eight sites were initially sampled, of which a large amount of genetic differentiation was observed between certain sites. As such, a further two sites were included to provide a larger study area. One of the two sites (Nap Nap) is within the Murrumbidgee River Catchment and the other (Murrin Bridge) is the most upstream location

of all sites on the Lachlan River (Figure 6.1). The Lachlan and Murrumbidgee rivers connect infrequently (O'Brien and Burne 1994; Meredith and Beesley 2009).

While river cooba has been grown as a forestry species (Boxshall and Jenkyn 2001), there is no evidence that either river cooba or tangled lignum have been used in habitat restoration in the lower Lachlan River Catchment and all sites are assumed to be remnant populations. Sex identification of tangled lignum plants was not possible as they were not flowering at the time of sampling. At each site fresh leaves from between 12-20 plants approximately 50 m apart to avoid sampling of clones were collected, immediately dried in silica gel and returned to the University of Canberra laboratory.
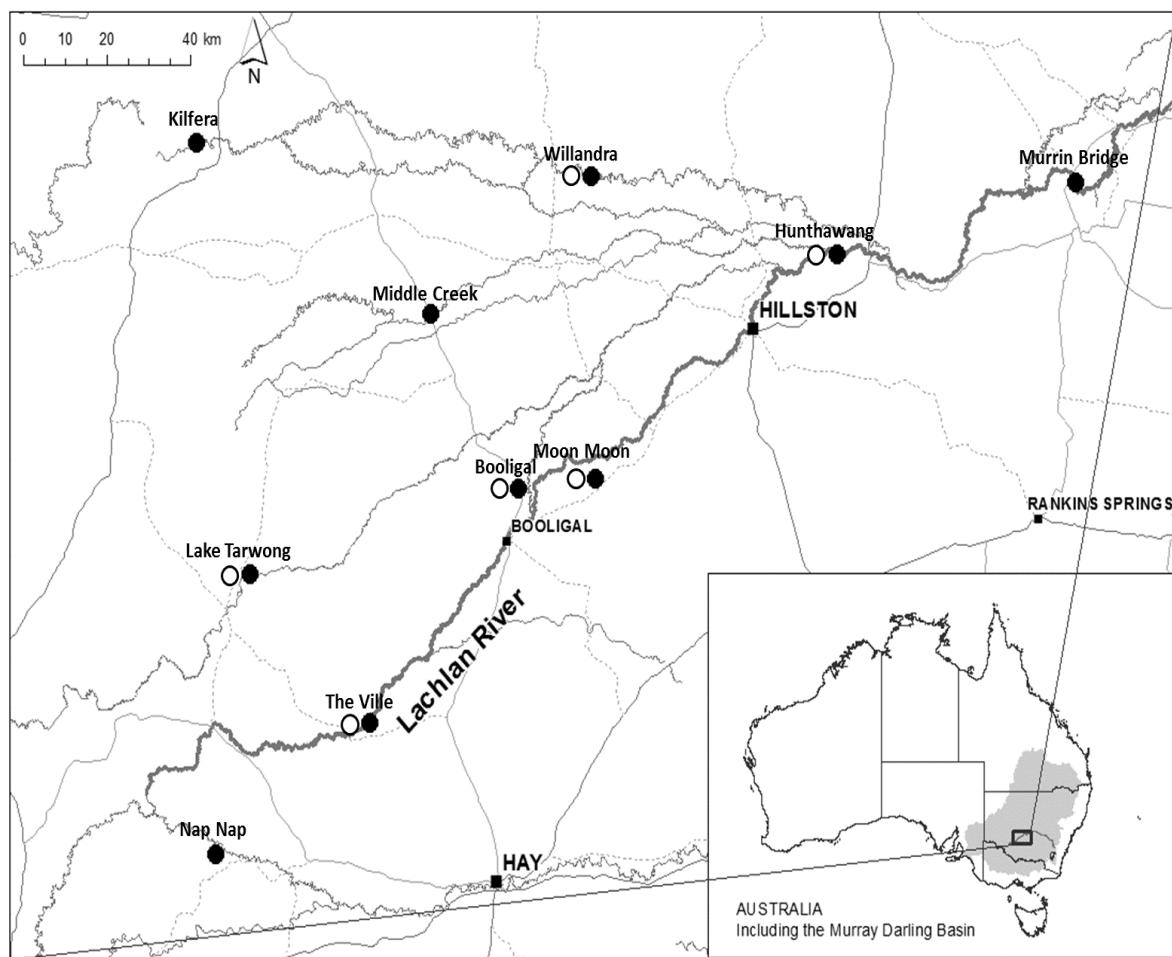
Figure 6.1 Locations where leaf samples of tangled lignum (all 10 sites; each site shown as a solid black circle) and river cooba (six sites; each site shown as an open (white) circle) were collected on the floodplain of the lower and mid Lachlan River, and Murrumbidgee River (Nap Nap) New South Wales, and location of the Murray-Darling Basin in south-eastern Australia.

6.3.2 Genotyping

DNA extraction was performed using protocols developed by Diversity Array Technology Pty Ltd (DArTseq[TM]). Tissue samples (25 mg) were incubated overnight at 56°C with lysis buffer and proteinase K. Lysed tissue was washed using washing buffer to remove impurities such as protein and polysaccharides and stored in elution buffer. Genomic analysis was undertaken on the 144 tangled lignum and 84 river cooba leaf samples using a genome wide profiling approach utilising restriction enzymes to produce DNA fragments, which were sequenced using next-generation platforms by Diversity Arrays Technology (http://www.diversityarrays.com). Their technique is similar to double-digest restriction associated DNA sequencing (Peterson *et al.* 2012), but has the advantages of accepting lower quantities of DNA, greater tolerance of lower quality DNA and yielding lower allelic dropout rates.

DArTseq™ represents a combination of a DArT complexity reduction method and next generation sequencing platforms (Kilian *et al.* 2012; Courtois *et al.* 2013; Cruz *et al.* 2013; Raman *et al.* 2014). Therefore, DArTseq™ represents an implementation of sequencing of complexity reduced representations (Altshuler *et al.* 2000) and more recent applications of this concept on the next generation sequencing platforms (Baird *et al.* 2008; Elshire *et al.* 2011). Similarly, to DArT methods based on array hybridisations the technology is optimized for each organism and application by selecting the most appropriate complexity reduction method (both the size of the representation and the fraction of a genome selected for assays). Four methods of complexity reduction were tested in each organism and the PstI-NspI method was selected. DNA samples were processed in digestion/ligation reactions principally as per Kilian *et al.* (2012) but replacing a single PstI-compatible adaptor with two different adaptors corresponding to two different Restriction Enzyme (RE) overhangs. The PstI-compatible adapter was designed to include Illumina flowcell attachment sequence, sequencing primer sequence and "staggered", varying length barcode region, similar to the sequence reported by Elshire *et al.* (2011). Reverse adapter contained flowcell attachment region and NspI-compatible overhang sequence.

Only 'mixed fragments' (PstI-NspI) were effectively amplified in 30 rounds of PCR using the following reaction conditions: 94°C for 1 min, 30 cycles of: 94°C for 20 sec, 58°C for 30 sec, 72°C for 45 sec, and 72°C for 7 min. After PCR equimolar amounts of amplification products from each sample of the 96-well microtiter plate were bulked and applied to c-Bot (Illumina)

bridge PCR followed by sequencing on Illumina Hiseq2500. The sequencing (single read) was run for 77 cycles.

Sequences generated from each lane were processed using proprietary DArT analytical pipelines. In the primary pipeline the fastq files were first processed to filter away poor-quality sequences, applying more stringent selection criteria to the barcode region compared to the rest of the sequence. In that way the assignments of the sequences to specific samples carried in the "barcode split" step was very reliable. Approximately 2,500,000 sequences per barcode/sample were identified and used in marker calling. Finally, identical sequences were collapsed into "fastqcoll files". The fastqcoll files were "groomed" using DArT PL's proprietary algorithm which corrects low quality base from singleton tag into a correct base using collapsed tags with multiple members as a template. The "groomed" fastqcoll files were used in the secondary pipeline for DArT PL's proprietary SNP and SilicoDArT (presence/absence of restriction fragments in representation) calling algorithms (DArTsoft14). For SNP calling all tags from all libraries included in theDArTsoft14 analysis are clustered using DArT PL's C++ algorithm at the threshold distance of 3, followed by parsing of the clusters into separate SNP loci using a range of technical parameters, especially the balance of read counts for the allelic pairs. Additional selection criteria were added to the algorithm based on analysis of approximately 1,000 controlled cross populations. Testing for Mendelian distribution of alleles in these populations facilitated selection of technical parameters discriminating well true allelic variants from paralogous sequences. In addition, multiple samples were processed from DNA to allelic calls as technical replicates and scoring consistency was used as the main selection criteria for high quality/low error rate markers. Calling quality was assured by high average read depth per locus (average across all markers was over 30 reads/locus).

DArTseq genotyping produced some 32,000 single nucleotide polymorphisms (SNPs) for tangled lignum and 26,000 SNPs for river cooba. For all analysis, the data sets supplied by DArTseq were filtered on rep average (threshold = 1) and loci call rate (threshold = 1) resulting in 1058 SNPs for tangled lignum and 9300 SNPs for river cooba, which were used in subsequent analyses.

6.3.3 Data analysis

All statistical analysis was conducted in R version 3.3.3 (R Core Team 2013). Genetic diversity: mean expected heterozygosity (H_e) (which describes what would be expected under the assumptions of Hardy Weinberg Equilibrium) and observed heterozygosity (H_o) (describing the deviation from the Hardy Weinberg Equilibrium) were calculated for each site using the R Package dartR version 0.94 (Gruber *et al.* 2018) for each species. An ANOVA of mean observed heterozygosity was conducted, to identify significant differences, in this measure a Tukey Honest Significance Differences (HSD) test was undertaken for each species in the R package stats (R Core Team 2013).

Population differentiation: For each species pairwise F_{ST} between sites (Weir and Cockerham 1984) were calculated using the R Package PopGenReport (Adamack and Gruber 2014) as well as Nei's genetic distance (D) (Nei 1972) using the R package StAMPP (Pembleton *et al.* 2013). F_{ST} describes the proportion of the genetic variance contained within a subpopulation relative to the total genetic variance within the population, and ranges from zero to one, with values closer to one showing a higher degree of differentiation.

Population genetic structure: Initially the most appropriate number of clusters (from 1 to 10) was identified, then individuals were assigned a cluster, through maximum-likelihood genetic clustering using snapclust in the R package Adegenet (Jombart 2008). Principal coordinates analysis (PCoA) was undertaken on all individuals within each species, in the R package dartR (Gruber *et al.* 2018).

The geographic distance being the pairwise (Euclidian) distance between each site was calculated. The river distance being the pairwise distance between each site along the network of rivers and distributaries was calculated. The proportion of time each site connects to the river by floodwaters (here after referred to as floodplain-river connection) was calculated using 120 years of modelled river gauge flow data from the nearest gauge to each site under a 'without development' scenario and the commence to flow (CTF) level required to connect the site to the river (see method section in chapter 2 for details). A site was assumed to be connected to the river if the CTF was exceeded. The 'without development' flow scenario was used for this analysis, as tangled lignum and river cooba are both long-lived perennial species (river cooba can live for >50 years) (Roberts and Marston 2011) and the hydrological changes related to water resource development which have occurred in the

Lachlan River Catchment since 1935 are thus unlikely to have resulted in detectable genetic effects.

For tangled lignum, one site (Nap Nap) was excluded from regression analysis of floodplain-river connection, as floodplain-river connection data was unavailable for this site. For river cooba, one site (Hunthawang) was excluded from floodplain-river connection analysis as most of the plants (16/20) which leaves were collected from at this site were growing along the Lachlan River and hence would not require floodplain-river connection for dispersal.

A (linear model) regression analysis comparing pairwise F_{ST} (Weir and Cockerham 1984) with geographic distance (lat/long), river distance, and Jaccard dissimilarity of floodplain-river connection was performed in the R package stats and plotted in the R package ggplot. The Jaccard coefficient was used for dissimilarity of floodplain-river connection so as, only days where at least one site was connected to the river were included in analysis. After preliminary analysis of tangled lignum using snapclust and PCoA, two highly differentiated clusters were identified, therefore a subset of eight sites were also analysed separately for regression analysis comparing F_{ST} with river distance, geographic distance and floodplain-river connection for tangled lignum. Floodplain-river connection and observed heterozygosity for each site were compared using (linear model) regression analysis.

6.4   Results

Genetic diversity

There were contrasting levels of genetic diversity between the two species (Table 6.1). For example, H_o in these tangled lignum sites ranged from 0.081 to 0.206 and was significantly different among sites ($F_{9, 134} = 21.072$, P <0.001) whereas in river cooba, H_o was less variable ranging from 0.161 to 0.172 and did not differ significantly among sites ($F_{5, 78} = 0.618$, P <0.686). For tangled lignum, H_o at Hunthawang and Middle Creek was significantly lower than all other sites (<0.001), while Willandra was significantly higher than Booligal (0.006), Lake Tarwong (0.020), Moon Moon (0.004), and Nap Nap (0.007). Although H_o in river cooba did not differ significantly it was highest at The Ville and lowest at Booligal. In tangled lignum mean H_e ranged from 0.060 to 0.154 while for river cooba the range was less variable (0.156 to 0.163) (Table 6.1).

Table 6.1 The number of plants genotyped per site, the percentage of days each site connects to the river by floodwaters, and observed and expected heterozygosity for tangled lignum (for the ten sites) and river cooba (for the six sites) in the Lachlan River Catchment and Murrumbidgee River Catchment. NA, not applicable.

Site name	*N* tangled lignum	*N* river cooba	% Days connected to the river by flooding tangled lignum	% Days connected to the river by flooding river cooba	H_o tangled lignum	H_o river cooba	H_e tangled lignum	H_e river cooba
Booligal	12	13	15.38	15.38	0.156 ± 0.002	0.161 ± 0.003	0.129	0.159
Hunthawang	20	20	1.57	11.6	0.081 ± 0.003	0.163 ± 0.003	0.060	0.160
Kilfera	12		6.79	NA	0.188 ± 0.008	NA	0.140	NA
Lake Tarwong	12	13	12.17	12.17	0.161 ± 0.003	0.164 ± 0.004	0.135	0.160
Middle Ck	12		4.51	NA	0.105 ± 0.014	NA	0.086	NA
Moon Moon	12	12	24.45	24.45	0.155 ± 0.002	0.165 ± 0.002	0.130	0.156
Murrin Bridge	20		11.6	NA	0.163 ± 0.007	NA	0.138	NA
Nap Nap	20		NA	NA	0.162 ± 0.006	NA	0.136	NA
The Ville	12	13	26.85	26.85	0.175 ± 0.015	0.172 ± 0.012	0.147	0.162
Willandra	12	13	19.18	19.18	0.206 ± 0.017	0.169 ± 0.001	0.154	0.163

Population differentiation

Pairwise F_{ST} for tangled lignum ranged from little differentiation between Booligal and The Ville (0.017) to high differentiation between Hunthawang and Nap Nap (0.442, Table 6.2). In contrast, Nei's genetic distance ranged from extremely low between Hunthawang and Middle Creek (0.003) to 0.177 between Hunthawang and Lake Tarwong (Table 6.2). In river cooba, pairwise F_{ST} values did not vary much ranging from 0.031 between Willandra and Booligal to 0.045 between Moon Moon and Lake Tarwong (Table 6.3). Differences in river cooba estimated using Nei's genetic distance were also low ranging from 0.014 between Willandra, Booligal, and The Ville to 0.020 between Lake Tarwong and Moon Moon (Table 6.3).

Table 6.2 Pairwise FST for tangled lignum sites in the bottom left diagonal and pairwise Nei's genetic distance in the top right diagonal of the matrix.

	Booligal	Hunthawang	Kilfera	Lake Tarwong	Middle Ck	Moon Moon	Murrin Bridge	Nap Nap	The Ville	Willandra
Booligal	0.000	0.174	0.012	0.009	0.157	0.008	0.013	0.007	0.008	0.010
Hunthawang	0.442	0.000	0.173	0.177	0.003	0.172	0.176	0.170	0.156	0.168
Kilfera	0.036	0.429	0.000	0.013	0.157	0.014	0.018	0.011	0.014	0.014
Lake Tarwong	0.030	0.438	0.039	0.000	0.160	0.010	0.016	0.008	0.008	0.012
Middle Ck	0.377	0.022	0.365	0.373	0.000	0.156	0.159	0.153	0.141	0.152
Moon Moon	0.027	0.438	0.042	0.033	0.374	0.000	0.013	0.008	0.009	0.012
Murrin Bridge	0.039	0.425	0.049	0.045	0.342	0.038	0.000	0.014	0.015	0.016
Nap Nap	0.021	0.416	0.031	0.022	0.332	0.024	0.043	0.000	0.006	0.009
The Ville	0.026	0.398	0.039	0.025	0.334	0.028	0.041	0.017	0.000	0.012
Willandra	0.031	0.405	0.040	0.033	0.343	0.032	0.041	0.025	0.029	0.000

Table 6.3 Pairwise FST for river cooba sites in the bottom left diagonal and Nei's genetic distance in the top right diagonal of the matrix.

	Booligal	Hunthawang	Lake Tarwong	Moon Moon	The Ville	Willandra
Booligal	0.000	0.016	0.017	0.017	0.014	0.014
Hunthawang	0.034	0.000	0.018	0.019	0.016	0.015
Lake Tarwong	0.037	0.039	0.000	0.020	0.016	0.017
Moon Moon	0.040	0.039	0.045	0.000	0.017	0.018
The Ville	0.032	0.035	0.035	0.040	0.000	0.014
Willandra	0.031	0.031	0.037	0.039	0.032	0.000

Floodplain-river connection did not have a significant positive effect on observed heterozygosity in tangled lignum ($F_{1,7} = 4.053$, $p = 0.084$; Figure 6.2a).Although non-significant observed heterozygosity in tangled lignum increased with increasing floodplain-river connection frequency (Figure 2a). Floodplain-river connection did not have a significant effect on observed heterozygosity in river cooba ($F_{1,3} = 2.412$, $p = 0.218$; Figure 6.2b). Although non-significant, there was an observable pattern where observed heterozygosity increased with increasing frequency of floodplain-river connection (Figure 6.2b).
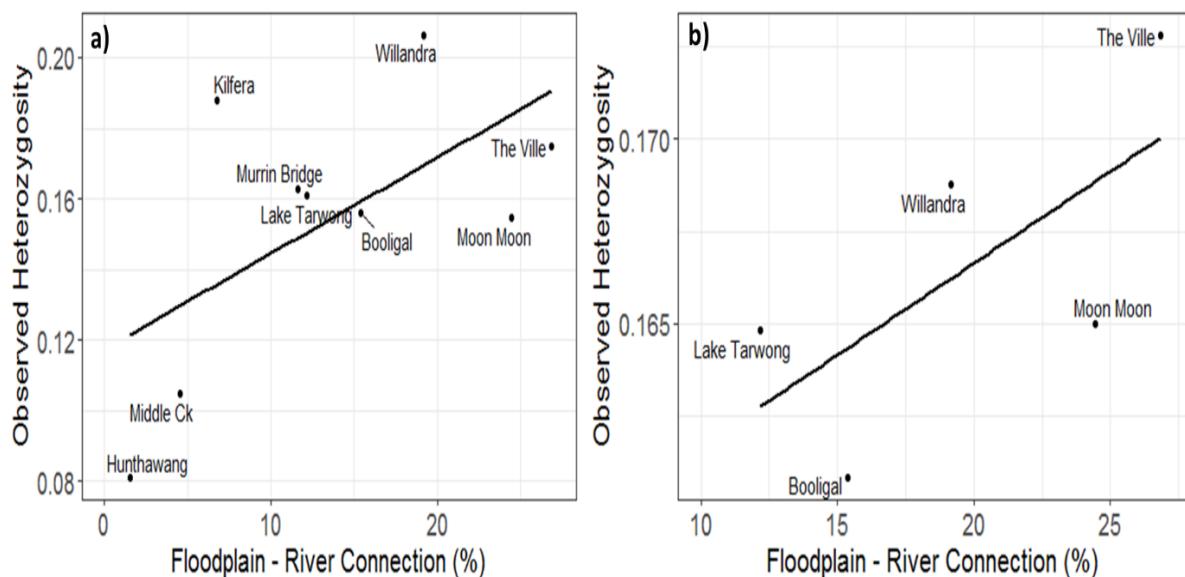
Figure 6.2 Regression analysis comparing the proportion of days each site is connected to the river (floodplain-river connection) and observed heterozygosity for (a) nine tangled lignum sites ($r^2 = 0.37$) and (b) five river cooba sites ($r^2 = 0.44$) on the floodplain of the mid and lower Lachlan River Catchment.

Population genetic structure

The first two PCoA axes accounted for 50% of the total variation in tangled lignum and highlighted strong differentiation among populations along axis 1 (47.2%) with a few plants falling between both groups (Figure 6.3). The maximum-likelihood genetic clustering using 'snapclust' confirmed these two genetic clusters with the largest cluster consisting of 113 plants consisting of all individuals from Booligal, Kilfera, Lake Tarwong, Moon Moon, Murrin Bridge, Nap Nap, The Ville, and Willandra and one individual from Middle Creek. The second cluster comprised the 20 Hunthawang plants and 11 of the 12 plants from Middle Creek. When this latter cluster of plants was removed and the PCoA analysis rerun, less than 10% of the total genetic variation was accounted for by the first two axes (Appendix C1). However, maximum-likelihood genetic clustering analysis did identify three sub-clusters consisting of 91 plants from Nap Nap, Lake Tarwong, Booligal, Kilfera, Willandra, and Moon Moon, the second consisting of 18 plants from Murrin Bridge, and the third cluster including four plants, one from each of Willandra, The Ville, Middle Creek, and Murrin Bridge.
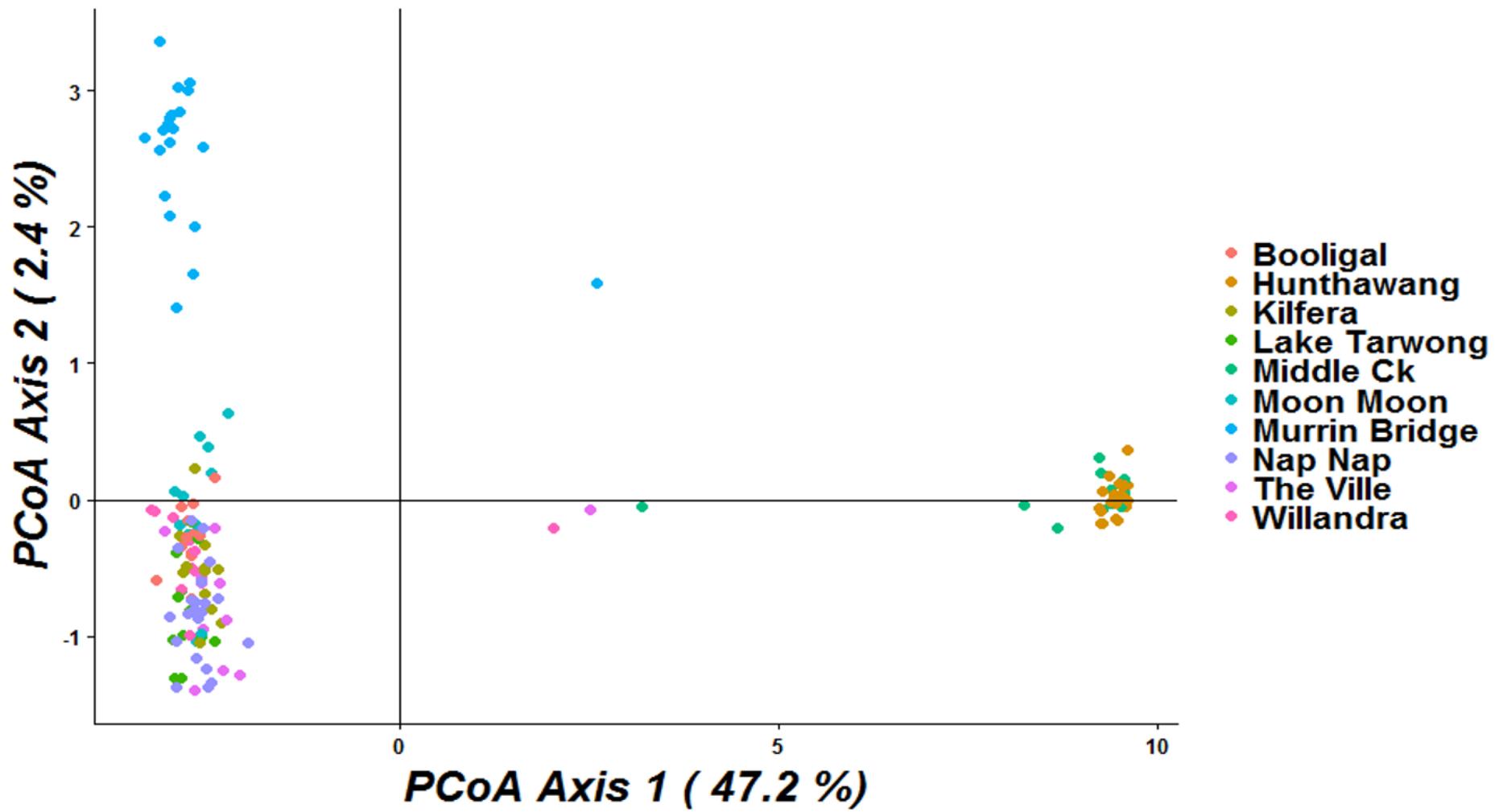
Figure 6.3 PCoA plot of 144 tangled lignum genotypes across all 10 sites in the mid and lower Lachlan River Catchment, and lower Murrumbidgee River Catchment.

In river cooba only 6.3% of the total variation was accounted for by the first two PCoA axes (Figure 6.4). Maximum likelihood clustering identified three likely genetic clusters with all plants from Booligal, Lake Tarwong, The Ville, and Willandra as well as seven plants from Moon Moon in the first cluster, all plants from Hunthawang being in the second cluster, and the third cluster comprising five individuals from Moon Moon (Figure 6.4).
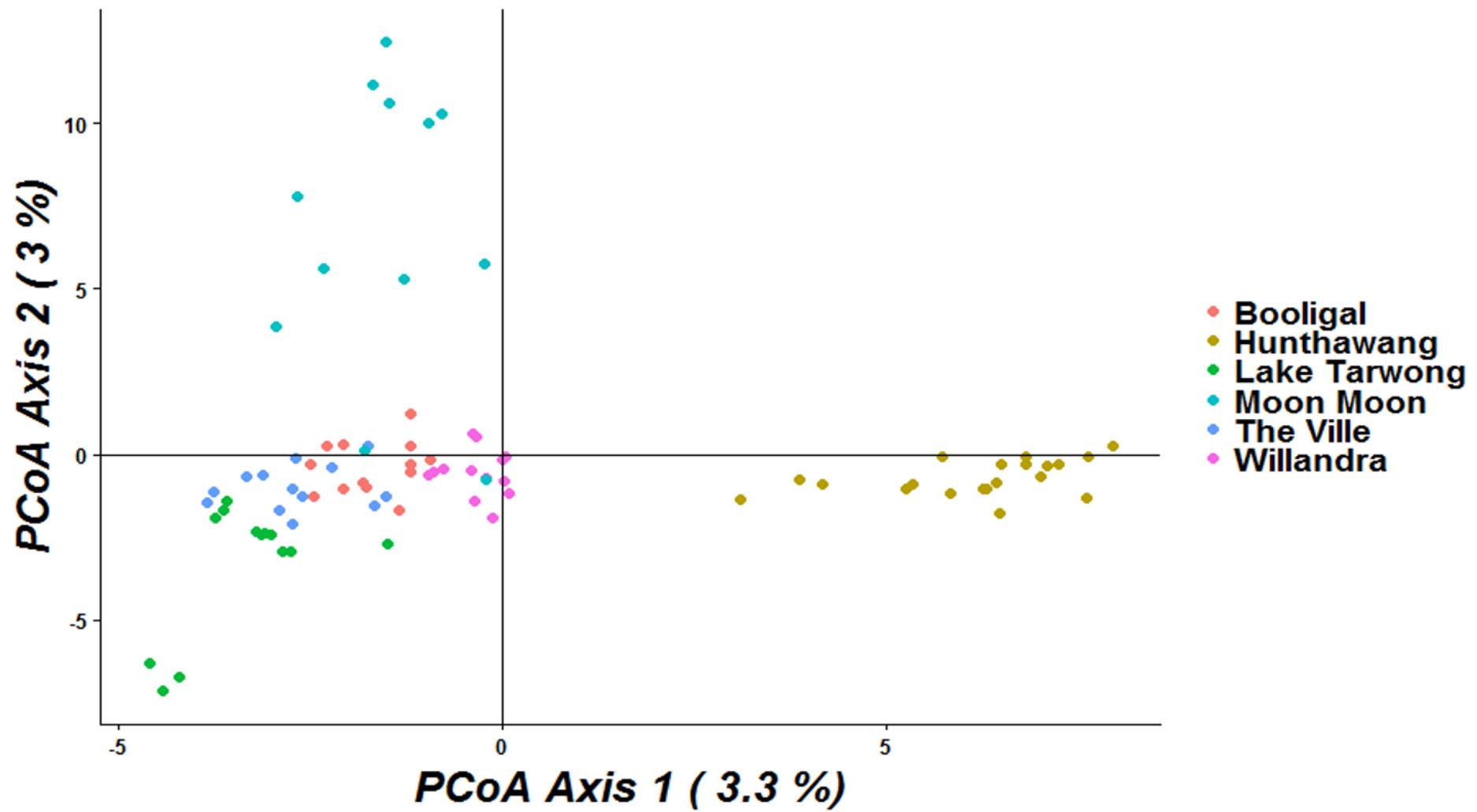
Figure 6.4 Plot from PCoA analysis on 84 river cooba genotypes across all six sites in the lower Lachlan River Catchment.

No significant relationship was observed between pairwise genetic distance (F_{ST}) and river distance for tangled lignum with all sites included in the analysis ($F_{1,43} = 0.164$, $p = 0.208$, $R^2 = .04$; Appendix C2). As Hunthawang and all but one individual from Middle Creek had vastly different F_{ST} values to all other sites, pairwise points representing Hunthawang and Middle Creek were removed (i.e., filter F_{ST} values >0.1). With the outliers removed, there was still no significant relationship between genetic distance and river distance ($F_{1,27} = 1.835$, $p = 0.187$, $R^2 = .06$) (Appendix C3).

There was no significant relationship between pairwise genetic distance (F_{ST}) and geographic (Euclidian) distance (lat/long) for tangled lignum with all sites included in the analysis ($F_{1,43} = 0.220$, $p = <0.641$, $R^2 = .01$; Appendix C4). Once the outlying individuals were removed (i.e., filter F_{ST} values >0.1), there was a significant relationship between pairwise genetic distance (F_{ST}) and geographic distance (lat/long) ($F_{1,27} = 21.79$, $p = <0.001$; Figure 6.5). Sites that were further apart were more genetically distinct compared to sites that were closer.

There was a significant relationship between pairwise genetic distance (F_{ST}) and Jaccard dissimilarity of flooding frequency for tangled lignum between sites ($F_{1,34} = 5.99$, $p = 0.0197$; Figure 6.6). This result was related to the large difference between Hunthawang and Middle Creek and all other sites in both floodplain-river connection frequency and genetic distance resulting in strong clustering of the pairwise points representing these outlying sites. Removing the outliers (i.e., F_{ST} values >0.1) reduced the difference between pairwise genetic distance (F_{ST}) and Jaccard dissimilarity of flooding frequency, demonstrating no significantly relationship ($F_{1,20} = 1.352$, $p = 0.2586$, $R^2 = .02$).

In river cooba, no relationship was observed between pairwise genetic distance (F_{ST}) and river distance ($F_{1,13} = 0.04$, $p = 0.839$, $R^2 = .01$; Appendix C5), geographic distance (lat/long) ($F_{1,13} = 1.991$, $p = 0.890$, $R^2 = <.01$; Appendix C6), or Jaccard dissimilarity of flooding frequency ($F_{1,8} = 0.095$, $p = 0.766$, r = 0.0012, $R^2 = <.01$) between sites (Appendix C7).
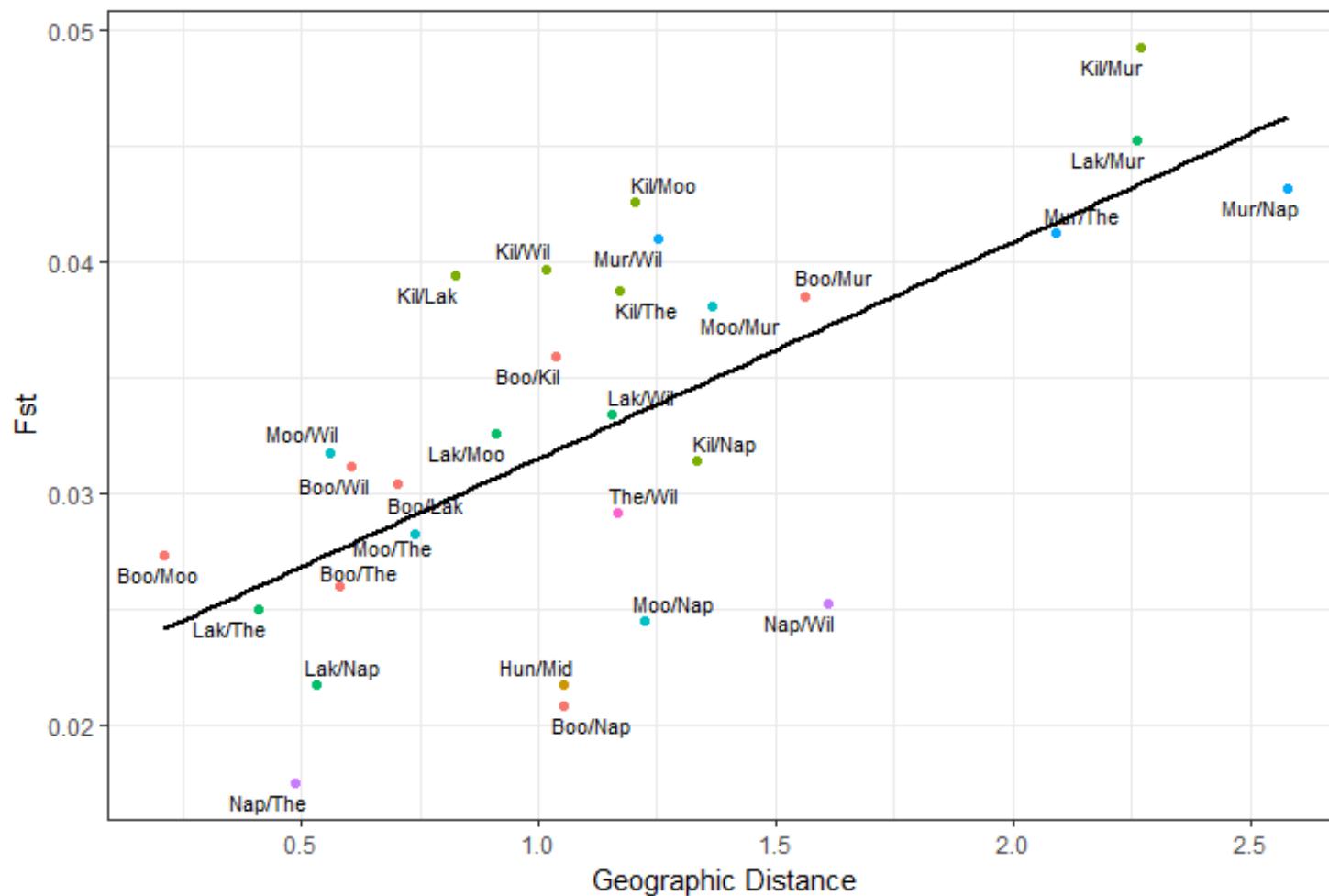
Figure 6.5 Pairwise F$_{ST}$ and geographic distance (lat/long) in tangled lignum with Hunthawang and Middle Creek removed. Solid line represents the linear regression. R^2 = .45.
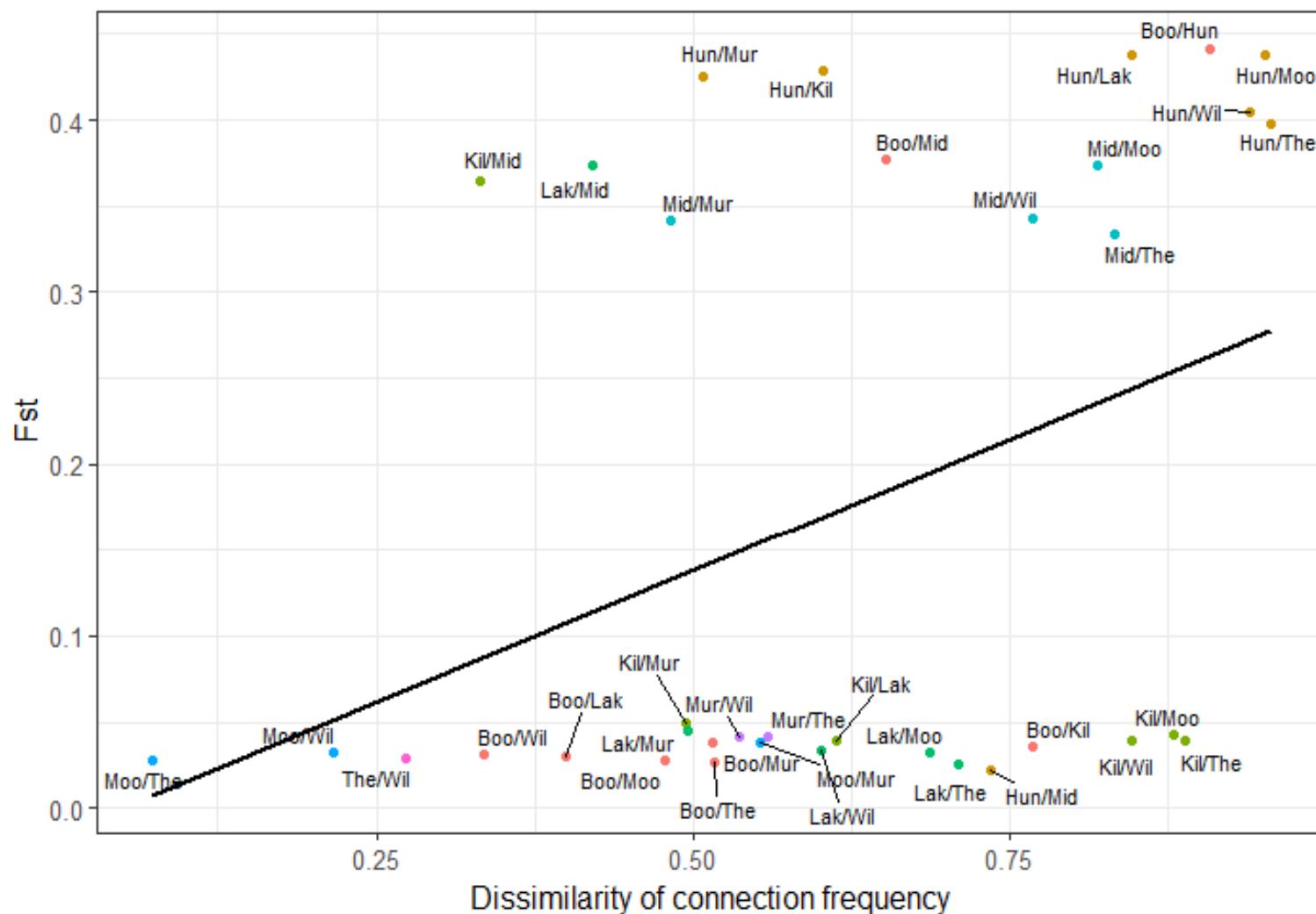
Figure 6.6 Pairwise F$_{ST}$ and Jaccard dissimilarity of flooding in tangled lignum. Solid line represents the linear regression. R^2 0.15.

6.5   Discussion

6.5.1 Tangled lignum

In tangled lignum, mean within-site genetic diversity (H$_e$ 0.13) was less than other plants which occur on floodplains, such as river red gum (H$_e$ 0.22) (Dillon *et al.* 2014). Variation in within-site genetic diversity was observed among sites in tangled lignum, particularly at two sites (Hunthawang and Middle Creek), which were found to have far less genetic diversity than all other sites. The two sites on Willandra Creek (Willandra and Kilfera) had the highest observed genetic diversity of all sites. As well as having lower mean observed heterozygosity, tangled lignum plants at Hunthawang and Middle Creek were also genetically differentiated from the other sites and were highly clustered. Within-site clustering has been observed in other plants which occur on floodplains such as *Triglochin procera* (water ribbons) (James *et al.* 2013), which also dispersers via hydrochory.

Dispersal and gene-flow in tangled lignum is possible through the movement of seeds, pollen and plant fragments (asexual reproduction) (Chong and Walker 2005; Roberts and Marston 2011). Chapter 4 showed that tangled lignum seeds can float for > 30 days. Very little is known about the pollination biology in tangled lignum, but it has been suggested that they may be pollinated by bees and other insects (Casanova 2015). Vegetative reproduction has been observed after flooding, but not after rain (Casanova 2015). The movement of plant fragments within and between populations by flooding, could reduce levels of genetic variation in tangled lignum between sites but no research has been conducted on the extent of clonality in tangled lignum. In species which disperse by hydrochory, genetic differentiation is often related to distance between patches along the river (Schleuning *et al.* 2011). Despite this, river distance was not correlated with genetic distance in tangled lignum, with and without the outlying individuals from Hunthawang and Middle Creek, and as such, this species doesn't fit the one directional stepping stone model proposed by Kudoh and Whigham (1997). Similarly, the stream hierarchy model (Meffe and Vrijenhoek 1988) hypothesis was not strongly supported in tangled lignum, and plants collected from Nap Nap on the Murrumbidgee River, were genetically very similar to plants collected from Booligal, Lake Tarwong, and Moon Moon (which are all on different distributary channels from each other and in a different river catchment to Nap Nap). One possible explanation is that historical climate fluctuations resulted in large, expansive flood events, promoting historical

movements of hydrochorous species, which has been observed in a freshwater decapod species (Carini and Hughes 2004). The recent Pleistocene (around 50,000 to 16,000 years before present) was the last period when higher discharges produced large quantalities of surface water and laterally active rivers in south-western NSW (Magee 1991).

Interestingly, genetic distance and geographic (Euclidean) distance between sites revealed isolation by distance once outlying individuals from Hunthawang and Middle Creek were removed. Isolation by distance has been observed in other species which disperse via hydrochory such as *Potamogeton pectinatus* (sago pondweed) (Mader *et al.* 1998). Thus, gene flow in tangled lignum depends on Euclidian distance between sites, and these sites fit the two dimensional stepping stone distance model (Kudoh and Whigham 1997). At sites such as Booligal, Moon Moon, and Lake Tarwong, river distance between sites is substantially greater than geographic distance as these sites are within a close proximity to each other while being situated on different rivers or distributary channels. The high correlation between genetic distance and geographic distance at these sites may be explained by lateral seed dispersal across the floodplain during floodplain inundation where hydrochory does not necessarily occur along the main channel of the river.

Waterbirds have been observed to fly between rivers and follow rivers where possible, to stop to rest and feed at suitable locations on rivers and floodplains (McGinness 2017), and known to use tangled lignum as nesting habitat (Roberts and Marston 2011). The large numbers of nomadic waterbirds such as straw-necked ibis that stop to rest, feed, and breed in lignum shrublands at Nap Nap, Booligal Swamp, and the Willandra Creek (McGinness 2017), may help to explain the higher genetic connectivity among sites on the lower Lachlan River, the two sites on Willandra Creek, and Nap Nap in the Murrumbidgee River Catchment than what would be expected solely through hydrochory. Consequently, nomadic waterbirds may have decreased the level of genetic differentiation between these sites. Waterbirds may play an important role in the dispersal of tangled lignum seeds and the maintenance of gene flow between patches.

The floodplain-river connection also had a significant effect on genetic distance (F_{ST}) in tangled lignum. This was particularly apparent at the two sites where geographic distance was not related to genetic distance (Hunthawang and Middle Creek). This result indicates that lateral floodplain inundation connects discrete patches and maintains gene flow. Topographic variability across floodplains influences the scale of connectivity of the floodplain with the

main channel (Ward and Stanford 1995). Tangled lignum occurs across a wide range of flooding regimes (Blanch *et al.* 1999) which was observed in this study (<2 % days connected at Hunthawang to 26 % days connected at The Ville; Table 6.1). The results suggest that variation in floodplain-river connection across tangled lignum patches on a floodplain relates to the level of genetic diversity that occurs within a site and genetic differentiation between sites.

The local geomorphology may have played a key role in the reduced genetic diversity and increased genetic differentiation of tangled lignum plants at Hunthawang and Middle Creek. These sites are hydrologically distinct from the other sites in this study, both being the least frequently connected to the river by flooding. It is also unlikely at these sites that floodwaters return to the main channel of the Lachlan River. The site at Hunthawang is located (approx. 1000 m) away from the Lachlan River in a black box woodland while Middle Creek is disconnected hydrologically from sites below the confluence of the Lachlan River and Middle Creek as it is a terminal creek and thus does not reconnect to the Lachlan River. The high river level required to inundate these sites and local geomorphology greatly reduces opportunities for hydrochorous gene flow both to and from these sites. These sites are not known to be used as nesting grounds for nomadic waterbirds, possibly related to the lack of regular water limiting bird visits (pers. coms Heather McGinness [1]).

Genetic diversity within patches and differentiation between patches reflects the founding number of individuals and dispersal rates (Boileau *et al.* 1992). The low genetic diversity and high genetic divergence at Hunthawang and Middle Creek may be a consequence of these populations being small or recently bottlenecked, as observed in a long-lived European fern species (Landergott *et al.* 2001). Hunthawang and Middle Creek, maybe low-quality ephemeral patches with very limited import of genetic material from other patches (Tero *et al.* 2003). As such these populations may be at the ecological range limits of this species. Populations occurring at their geographic or ecological range limits, are often small and isolated, and as such they are often genetically divergent (through reduced rates of gene flow) and exhibit low genetic diversity (resulting from founder effects, genetic bottlenecks, and inbreeding) (Lesica and Allendorf 1995; Dorken and Eckert 2001; Willi *et al.* 2018). As the main selection pressure in drier environments is water availability (Ward *et al.* 2011) selection for suitable genotypes may also play a role. While local adaptation in plants may be

---

[1] CSIRO Ecosystem Sciences, GPO Box 1700, Canberra ACT 2601, Australia

less wide spread than once thought (Leimu and Fischer 2008), there are many examples of locally adapted genotypes increasing a plants performance and relative fitness under local conditions (Prati and Schmid 2000; Karrenberg *et al.* 2002; Kawecki and Ebert 2004).

On the chance seeds do arrive at a given site, sites such as Hunthawang may rarely provide suitable conditions for seed germination and seedling establishment (Chapter 4; Capon *et al.* 2009). The phylogenetic tree revealed identical genotypes at Hunthawang (three pairs) and at Willandra (one pair) that are likely to be clones (Appendix C8). This analysis is the first genetic approach to confirm tangled lignum reproduces and disperses asexually across at least several hundred metres within a site (as plants were collected >50 m apart and one pair of clones were approximately 200 metres apart). While it is unlikely that these clones are connected by root suckers, it is recommended that clonal extent in tangled lignum is confirmed. Clonality was only observed within and not among sites, suggesting vegetative dispersal within patches. The colonisation of a site may occur with a variety of new genotypes from seeds and continue with the spreading of suitable clones under local conditions, as observed in another clonal species *Phragmites australis* (Koppitz *et al.* 1997). James *et al.* (2013) found identical genotypes up to 3 km apart in *Phragmites australis*. Clones were primarily observed at Hunthawang which was the site least frequently flooded, thus, clonality may enable tangled lignum to occur in the absence of suitable conditions for seed germination and seedling establishment. The lack of clones at the other more frequently flooded sites suggests that tangled lignum does not spread predominantly by vegetative growth in more frequently flooded parts of floodplains as predicted by others (Capon *et al.* 2009).

6.5.2 River cooba

Within-site genetic diversity in river cooba (H_o 0.17) was similar to that observed in other Australian acacia species such as *Acacia anomala* (grass wattle) (H_o 0.15) (Coates 1988) and *Acacia mearnsii* (black wattle) (H_o 0.16) (Coates 1988). In river cooba, mean observed heterozygosity was very similar among sites. This study revealed a moderate level of within-site clustering, with individuals clustering within the site they were collected. No strong geographic or environmental barriers were detected. The results suggest gene flow between river cooba patches and a panmictic population structure (Tero *et al.* 2003) within the lower

148

Lachlan River Catchment. Within-site clustering at these sites may be related to site specific geomorphology and position of sites on the floodplain. All three possible models of pathways of gene flow propossed by Kudoh and Whigham (1997) were not strongly supported in river cooba. The stream hierarchy model (Meffe and Vrijenhoek 1988) hypothesis was also not strongly supported in river cooba.

Pollination in river cooba is suggested to occur primarily by insects (Casanova, 2015). Long distance dispersal of pollen by insects has been observed to occur up to a distance of > 6 km (Noreen, et al. 2016). While the six sites used as part of this study were > 20 km apart, insect mediated gene-flow may play an important role in maintaining population genetic structure in river cooba. River cooba seeds do not have an aril (Entwisle *et al.* 1996) which is a fatty appendage of the seed which promotes seed dispersal by birds and ants in most other Australian acacia species (O'Dowd and Gill 1986; Willson and Traveset 2000). This suggests that zoochorous seed dispersal is unlikely. The seed pods of river cooba disarticulate into one seeded sections capable of dispersing individually (Roberts and Marston 2011) which is an effective morphological adaptation to floatation. The results demonstrate that river cooba appears to be dispersing effectively within patches and to a lesser extent between patches along rivers and across floodplains. The fact that river distance was not strongly correlated with genetic distance may indicate that river cooba may not rely on hydrochory as its primary dispersal mechanism.

Although river cooba can regenerate by suckering from the roots of mature trees (Roberts and Marston 2011), as in other Australian acacia species such as *Acacia anomala* (Coates 1988), I found no evidence that river cooba disperses asexual clones (50 m) away from the parent plant, as no clonality was present (Appendix C9).

6.6   Implications

This study demonstrates that plants which occur primarily on floodplains in arid and semi-arid environments such as tangled lignum, depend on floodplain inundation for dispersal (by floodwaters and possibly visiting waterbirds) between patches and to maintain genetic structure. Thus, highlighting the importance of flooding for connecting spatially isolated patches on floodplains (Bishop-Taylor *et al.* 2015) and maintaining genetic diversity. In

tangled lignum, sites that experienced more frequent flooding had higher levels of genetic diversity and were more genetically homogenous. There was also an isolation by distance effect where increasing geographic distance correlated with increasing genetic differentiation. Conversely, no strong correlation was found between genetic distance, and river or geographic distance, or floodplain-river connection frequency in river cooba. In river cooba within site genetic diversity was far less variable between sites than in tangled lignum and river cooba had less genetic differentiation between sites. The genetic differences observed between tangled lignum and river cooba may be related to the spatial location of each species in relation to rivers and floodplains. River cooba commonly occurs along rivers and creek banks (Cunningham *et al.* 1981) whilst tangled lignum typically occurs away from the main river channel on floodplains (Roberts *et al.* 2016). The distribution of river cooba along rivers facilitates regular dispersal of seeds via hydrochory regardless of river level, whilst the dispersal of seeds of tangled lignum between patches is dependent on flooding events and the distance these floodwaters travel. As tangled lignum was found to be more reliant on floodplain inundation than river cooba for dispersal, the genetic impact of water resource development may be greater for species which occur on floodplains compared to species along river channels.

This study demonstrated the likely importance of nomadic waterbird movement to gene flow between patches for tangled lignum. While tangled lignum plays a vital role as nesting habitat for nomadic waterbirds, nomadic waterbirds may play a vital role in maintaining tangled lignum in the landscape and enabling gene flow between patches. Further research should be conducted on the importance of bird movement to the dispersal of plants on floodplains and connection between patches. Further research is recommended on the ability of river cooba to disperse by hydrochory. Further research is recommended on the proportion of male and female plants of tangled lignum reproducing clonally.

7. Synthesis

7.1   Importance

Floodplain wetlands are a common feature of the inland Australian landscape (Kingsford *et al.* 2004). These landscape features are characterised by a highly variable hydrological regime, cycling between often unpredictable wet and dry phases (Walker *et al.* 1997). This natural variability structures the physical template of the floodplain (Southwood 1988) which in turn structures the vegetation (Blom *et al.* 1990; Reid *et al.* 2016). Consequently, the hydrological regime, is of great ecological importance (Walker *et al.* 1995). World-wide, the patterns of flow in rivers have been, and continue to be, affected by water resource development (Nilsson *et al.* 2005; Poff *et al.* 2007; Haddeland *et al.* 2014; Grill *et al.* 2015).

Within the Lachlan River Catchment, regulation and flow extraction has reduced the flow of the Lachlan River and changed the behaviour and distribution of floodwaters (Driver *et al.* 2004; Driver *et al*. 2011). The vegetation which occurs on the floodplain of the lower Lachlan River Catchment supports or is capable of supporting a range of fauna, including a large number of water bird species (Kingsford *et al.* 1999; McGinness *et al.* 2010; McGinness *et al.* 2013;), including species listed as threatened under Commonwealth and state legislation as well as species which are recognized in international migratory bird agreements (MDBA 2012). As such, changes to the flow of the Lachlan River have widespread ecological implications.

There is a lack of information on the role of the hydrological regime in structuring the distribution and abundance of the plants which occur on the semi-arid floodplains of the Murray-Darling Basin. This information is required to establish specific flow requirements for plant species, and when considering the possible consequences of changes to the way in which water is managed within the landscape. This thesis addressed some of the current gaps in our understanding of floodplain vegetation by assessing the impacts of water resource development on the hydrological connectivity to different floodplain habitat types which are common in the southern parts of the Murray-Darling Basin and investigating the influence of depth, duration and frequency of flooding on the growth, germination, and dispersal of plants which occur on the floodplain of the lower Lachlan River.

7.2   Modification of river-floodplain hydrology

This thesis began (chapter 2) with an analysis of the effects of water resource development on the connection regime between the river and the floodplain of the lower Lachlan River. The results from chapter 2 show the Lachlan River like many of Australia's inland rivers (Walker *et al.* 1995) experiences extreme variability in flow. The floodplains of the lower Lachlan River naturally experience extreme and extended drought conditions and large floods. Through hydrological modelling, chapter 2 identified the connection regime required to sustain the floodplain habitat types which occur on the lower Lachlan River, with the assumption that 'without development' conditions is the desired state. Water resource development has substantially changed the flow pattern of the Lachlan River and reduced the frequency of large flow events required to connect the floodplain to the river. The floodplains of the lower Lachlan River connect to the river under current flow conditions half as often as they had 'without development' which has substantially increased the number of days between connection events. These changes to the floodplain-river connection regime are a common theme across the Murray-Darling Basin (Bren 1991; Maheshwari *et al.* 1995; Dyer 2002; Catford *et al.* 2011).

7.3   Variation in the soil seed bank

The soil seed bank on the floodplain contributes to a diverse range of mostly annual or short-lived perennial species. These seed bank species germinate in response to certain hydrological conditions, germinating in response to rainfall, submergence, flood recession, and on waterlogged soil. The high germination rates observed in conditions typical on a floodplain during and following flooding, most notable on waterlogged soil, results in a great deal of productivity from the soil seed bank. These species rely on a long-lived seed bank as a means for surviving through the dry phase and recover following re wetting. The results suggest that the seed bank regularly fluctuates in response to hydrological conditions on the floodplain. While the locations where soil was collected in 2017, following the fourth largest flood on record in the lower Lachlan River had a significantly lower seed bank density and species richness compared to locations collected in 2016, this was likely attributable to the loss of terrestrial species from the seed bank. The aquatic respondent WPFGs were either present in both years or more diverse in 2017. The seed bank of these species are not

152

exhausted after one flooding event (Brock *et al.* 2003) and the flooding conditions provide the opportunity for replenishment.

The number of aquatic plant species remaining viable in the soil seed bank has been observed to reduce with increasing time since flooding (Brock *et al.* 2003). Based on the significant and long-term changes in flow of the Lachlan River, the species observed from the soil seed bank, especially the aquatic respondent annual and short-lived perennial species, may be a reduced sub-set of the species which occurred 'without development' while many of the terrestrial species may have increased in density and diversity. The areas on the floodplain which rarely connect, probably receive fewer additions via hydrochory and less opportunities for replenishment from flood induced germination events (Capon and Brock 2006). These rarely flooded parts of floodplains may have lost or will continue to lose aquatic respondent species, resulting from insufficient replenishment via hydrochory and local germination.

7.4   Seed germination of tangled lignum and nitre goosefoot under experimental hydrological regime

As tangled lignum and nitre goosefoot were not observed from the soil seed bank during the seedling emergence experiments (chapter 3), despite being widely distributed on the floodplains of the lower Lachlan River. Chapter 4 investigated the possible influence of duration of inundation on seed germination and seed dispersal in tangled lignum and nitre goosefoot.

Tangled lignum and nitre goosefoot depend on available moisture to induce germination. Seeds of tangled lignum germinated best on waterlogged soil and required an extended contact with moisture to germinate (16 - 20 days). Few seeds of tangled lignum germinated with rainfall alone. This suggests that seed germination and seedling recruitment in tangled lignum is episodic and associated with flooding events. The fact that tangled lignum seeds germinated under a range of durations of inundation shows that this species has adapted plasticity in seed germination, enabling it to cope with the temporally and spatially variable nature of flooding of Australian inland floodplains. Tangled lignum seeds floated for up to 30 days and are likely to disperse by floodwaters during flooding events, with gene flow related to distance between patches on the floodplain and floodplain-river connection frequency

(chapter 6). This dependence of tangled lignum for flooding means that drying poses a significant impediment to dispersal and germination. While, tangled lignum seedlings have considerable tolerance to drought conditions (Capon *et al.* 2009), the increased dry spell and reduced number of floodplain-river connection events under current flow conditions (chapter 2), reduce the opportunity for seedling establishment, and may influence the current distribution and persistence of tangled lignum on the floodplain. Reduced floodplain-river connection could result in tangled lignum disappearing from drier areas on floodplains while encroaching on to more frequently flooded parts of floodplains such as temporary floodplain lakes. Encroachment has been observed in other species which occur on floodplains such as river red gum (Bren 1992).

Nitre goosefoot germinated best in response to five days of flooding, and on wet soils such as occur on higher parts of floodplains during flooding and following flood recession. Effective germination reduced considerably after 10 days inundation. Thus, nitre goosefoot is unlikely to occur in locations where prolonged flooding occurs. Whilst almost all seeds of nitre goosefoot had sunk within 1 week, and hydrochory is unlikely to be an important dispersal mechanism for seeds of nitre goosefoot (see Nilsson *et al.* 1991b), hydrochorous seed dispersal may occur across short distances on floodplains. Although nitre goosefoot requires short periods of inundation to germinate, its position at the edge of floodplains means that inundation of the floodplain needs to be of sufficient duration for water to reach the higher areas, usually edges, of floodplains where nitre goosefoot occurs. The changes in flow regime which have occurred in the Lachlan River over the past 80 years, may have, and continue to result in nitre goosefoot expanding into lower areas of floodplains and disappearing from the edges of floodplains.

7.5   Responses of nitre goosefoot to simulated rainfall, and depth and duration of flooding

Nitre goosefoot is not currently recognised as a flood dependent species, and the tolerances and requirements to flooding haven't been established. As such, chapter 5 explored the tolerance and responses of nitre goosefoot to depth and duration of flooding. Nitre goosefoot can tolerate partial submergence for at least 40 days and total submergence for 20 days. Leaf production, biomass and seed set in nitre goosefoot were highest under shallow flooding for

20 - 40 days. The results from chapters 4 and 5, suggest that nitre goosefoot increases growth and leaf production, and produces seed during flooding events, the seed matures and will fall on the receding flood waters, and the seeds germinate on the waterlogged soil following flood recession. The naturally variable hydrological regime on semi-arid floodplains means that overtime nitre goosefoot (like other perennial species) may shift in distribution and abundance across the floodplain, retreating from some areas of the floodplain while colonising others. Being a perennial species, nitre goosefoot can afford years of non-establishment and needs the hydrological conditions which allows reproduction infrequently. Changes to the hydrological regime related to water resource development have the potential to influence the condition of current populations of nitre goosefoot, reducing vigour, size, and reproductive capabilities. Reduced floodplain-river connection may result in a shift in the distribution of nitre goosefoot to areas more frequently flooded. This shift may have already occurred in parts of the Murray-Darling Basin that have seen long-term flow regulation. The results from chapters 4 and 5 provide the information to factor the hydrological requirements of nitre goosefoot into environmental flow regimes.

7.6 Genetic diversity and gene flow patterns in tangled lignum and river cooba

Little is known about population structure and gene flow in plants which occur on floodplains or wetlands and whether these patterns reflect landscape features such as river architecture, abiotic variables including hydrology and floodplain inundation, and biotic influences such as bird movements. Chapter 6 investigated the population genetic structure of tangled lignum and river cooba. Tangled lignum and river cooba had varying population genetic structure. In tangled lignum, sites that experienced more frequent flooding had higher levels of genetic diversity and were more genetically homogenous. There was also an isolation by distance effect where increasing geographic distance correlated with increasing genetic differentiation. This may be explained by lateral seed dispersal across the floodplain during floodplain inundation where hydrochory does not necessarily occur along the main channel of rivers. Waterbirds may also play an important role in the dispersal of tangled lignum, dispersing seeds between habitat patches, as water dispersal alone did not explain connectivity between patches. In river cooba, mean observed heterozygosity was very similar among sites and no

strong correlation was found between genetic distance and river or geographic distance, or floodplain-river connection frequency.

The genetic differences observed between tangled lignum and river cooba may be related to the spatial location of each species in relation to rivers and floodplains. River cooba commonly occurs along rivers and creek banks (Cunningham *et al.* 1981) whilst tangled lignum typically occurs away from the main river channel on floodplains (Roberts *et al.* 2016). The distribution of river cooba along rivers, facilitates regular dispersal of seeds via hydrochory regardless of river level, whilst the dispersal of seeds of tangled lignum between patches is dependent on flooding events and the distance these flood waters travel. As tangled lignum was found to be more reliant on floodplain inundation than river cooba for dispersal, the genetic impact of water resource development may be greater for species which occur on floodplains compared to species along river channels.

7.7   Discussion

The life-history traits of each species and the hydrological conditions strongly influence the composition, distribution and abundance of plants on floodplains (chapter 1.5 and Figure 1.2). Chapters 3-6 shed light on some of the life-history traits (such as germination, dispersal, seed production and growth) important to plants which occur on floodplains and their responses to characteristics of the flooding regime (such as depth, duration, and frequency). In systems, such as floodplains, which are maintained by natural disturbance (i.e. the flood regime) (Ward and Stanford 1995), the success of a life-history trait is dependent on the 'heterogeneity in time', that is the length of the favourable period (permits reproduction) and the length of the unfavourable period (permits existence) (Southwood 1977).

The observed changes to the hydrological regime under current flow conditions, change the 'heterogeneity in time'. If we consider the favourable period for aquatic or semi-aquatic plants, the period when a floodplain is connected to the river (i.e. duration of floodplain-river connection), and the unfavourable period the dry phase (while acknowledging that floodwaters can persist on lower lying parts of floodplains), the hydrological study (Chapter 2) shows that the duration of the favourable period hasn't changed considerable under current flow conditions, but there has been a substantial increase in the length of the unfavourable

period. This means that the favourable periods occur less frequently (Figure 7.1). These changes to the hydrological regime have fundamentally changed the character of the habitat. The reduced frequency of floods and increased duration of dry phases influences the frequency of the ecological processes which occur during flooding such as the changes in resource availability and productivity shifts, landscape connectivity and habitat heterogeneity (Figure 7.1). These ecological processes determine the success of species, which will be a function of their life-history traits, and are likely to be more beneficial to terrestrial species and deleterious to aquatic species.

Ecological processes on the floodplain during flooding
Creation of habitat
Water and nutrient availability
Increased connectivity
Stresses related to flooding

Plant responses to flooding
Aquatic species: Increased growth, seed production, dispersal and germination
Terrestrial species: reduced density and diversity

Increased duration of dry phases under current flow conditions

Figure 7.1 Heterogeneity of habitats in time; F, length of favourable period (permits reproduction); L, length of unfavourable period (permits existence) (Adapted from Southwood 1977) representing conditions under: a) 'without development' and b) current flow conditions.

The life-history traits of floodplain plants have been observed to vary with elevation on the floodplain, with more flood tolerant species characterised by fast turnover rates (i.e., completing their life-cycle between flooding events) occurring on frequently inundated, near-channel sites on the floodplain while the higher elevated parts of floodplains which are flooded less frequently are dominated more so by perennial species (Hughes 1988; Blom and

157

Voesenek 1996; Capon 2003; Capon 2005; Mommer *et al.* 2006; Figure 1.4). The ecological effects of these changes to the hydrological regime may include a change in the composition of the vegetation to species less tolerant of flooding, a shift of habitat types toward the more frequently flooded parts of the floodplain, and a loss of spatial and temporal heterogeneity amongst the floodplain habitat types (some of which have been observed in parts of the Murray-Darling Basin (Eamus *et al.* 2006; Roberts and Marston 2011)).

Van Looy *et al.* (2018) proposed that there are three mechanisms driving community resilience in river ecosystems: resource competition/facilitation, dispersal-based recruitment recovery, and refuge-mediated functional redundancy (Figure 7.2). This framework is helpful in determining the sensitivity of a community to changes in flow conditions and builds on the conceptual model presented in chapter 1 (Figure 1.2), in regards to the ecological processes which occur during flooding and how they operate as filters depending on the life-history traits of the plants (Figure 1.2). In plants, an increase in resource availability may benefit competitive species at the expense of stress tolerant or ruderal species which may be overgrown (Grime 1977). Tamarix (*Tamarix ramosissima*) has naturalised, and established widely in riparian corridors and floodplains in arid and semi-arid North America, owing to it being a competitive generalist species, tolerant to the changed hydrological conditions, while the native cottonwood (*Populus deltoides*) has declined (Merritt and Poff 2010).
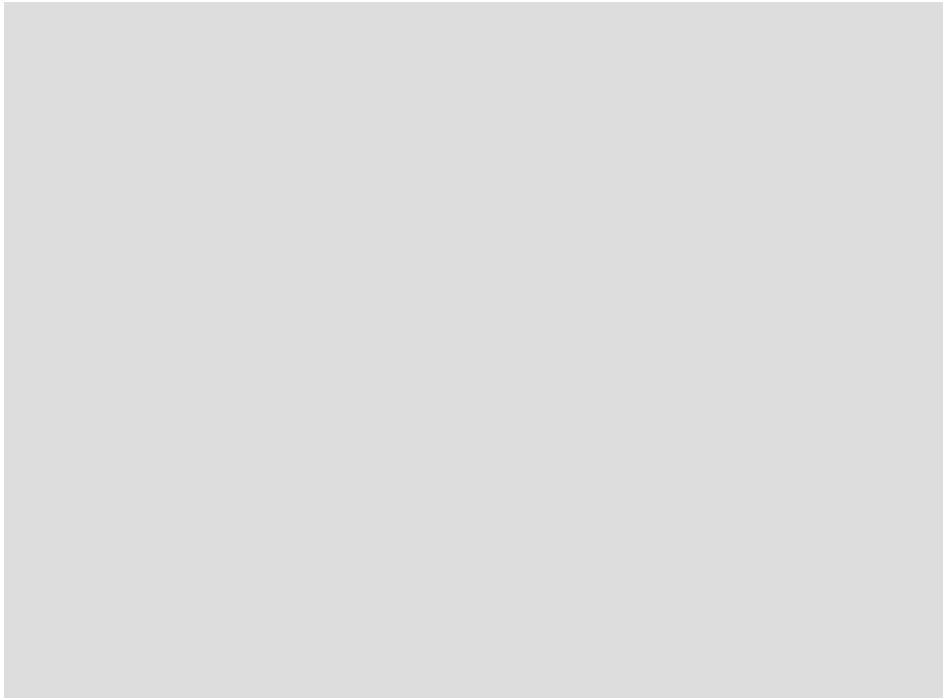
Figure 7.2 Conceptual river resilience framework representing the three mechanisms driving resilience of communities: resource competition/facilitation, dispersal-based recruitment recovery, and refuge-mediated functional redundancy. The extrinsic drivers at the ecosystem scale (arrows) that steer the prevalence of specific mechanisms (Van Looy *et al.* 2018).

The semi-arid floodplains of the lower Lachlan River primarily consist of ruderal (seed bank) species and stress tolerant woody species, as well as terrestrial species which may establish on the floodplain during dry phases (Chapter 3). Many of the ruderal (seed bank) species germinate from the soil seed bank during flooding and following flood recession. These species are refugia communities with a diverse range of habitat specialists which capitalise on the changing hydrological conditions on the floodplain and consequently require habitat heterogeneity to persist (Figure 7.2). Both spatial and temporal heterogeneity are likely to be important to these species.

The stress tolerant woody species (for example river cooba, nitre goosefoot, and tangled lignum) which occur on the floodplain of the lower Lachlan River, are tolerant of intermittent flooding and require flooding to maintain condition, produce seed, disperse and for seedling establishment (Chapters 4 and 5). For these species, flooding is required to provide connection between patches by hydrochory (e.g. for tangled lignum as shown in Chapter 6), to increase resource availability for growth and condition (nitre goosefoot as shown in Chapter 5), and to provide habitat heterogeneity which occurs during and following flooding

to provide conditions for establishment (e.g. tangled lignum and nitre goosefoot germinate during flood recession as shown in Chapters 4).

On the floodplains of the lower Lachlan River, African boxthorn (*Lycium ferocissimum*) may be a comparable species to tamarix in North America, being a widely spread noxious weed in NSW (Cunningham *et al.* 1981) and observed to occur on floodplains (pers.obs.). This species may become more prevalent on floodplains with the reduced occurrence of flooding at the expense of flood tolerant or ruderal species.

Plants on floodplains depend on flooding to provide habitat, resources and connectivity and the results show that they are likely to be sensitive to changes in flow regime. To maintain a diverse and species rich community of ruderal species which colonise the floodplain during flooding and following flood recession, and provide the conditions for dispersal, germination and growth of long-lived flood tolerant species, requires wet and dry phases are maintained. The results suggest that, environmental flow would be best used to increase the frequency of flooding events, replacing the flooding events which have been removed by water resource development, especially those during extended dry periods.

7.8 Opportunities for further research

This thesis provides fundamental knowledge on the hydrological requirements of floodplain plants and contributes to the growing breadth of research on floodplain ecology. While this thesis was focused on the influence of flooding on floodplain vegetation, the increased length of the dry phase shown by the hydrological modelling (chapter 2), suggests further research must focus on the ecological impacts of increased duration of dry phases. The results of this thesis suggest that the response of the vegetation to floodplain inundation mean that changes in flow regime and floodplain inundation resulting from water resource development in the Lachlan River Catchment over the past 80 years or so are likely to have resulted in changes in the distribution and abundance of perennial trees and shrubs (such as tangled lignum, nitre goosefoot and river cooba) along with annual species (such as those observed in the soil seed bank). It is recommended that further research be conducted to investigate potential changes in the distribution and abundance of floodplain plants in response to changes in flow regime resulting from water resource development to confirm the predictions made in this thesis.

160

Currently, there is still a lack of information on floodplain plants and their response to local environmental factors. For example, a range of important species which occur on floodplains in the Murray-Darling Basin have little or no information on their hydrological requirements (for germination, establishment, growth and dispersal), including *Eleocharis acuta* (common spike-rush) and *Marsilea drummondii* (common nardoo) which are both wide spread on floodplains, re-establishing during flooding and undoubtedly providing a range of important ecological roles.

There is currently insufficient knowledge on the sustainable population growth of flood dependent species (such as river red gum, black box, river cooba and tangled lignum) which germinate and establish following flood recession (see Roberts and Marston 2011). These species currently experience fewer opportunities for seed germination and seedling establishment under current flow regimes and are often impacted by grazing by feral animals. Determining the number of seedlings which are reaching reproductive maturity under current conditions and number required to maintain a sustainable population will enable us to be better able to predict and mitigate the impacts of water resource development and the ecological responses from management interventions to ensure the long-term persistence of these important species.

Floodplain trees such as river red gums use groundwater to sustain vigor and growth (Capon et al. 2016). The depth of groundwater has been observed to be a strong predictor of stand condition in river red gums (Cunningham et al. 2011). Currently there is little known of the relationship between groundwater depth and the vegetation condition in the lower Lachlan River Catchment and the relative importance of groundwater, floodwater and rainfall. It is recommended that further research be undertaken on the influence of groundwater on the distribution and condition of floodplain trees in the lower Lachlan River Catchment.

The soil seed bank chapter (Chapter 3) of this thesis was exploratory and has yielded interesting results. It suggests that recent flooding reduces the seed bank density and diversity on the floodplain, driven by a loss of terrestrial species, but deficiencies in the sampling strategy and site-based flooding information means these are preliminary findings that should be tested. It is recommended that a subsample of the locations where soil was collected as part of chapter 3 should be resampled following flooding, to test the model developed in Chapter 3 (Figure 3.7) and to build on the findings of this research.

Chapter 6 showed the possible importance of birds in the movement of seeds of tangled lignum between hydrological disconnected patches on the floodplain. Further research is needed to investigate the possible role of birds in maintaining gene flow and genetic diversity in plants which occur on floodplains. Undertaking a population genetic study on plants collected from known bird breeding areas, known non-breeding areas, and from the feathers and feet of birds would more definitively identify the role of waterbirds in the dispersal of floodplain plants. Birds may also increase seed germination rates through internal digestion processes. Birds may play an important role in the distribution and abundance of floodplain plant species.

7.9   Conclusions

This thesis combined hydrological modelling with experimental studies on plant responses to variation in hydrological attributes, and a population genetics study. This multi-disciplinary approach has enabled an in-depth understanding of the vegetation of a semi-arid floodplain. This thesis describes how semi-arid floodplains such as the floodplain of the lower Lachlan River are temporally and spatially dynamic and complex. The vegetation on the floodplain has evolved to persist through extended drying and wetting, and to capitalise on available resources and habitat when the hydrological conditions permit. The organisation of vegetation on the floodplain is strongly related to the hydrological regime and the requirements and tolerances of each species.

 Hydrological changes in floodplain-river connection regimes translate to changing conditions on the floodplain. The dependency of floodplain vegetation for flooding means hydrological changes are likely to affect floodplain vegetation by reducing opportunities for life history strategies such as growth, dispersal, seed production, seed germination and establishment. The results suggest changes to the flow regime will influence the distribution and abundance of species, genetic diversity and genetic structure, and the distribution of populations and communities across floodplains.

To maintain the diversity of vegetation and habitat types on floodplains, environmental flow must consider the life-history traits of the plants, primarily growth, seed production, dispersal and germination. This involves consideration of hydrological characteristics such as frequency, timing, duration and predictability of flow events (Poff *et al.* 1997; Arthington *et*

al. 2006) which will meet the requirements of the species. Water actions may have to be coordinated with complimentary management actions (such as live-stock removal and feral herbivore control) to ensure the desired outcomes from natural unregulated and managed flooding events are occurring.

While in the last few decades the environment has been considered in the allocation of water in the Murray-Darling Basin (SEWPaC 2011) and other parts of the world (Postel and Richter 2012). The issue raised in this thesis regarding the dichotomy between the dependency of floodplain plants for flooding and the changes to flow resulting from the human requirements of freshwater is a great and current challenge. For example, the lower Darling River has been subject to three fish death events in December 2018 and January 2019, resulting from lack of water and water quality issues (https://www.mdba.gov.au/managing-water/drought-murray-darling-basin/fish-deaths-lower-darling), which has received a great deal of media attention. The results of this thesis show that the vegetation is also likely to be affected by the reduced availability of water for the environment. The species assemblages are likely to change and the position in which they occur in the landscape may also change.

Australia's inland rivers have and will continue to be a challenge to manage effectively because of the climate variability, climate change and the associated changes in rainfall (Argent 2017). 80% of the world's population lives in areas with threats to human water security (Vörösmarty *et al.* 2010) and further increases are expected in irrigation water requirements to supply increasing global food production (Tilman *et al.* 2001). As proposed by Postel and Richter (2012), water allocation in the 21st century must initially consider the quantity and timing of flows needed to support freshwater ecosystems, and a sustainability boundary be established, of which human consumption cannot exceed.

8. References

Adamack, A.T., and Gruber, B. (2014). PopGenReport: simplifying basic population genetic analyses in R. *Methods in Ecology and Evolution,* **5**, 384-387.

Aguilar, R., Quesada, M., Ashworth, L., Herrerias-Diego, Y., and Lobo, J. (2008). Genetic consequences of habitat fragmentation in plant populations: susceptible signals in plant traits and methodological approaches. *Molecular Ecology,* **17**, 5177-5188.

Altshuler, D., Pollara, V.J., Cowles, C.R., Van Etten, W.J., Baldwin, J., Linton, L., and Lander, E.S. (2000). An SNP map of the human genome generated by reduced representation shotgun sequencing. *Nature,* **407**, 513-516.

Amoros, C., and Bornette, G. (2002). Connectivity and biocomplexity in waterbodies of riverine floodplains. *Freshwater Biology,* **47**, 761-776.

Argent, R. (2017). Australia state of the environment 2016: inland water, independent report to the Australian Government Minister for the Environment and Energy. (Australian Government Department of the Environment and Energy, Canberra.)

Argus, R., Colmer, T., and Grierson, P. (2015). Early physiological flood tolerance is followed by slow post-flooding root recovery in the dryland riparian tree E ucalyptus camaldulensis subsp. refulgens. *Plant, Cell & Environment,* **38**, 1189-1199.

Armstrong, J., Kingsford, R., and Jenkins, K. (2009). The effect of regulating the Lachlan River on the Booligal Wetlands – the floodplain red gum swamps. (Report from University of New South Wales, Sydney.) www.wetrivers.unsw.edu.au/Booligal_Lachlan_River_Report.pdf

Armstrong, W. (1979). Aeration in higher plants. *Advances in Botanical Research,* **7**, 225-332.

Armstrong, W., Brändle, R., and Jackson, M.B. (1994). Mechanisms of flood tolerance in plants. *Acta Botanica Neerlandica,* **43**, 307-358.

Arthington, A.H., Bunn, S.E., Poff, N.L., and Naiman, R.J. (2006). The challenge of providing environmental flow rules to sustain river ecosystems. *Ecological Applications,* **16**, 1311-1318.

Auchincloss, L., Richards, J., Young, C., and Tansey, M. (2012). Inundation depth, duration, and temperature influence Fremont cottonwood (Populus fremontii) seedling growth and survival. *Western North American Naturalist,* **72**, 323-333.

AVH (2016). 'Australia's Virtual Herbarium, Council of Heads of Australasian Herbaria'. Available at http://bie.ala.org.au/species/http://id.biodiversity.org.au/node/apni/2901190 [accessed 12-07-2016].

Baird, N.A., Etter, P.D., Atwood, T.S., Currey, M.C., Shiver, A.L., Lewis, Z.A., Selker, E.U., Cresko, W.A., and Johnson, E.A. (2008). Rapid SNP discovery and genetic mapping using sequenced RAD markers. *PloS one,* **3**, e3376.

Baldwin, D.S., Rees, G.N., Wilson, J.S., Colloff, M.J., Whitworth, K.L., Pitman, T.L., and Wallace, T.A. (2013). Provisioning of bioavailable carbon between the wet and dry phases in a semi-arid floodplain. *Oecologia,* **172**, 539-550.

Ballinger, A., and Lake, P. (2006). Energy and nutrient fluxes from rivers and streams into terrestrial food webs. *Marine and Freshwater Research,* **57**, 15-28.

Barbier, E.B., and Thompson, J.R. (1998). The value of water: floodplain versus large-scale irrigation benefits in northern Nigeria. *Ambio,* **27**, 434-440.

Barrett, R.D., and Schluter, D. (2008). Adaptation from standing genetic variation. *Trends in Ecology & Evolution,* **23**, 38-44.

Baskin, C.C., and Baskin, J.M. (1998). 'Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination.' (Academic Press: San Diego.)

Bayley, P.B. (1995). Understanding large river: floodplain ecosystems. *BioScience,* **45**, 153-158.

Benson, J., Allen, C., Togher, C., and Lemmon, J. (2006). New South Wales vegetation classification and assessment: part 1 plant communities of the NSW Western Plains. *Cunninghamia, 9*, 383-450.

Bishop-Taylor, R., Tulbure, M.G., and Broich, M. (2015). Surface water network structure, landscape resistance to movement and flooding vital for maintaining ecological connectivity across Australia's largest river basin. *Landscape Ecology, 30*, 2045-2065.

Blakey, R.V., Kingsford, R.T., Law, B.S., and Stoklosa, J. (2017). Floodplain habitat is disproportionately important for bats in a large river basin. *Biological Conservation, 215*, 1-10.

Blanch, S.J., Ganf, G.G., and Walker, K.F. (1999). Tolerance of riverine plants to flooding and exposure indicated by water regime. *Regulated Rivers: Research & Management: An International Journal Devoted to River Research and Management, 15*, 43-62.

Blom, C., Bögemann, G., Laan, P., Van der Sman, A., Van de Steeg, H., and Voesenek, L. (1990). Adaptations to flooding in plants from river areas. *Aquatic Botany, 38*, 29-47.

Blom, C., and Voesenek, L. (1996). Flooding: the survival strategies of plants. *Trends in Ecology & Evolution, 11*, 290-295.

Boileau, M.G., Hebert, P.D., and Schwartz, S.S. (1992). Non-equilibrium gene frequency divergence: persistent founder effects in natural populations. *Journal of Evolutionary Biology, 5*, 25-39.

Brandis, K., Nairn, L., Porter, J., and Kingsford, R. (2009). Preliminary assessment for the environmental water requirements of waterbird species in the Murray Darling Basin. (University of New South Wales Sydney.)

Bren, L. (1988). Effects of river regulation on flooding of a riparian red gum forest on the River Murray, Australia. *River Research and Applications, 2*, 65-77.

Bren, L. (1991). Modelling the influence of River Murray management on the Barmah river red gum forests. *Australian Forestry, 54*, 9-15.

Bren, L. (1992). Tree invasion of an intermittent wetland in relation to changes in the flooding frequency of the River Murray, Australia. *Austral Ecology,* **17**, 395-408.

Briggs, S., Hodgson, P., and Ewin, P. (1994). Changes in populations of waterbirds on a wetland following water storage. *Wetlands (Australia),* **13**, 36-48.

Briggs, S., Seddon, J., and Thornton, S. (2000). Wildlife in dry lake and associated habitats in western New South Wales. *The Rangeland Journal,* **22**, 256-271.

Brock, M., and Casanova, M. (1997). Plant life at the edge of wetlands: ecological responses to wetting and drying patterns. In (pp. 181-192). Elsevier Science Ltd., Oxford,

Brock, M., Nielsen, D.L., Shiel, R.J., Green, J.D., and Langley, J.D. (2003). Drought and aquatic community resilience: the role of eggs and seeds in sediments of temporary wetlands. *Freshwater Biology,* **48**, 1207-1218.

Brock, M., Capon, S., and Porter, J. (2006). Disturbance of plant communities dependent on desert rivers. In Ecology of desert rivers. (Ed. R.T. Kingsford) pp. 100-132. (Cambridge University Press: Cambridge.)

Brock, M.A., and Rogers, K. (1998). The regeneration potential of the seed bank of an ephemeral floodplain in South Africa. *Aquatic Botany,* **61**, 123-135.

Brock, M.A. (2011). Persistence of seed banks in Australian temporary wetlands. *Freshwater Biology,* **56**, 1312-1327.

Brooks, S., Cottingham, P., Butcher, R., and Hale, J. (2013). Murray-Darling Basin aquatic ecosystem classification: Stage 2 report. *Peter Cottingham & Associates report to the Commonwealth Environmental Water Office and Murray-Darling Basin Authority, Canberra.*

Bunn, S.E., and Arthington, A.H. (2002). Basic principles and ecological consequences of altered flow regimes for aquatic biodiversity. *Environmental Management,* **30**, 492-507.

Bureau of Meteorology (2017). 'Climate statistics for Australian locations, Summary statistics Hillston Airport'. Available at http://www.bom.gov.au/climate/data-services/station-data.shtml [accessed 24-11-2018].

Campbell, C., Johns, C., and Nielsen, D. (2014). The value of plant functional groups in demonstrating and communicating vegetation responses to environmental flows. *Freshwater Biology, 59*, 858-869.

Campbell, D. (1973). Living with lignum. *Agricultural Gazette of New South Wales, 84*, 290-292.

Capon, S. (2005). Flood variability and spatial variation in plant community composition and structure on a large arid floodplain. *Journal of Arid Environments, 60*, 283-302.

Capon, S., James, C.S., Williams, L., and Quinn, G. (2009). Responses to flooding and drying in seedlings of a common Australian desert floodplain shrub: *Muehlenbeckia florulenta* Meisn.(tangled lignum). *Environmental and Experimental Botany, 66*, 178-185.

Capon, S., Rolls, R., James, C., and Mackay, S. (2012). Regeneration of Floodplain Vegetation in Response to Large-scale Flooding in the Condamine-Balonne and Border Rivers. (Australian Rivers Institute, Griffith University, Nathan, Queensland.)

Capon, S., James, C., and George, A. (2016). Riverine trees and shrubs. In Vegetation of Australian Riverine Landscapes: Biology, Ecology and Management. (Eds S. Capon, C. James, & M. Reid) pp. 119-135. (CSIRO Publishing: Clayton.)

Capon, S.J. (2003). Plant community responses to wetting and drying in a large arid floodplain. *River Research and Applications, 19*, 509-520.

Capon, S.J., and Brock, M.A. (2006). Flooding, soil seed bank dynamics and vegetation resilience of a hydrologically variable desert floodplain. *Freshwater Biology, 51*, 206-223.

Capon, S.J. (2007). Effects of flooding on seedling emergence from the soil seed bank of a large desert floodplain. *Wetlands, 27*, 904-914.

Carini, G., and Hughes, J. (2004). Population structure of Macrobrachium australiense (Decapoda: Palaemonidae) in Western Queensland, Australia: the role of contemporary and historical processes. *Heredity, 93*, 350.

Casanova, M.T., and Brock, M.A. (2000). How do depth, duration and frequency of flooding influence the establishment of wetland plant communities? *Plant Ecology,* **147**, 237-250.

Casanova, M.T. (2011). Using water plant functional groups to investigate environmental water requirements. *Freshwater Biology,* **56**, 2637-2652.

Casanova, M.T. (2015). Review of water requirements for key floodplain vegetation for the Northern Basin: literature review and expert knowledge assessment. Report to the Murray–Darling Basin Authority. (Charophyte Services, Lake Bolac, Vic. Australia.) https://www.mdba.gov.au/sites/default/files/pubs/Review%20of%20Water%20Requirements%20for%20Floodplain%20Vegetation_final.docx.

Caskey, S.T., Blaschak, T.S., Wohl, E., Schnackenberg, E., Merritt, D.M., and Dwire, K.A. (2015). Downstream effects of stream flow diversion on channel characteristics and riparian vegetation in the Colorado Rocky Mountains, USA. *Earth Surface Processes and Landforms,* **40**, 586-598.

Catelotti, K., Kingsford, R., Bino, G., and Bacon, P. (2015). Inundation requirements for persistence and recovery of river red gums (*Eucalyptus camaldulensis*) in semi-arid Australia. *Biological Conservation,* **184**, 346-356.

Catford, J.A., Downes, B.J., Gippel, C.J., and Vesk, P.A. (2011). Flow regulation reduces native plant cover and facilitates exotic invasion in riparian wetlands. *Journal of Applied Ecology,* **48**, 432-442.

Catford, J.A., and Jansson, R. (2014). Drowned, buried and carried away: effects of plant traits on the distribution of native and alien species in riparian ecosystems. *New Phytologist,* **204**, 19-36.

Chaves, M.M., Maroco, J.P., and Pereira, J.S. (2003). Understanding plant responses to drought - from genes to the whole plant. *Functional Plant Biology,* **30**, 239-264.

Chesson, P., and Huntly, N. (1989). Short-term instabilities and long-term community dynamics. *Trends in Ecology & Evolution,* **4**, 293-298.

Chong, C., and Walker, K.F. (2005). Does lignum rely on a soil seed bank? Germination and reproductive phenology of *Muehlenbeckia florulenta* (Polygonaceae). *Australian Journal of Botany,* **53**, 407-415.

Coates, D.J. (1988). Genetic diversity and population genetic structure in the rare chittering grass wattle, *Acacia anomala* Court. *Australian Journal of Botany,* **36**, 273-286.

Coleman, M., Clark, J., Doblin, M., Bishop, M., and Kelaher, B. (2018). Genetic differentiation between estuarine and open coast ecotypes of a dominant ecosystem engineer. *Marine and Freshwater Research*

Colloff, M.J., and Baldwin, D.S. (2010). Resilience of floodplain ecosystems in a semi-arid environment. *The Rangeland Journal,* **32**, 305-314.

Conover, W.J., and Iman, R.L. (1981). Rank transformations as a bridge between parametric and nonparametric statistics. *The American Statistician,* **35**, 124-129.

Copp, G.H., Guti, G., Rovný, B., and Černý, J. (1994). Hierarchical analysis of habitat use by 0+ juvenile fish in Hungarian/Slovak flood plain of the Danube River. *Environmental Biology of Fishes,* **40**, 329-348.

Costanza, R., d'Arge, R., De Groot, R., Farber, S., Grasso, M., Hannon, B., Limburg, K., Naeem, S., O'Neill, R.V., and Paruelo, J. (1997). The value of the world's ecosystem services and natural capital. *Nature,* **387**, 253.

Costelloe, J., Payne, E., Woodrow, I., Irvine, E., Western, A. (2008). Water sources accessed by arid zone riparian trees in highly saline environments, Australia. *Ocecologia*, **156**, 43-52.

Courtois, B., Audebert, A., Dardou, A., Roques, S., Ghneim-Herrera, T., Droc, G., Frouin, J., Rouan, L., Gozé, E., and Kilian, A. (2013). Genome-wide association mapping of root traits in a japonica rice panel. *PloS one,* **8**, e78037.

Craig, A., Walker, K., and Boulton, A. (1991). Effects of edaphic factors and flood frequency on the abundance of lignum (*Muehlenbeckia florulenta* Meissner)(Polygonaceae) on the River Murray floodplain, South Australia. *Australian Journal of Botany,* **39**, 431-443.

Cruz, V.M., Kilian, A., and Dierig, D.A. (2013). Development of DArT marker platforms and genetic diversity assessment of the US collection of the new oilseed crop lesquerella and related species. *PloS one,* **8**, e64062.

Cunningham, G., Mulham, W., Milthorpe, P., and Leigh, J. (1981). 'Plants of Western New South Wales.' (CSIRO Publishing: Melbourne, Vic., Australia.)

Cunningham, S., Thomson, J., Mac Nally, R., Read, J., Baker, P. (2011) Groundwater change forecasts widespread forest dieback across an extensive floodplain system. *Freshwater Biology*, **56**, 1494-1508.

Daniels, J.M. (2003). Floodplain aggradation and pedogenesis in a semiarid environment. *Geomorphology,* **56**, 225-242.

Dillon, S., McEvoy, R., Baldwin, D.S., Rees, G.N., Parsons, Y., and Southerton, S. (2014). Characterisation of adaptive genetic diversity in environmentally contrasted populations of *Eucalyptus camaldulensis* Dehnh.(River Red Gum). *PloS one,* **9**, e103515.

Dillon, S., McEvoy, R., Baldwin, D.S., Southerton, S., Campbell, C., Parsons, Y., and Rees, G.N. (2015). Genetic diversity of *Eucalyptus camaldulensis* Dehnh. following population decline in response to drought and altered hydrological regime. *Austral Ecology,* **40**, 558-572.

Dollar, E.S. (2004). Fluvial geomorphology. *Progress in Physical Geography,* **28**, 405.

Doody, T., and Overton, I. (2009). Environmental management of riparian tree health in the Murray-Darling Basin, Australia. In River Basin Management V. (Ed. C.A. Brebbia) pp. 197. (WIT Press: Boston, UK.)

Doody, T.M., Benger, S.N., Pritchard, J.L., and Overton, I.C. (2014). Ecological response of Eucalyptus camaldulensis (river red gum) to extended drought and flooding along the River Murray, South Australia (1997–2011) and implications for environmental flow management. *Marine and Freshwater Research,* **65**, 1082-1093.

Dorken, M.E., and Eckert, C.G. (2001). Severely reduced sexual reproduction in northern populations of a clonal plant, Decodonverticillatus (Lythraceae). *Journal of Ecology,* **89**, 339-350.

Driver, P., Lloyd-Jones, P., and Unthank, S. (2000). Responses of Lachlan wetlands and distributaries to 1999 environmental flows. (Paper presented to the Lachlan River Management Committee Forbes NSW.) Available at https://www.researchgate.net/publication/236247528_Responses_of_Lachlan_wetlands_and_distributaries_to_1999_environmental_flows_Paper_presented_to_the_Lachlan_River_Management_Committee_Forbes_NSW

Driver, P., Chowdhury, S., Wettin, P., and Jones, H. (2004a). Models to predict the effects of environmental flow releases on wetland inundation and the success of colonial bird breeding in the Lachlan River, NSW, 'Proceedings of the 4th Annual Stream Management Conference: linking rivers to landscapes, Launceston 19-22 October 2004' (Eds. I.W. ID Rutherford, MJ Askey-Doran and R Glazik), pp. 192-198, (Tasmanian Dept of Primary Industries, Water and Environment).

Driver, P., Chowdhury, S.H., T , Lloyd-Jones, P., Raisin, G., Ribbons, C., Singh, G., and Wettin, P. (2004b). Natural and modified flows of the Lachlan Valley wetlands. (Resource Analysis Unit, NSW Department of Infrastructure, Planning and Natural Resources, Central West Region, Forbes, NSW, Australia.)

Driver, P., Hameed, T., Smith, T., Wettin, P., and Raisin, G. (2005). Rehabilitation of wetland inundation regimes in the Lachlan River, NSW, Australia: achievements and ongoing challenges, Proceedings of the 8th International River Symposium, Brisbane, Queensland 6-9 September.

Driver, P., Chowdhury, S., Hameed, T., O'Rourke, M., and Shaikh, M. (2010). Ecosystem response models for lower Calare (Lachlan River) floodplain wetlands: managing wetland biota and climate change modelling. *Ecosystem response modelling in the Murray-Darling Basin (Overton, I. and Saintilan, N., Eds.), CSIRO Publishing*, 183-196.

Driver, P., Barbour, E., Lenehan, J., Kumar, S., Merritt, W., and Wise, N. (2013a). Lachlan wetland flow-response status: Field observations, university research update and

ecological condition assessment from the 18-22 February 2013 field trip for the Lachlan Riverine Working Group. (Department of Industry and Investment NSW, Sydney, NSW, Australia.) Available at https://www.researchgate.net/publication/260294101_Lachlan_wetland_flow-response_status

Driver, P.D., Raine, A., Foster, N.D., and Williams, S.A. (2013b). Ecological monitoring to support Water Sharing Plan evaluation and protect wetlands of inland New South Wales, Australia. *Ecological Management & Restoration,* **14**, 187-193.

Dudaniec, R.Y., Rhodes, J.R., Worthington Wilmer, J., Lyons, M., Lee, K.E., McAlpine, C.A., and Carrick, F.N. (2013). Using multilevel models to identify drivers of landscape-genetic structure among management areas. *Molecular Ecology,* **22**, 3752-3765.

Dugan, P.J. (1990). 'Wetland conservation: A review of current issues and required action.' (IUCN: Gland, Switzerland.)

Dunkerley, D. (2008). Flow chutes in Fowlers Creek, arid western New South Wales, Australia: evidence for the diversity in influence of trees on ephemeral channel form and process. *Geomorphology*, **102**, 232-241.

Dunlop, M., and Brown, P.R. (2008). Implications of climate change for Australia's national reserve system-a preliminary assessment. (Department of Climate Change, Canberra, Australia.)

Dyer, F., Broadhurst, B., Thiem, J., Thompson, R., Driver, P., Bowen, S., Asmus, M., and Lenehan, J. (2015). Commonwealth Environmental Water Office Long Term Intervention Monitoring Project Lower Lachlan river system 2015 Annual Report. University of Canberra: Canberra,

Dyer, F.J. (2002). Assessing the hydrological changes to flood plain wetland inundation caused by river regulation. In F. Dyer, M. Thoms, & J. Olley (Eds.), *International Symposium on the Structure, Function and Management Implications of Fluvial Sedimentary Systems* (pp. 245-253). IAHS Publication, Alice Springs.

Eamus, D., Hatton, T., Cook, P., and Colvin, C. (2006). 'Ecohydrology: Vegetation Function, Water and Resource Management.' (CSIRO Publishing: Victoria.)

Ellstrand, N.C. (2014). Is gene flow the most important evolutionary force in plants? *American Journal of Botany,* **101**, 737-753.

Elshire, R.J., Glaubitz, J.C., Sun, Q., Poland, J.A., Kawamoto, K., Buckler, E.S., and Mitchell, S.E. (2011). A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. *PloS one,* **6**, e19379.

Entwisle, T.J., Maslin, B.R., Cowan, R.S., and Court, A.B. (1996). 'Mimosaceae. In: Flora of Victoria Vol. 3, Dicotyledons Winteraceae to Myrtaceae.'. (Inkata Press: Melbourne.)

Environment Australia (2001). A Directory of Important Wetlands in Australia. (Environment Australia, Canberra.)

Falconer, D.S., Mackay, T.F., and Frankham, R. (1996). Introduction to quantitative genetics (4th edn). *Trends in Genetics,* **12**, 280.

Field, A. (2013). 'Discovering statistics using IBM SPSS statistics.' (4th ed.). (Sage: London.)

Fisher, A., Flood, N., and Danaher, T. (2016). Comparing Landsat water index methods for automated water classification in eastern Australia. *Remote Sensing of Environment,* **175**, 167-182.

Flood, N., Danaher, T., Gill, T., and Gillingham, S. (2013). An operational scheme for deriving standardised surface reflectance from Landsat TM/ETM+ and SPOT HRG imagery for Eastern Australia. *Remote Sensing,* **5**, 83-109.

Frankham, R. (2005). Genetics and extinction. *Biological Conservation,* **126**, 131-140.

Frankham, R., Ballou, J.D., Ralls, K., Eldridge, M., Dudash, M.R., Fenster, C.B., Lacy, R.C., and Sunnucks, P. (2017). 'Genetic management of fragmented animal and plant populations.' (Oxford University Press: Oxford, UK.)

Freestone, F., Brown, P., Campbell, C., Wood, D., Nielsen, D., and Henderson, M. (2017). Return of the lignum dead: Resilience of an arid floodplain shrub to drought. *Journal of Arid Environments,* **138**, 9-17.

Froend, R., and McComb, A. (1994). Distribution, productivity and reproductive phenology of emergent macrophytes in relation to water regimes at wetlands of south-western Australia. *Marine and Freshwater Research,* **45**, 1491-1508.

Galat, D.L., Fredrickson, L.H., Humburg, D.D., Bataille, K.J., Bodie, J.R., Dohrenwend, J., Gelwicks, G.T., Havel, J.E., Helmers, D.L., and Hooker, J.B. (1998). Flooding to restore connectivity of regulated, large-river wetlands natural and controlled flooding as complementary processes along the lower Missouri River. *BioScience,* **48**, 721-733.

Garner, B.A., Hand, B.K., Amish, S.J., Bernatchez, L., Foster, J.T., Miller, K.M., Morin, P.A., Narum, S.R., O'Brien, S.J., and Roffler, G. (2016). Genomics in conservation: case studies and bridging the gap between data and application. *Trends in Ecology & Evolution,* **31**, 81-83.

George, A.K. (2004). Eucalypt regeneration on the Lower Murray floodplain, South Australia. PhD Thesis, *School of Earth and Environmental Sciences* University of Adelaide.

George, A.K., Walker, K.F., and Lewis, M.M. (2005). Population status of eucalypt trees on the River Murray floodplain, South Australia. *River Research and Applications,* **21**, 271-282.

Gouskov, A., Reyes, M., Wirthner-Bitterlin, L., and Vorburger, C. (2016). Fish population genetic structure shaped by hydroelectric power plants in the upper Rhine catchment. *Evolutionary Applications,* **9**, 394-408.

Green, A.J., Jenkins, K., Bell, D., Morris, P., and Kingsford, R. (2008). The potential role of waterbirds in dispersing invertebrates and plants in arid Australia. *Freshwater Biology,* **53**, 380-392.

Green, D., Petrovic, J., Moss, P., and Burrell, M. (2011). Water resources and management overview: Lachlan Catchment. *NSW Office of Water, Sydney*

Greet, J. (2015). The marked flooding tolerance of seedlings of a threatened swamp gum: implications for the restoration of critical wetland forests. *Australian Journal of Botany,* **63**, 669-678.

Greet, J. (2016). The potential of soil seed banks of a eucalypt wetland forest to aid restoration. *Wetlands Ecology and Management,* **24**, 565-577.

Grill, G., Lehner, B., Lumsdon, A.E., MacDonald, G.K., Zarfl, C., and Liermann, C.R. (2015). An index-based framework for assessing patterns and trends in river fragmentation and flow regulation by global dams at multiple scales. *Environmental Research Letters,* **10**, 015001.

Grime, J. (1989). The stress debate: symptom of impending synthesis? *Biological Journal of the Linnean Society,* **37**, 3-17.

Grime, J.P. (1977). Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. *The American Naturalist,* **111**, 1169-1194.

Gruber, B., Unmack, P.J., Berry, O.F., and Georges, A. (2018). dartr: An r package to facilitate analysis of SNP data generated from reduced representation genome sequencing. *Molecular Ecology Resources,* **18**, 691-699.

Haddeland, I., Heinke, J., Biemans, H., Eisner, S., Flörke, M., Hanasaki, N., Konzmann, M., Ludwig, F., Masaki, Y., and Schewe, J. (2014). Global water resources affected by human interventions and climate change. *Proceedings of the National Academy of Sciences,* **111**, 3251-3256.

Hameed, T., and Podger, G. (2001). Use of the IQQM simulation model for planning and management of a regulated river system. In M. Marino, & S. Simonovic (Eds.), *International Symposium on Integrated Water Resources Management* (pp. 83-90). IAHS Publication,Davis, California, USA.

Higgins, S.I., Rogers, K.H., and Kemper, J. (1997). A description of the functional vegetation pattern of a semi-arid floodplain, South Africa. *Plant Ecology,* **129**, 95-101.

Hillman, M., and Brierley, G. (2002). Information needs for environmental-flow allocation: a case study from the Lachlan River, New South Wales, Australia. *Annals of the Association of American Geographers,* **92**, 617-630.

Hoffmann, A., Griffin, P., Dillon, S., Catullo, R., Rane, R., Byrne, M., Jordan, R., Oakeshott, J., Weeks, A., and Joseph, L. (2015). A framework for incorporating evolutionary genomics into biodiversity conservation and management. *Climate Change Responses,* **2**, 1-23.

Holland, K., Turnadge, C., Nicol, J., Gehrig, S., and Strawbridge, A. (2013). Floodplain response and recovery: comparison between natural and artificial floods. (Goyder Institute for Water Research Technical Report Series, 13/4, Adelaide, South Australia.)

Holland, K.L., Charles, A.H., Jolly, I.D., Overton, I.C., Gehrig, S., and Simmons, C.T. (2009). Effectiveness of artificial watering of a semi-arid saline wetland for managing riparian vegetation health. *Hydrological Processes: An International Journal,* **23**, 3474-3484.

Hölzel, N., and Otte, A. (2004). Inter-annual variation in the soil seed bank of flood-meadows over two years with different flooding patterns. *Plant Ecology,* **174**, 279-291.

Howe, H.F., and Smallwood, J. (1982). Ecology of seed dispersal. *Annual Review of Ecology and Systematics,* **13**, 201-228.

Hughes, F. (1988). The ecology of African floodplain forests in semi-arid and arid zones: a review. *Journal of Biogeography,* **15**, 127-140.

Hughes, J., and Hillyer, M. (2006). Mitochondrial DNA and allozymes reveal high dispersal abilities and historical movement across drainage boundaries in two species of freshwater fishes from inland rivers in Queensland, Australia. *Journal of Fish Biology,* **68**, 270-291.

Hupp, C.R., and Bornette, G. (2003). Vegetation as a tool in the interpretation of fluvial geomorphic processes and landforms in humid temperate areas. *Tools in Fluvial Geomorphology*, 269-288.

Isbell, R. (2016). 'The Australian soil classification.' (CSIRO publishing: Victoria.)

Iwaizumi, M.G., Takahashi, M., Isoda, K., and Austerlitz, F. (2013). Consecutive five-year analysis of paternal and maternal gene flow and contributions of gametic

heterogeneities to overall genetic composition of dispersed seeds of *Pinus densiflora* (Pinaceae). *American Journal of Botany,* **100**, 1896-1904.

Jackson, M., and Colmer, T. (2005). Response and adaptation by plants to flooding stress. *Annals of botany,* **96**, 501-505.

Jackson, R.B., Carpenter, S.R., Dahm, C.N., McKnight, D.M., Naiman, R.J., Postel, S.L., and Running, S.W. (2001). Water in a changing world. *Ecological Applications,* **11**, 1027-1045.

James, C.S., Capon, S.J., White, M.G., Rayburg, S.C., and Thoms, M.C. (2007). Spatial variability of the soil seed bank in a heterogeneous ephemeral wetland system in semi-arid Australia. *Plant Ecology,* **190**, 205-217.

James, E.A., Jordan, R., and Griffin, P.C. (2013). Spatial genetic analysis of two polyploid macrophytes reveals high connectivity in a modified wetland. *Freshwater Biology,* **58**, 2102-2113.

Jansson, R., Nilsson, C., and Renöfält, B. (2000). Fragmentation of riparian floras in rivers with multiple dams. *Ecology,* **81**, 899-903.

Jensen, A.E. (2008). The roles of seed banks and soil moisture in recruitment of semi-arid floodplain plants: the River Murray, Australia. PhD Thesis, University of Adelaide.

Jensen, A.E., Walker, K.F., and Paton, D.C. (2008). The role of seedbanks in restoration of floodplain woodlands. *River Research and Applications,* **24**, 632-649.

Johansson, M.E., Nilsson, C., and Nilsson, E. (1996). Do rivers function as corridors for plant dispersal? *Journal of Vegetation Science,* **7**, 593-598.

Johns, C., Reid, C., Roberts, J., Sims, N., Doody, T., Overton, I., McGinness, H., Rogers, K., Campbell, C., and Gawne, B. (2009). Native trees of the River Murray floodplain: literature review and experimental designs to examine effects of flow enhancement and floodwater retention. *Final Report prepared for the MDBA.(Murray–Darling Freshwater Research Centre: Wodonga.)*

Jombart, T. (2008). adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics,* **24**, 1403-1405.

Junk, W.J., Bayley, P.B., and Sparks, R.E. (1989). The flood pulse concept in river-floodplain systems. *Canadian Special Publication of Fisheries and Aquatic Sciences,* **106**, 110-127.

Junk, W.J., An, S., Finlayson, C., Gopal, B., Kvĕt, J., Mitchell, S.A., Mitsch, W.J., and Robarts, R.D. (2013). Current state of knowledge regarding the world's wetlands and their future under global climate change: a synthesis. *Aquatic Sciences,* **75**, 151-167.

Karrenberg, S., Edwards, P., and Kollmann, J. (2002). The life history of Salicaceae living in the active zone of floodplains. *Freshwater Biology,* **47**, 733-748.

Kawecki, T.J., and Ebert, D. (2004). Conceptual issues in local adaptation. *Ecology letters,* **7**, 1225-1241.

Keith, D.A. (2004). 'Ocean shores to desert dunes: the native vegetation of NSW and the ACT (Selected Extracts).' (Department of Environment and Conservation (NSW): Sydney.)

Kelleway, J., Mazumder, D., Wilson, G.G., Saintilan, N., Knowles, L., Iles, J., and Kobayashi, T. (2010). Trophic structure of benthic resources and consumers varies across a regulated floodplain wetland. *Marine and Freshwater Research,* **61**, 430-440.

Kemp, J. (2004). Flood channel morphology of a quiet river, the Lachlan downstream from Cowra, southeastern Australia. *Geomorphology,* **60**, 171-190.

Kennard, M.J., Mackay, S.J., Pusey, B.J., Olden, J.D., and Marsh, N. (2010). Quantifying uncertainty in estimation of hydrologic metrics for ecohydrological studies. *River Research and Applications,* **26**, 137-156.

Kilian, A., Wenzl, P., Huttner, E., Carling, J., Xia, L., Blois, H., Caig, V., Heller-Uszynska, K., Jaccoud, D., and Hopper, C. (2012). Diversity arrays technology: a generic genome profiling technology on open platforms. *Methods in Molecular Biology,* **888**, 67-89.

King, A., Ward, K., O'connor, P., Green, D., Tonkin, Z., and Mahoney, J. (2010). Adaptive management of an environmental watering event to enhance native fish spawning and recruitment. *Freshwater Biology,* **55**, 17-31.

Kingsford, R., and Johnson, W. (1998). Impact of water diversions on colonially-nesting waterbirds in the Macquarie Marshes of arid Australia. *Colonial Waterbirds*, **21**, 159-170.

Kingsford, R., Curtin, A., and Porter, J. (1999). Water flows on Cooper Creek in arid Australia determine 'boom'and 'bust'periods for waterbirds. *Biological Conservation,* **88**, 231-248.

Kingsford, R., Brandis, K., Thomas, R., Crighton, P., Knowles, E., and Gale, E. (2004). Classifying landform at broad spatial scales: the distribution and conservation of wetlands in New South Wales, Australia. *Marine and Freshwater Research,* **55**, 17-31.

Kingsford, R.T. (2000a). Ecological impacts of dams, water diversions and river management on floodplain wetlands in Australia. *Austral Ecology,* **25**, 109-127.

Kingsford, R.T. (2000b). Protecting rivers in arid regions or pumping them dry? *Hydrobiologia,* **427**, 1-11.

Knaapen, J.P., Scheffer, M., and Harms, B. (1992). Estimating habitat isolation in landscape planning. *Landscape and Urban Planning,* **23**, 1-16.

Koppitz, H., Kühl, H., Hesse, K., and Kohl, J.G. (1997). Some aspects of the importance of genetic diversity in *Phragmites australis* (Cav.) Trin. ex Steudel for the development of reed stands. *Plant Biology,* **110**, 217-223.

Kozlowski, T. (1984). Plant responses to flooding of soil. *BioScience,* **34**, 162-167.

Kozlowski, T. (1997). Responses of woody plants to flooding and salinity. *Tree Physiology,* **17**, 1-29.

Kudoh, H., and Whigham, D.F. (1997). Microgeographic genetic structure and gene flow in Hibiscus moscheutos (Malvaceae) populations. *American Journal of Botany,* **84**, 1285-1293.

Kuiper, J.J., Janse, J.H., Teurlincx, S., Verhoeven, J.T., and Alkemade, R. (2014). The impact of river regulation on the biodiversity intactness of floodplain wetlands. *Wetlands Ecology and Management,* **22**, 647-658.

Laan, P., and Blom, C. (1990). Growth and survival responses of Rumex species to flooded and submerged conditions: the importance of shoot elongation, underwater photosynthesis and reserve carbohydrates. *Journal of Experimental Botany,* **41**, 775-783.

Lake, P. (2000). Disturbance, patchiness, and diversity in streams. *Journal of the North American Benthological Society,* **19**, 573-592.

Landergott, U., Holderegger, R., Kozlowski, G. and Schneller, J.J. (2001). Historical bottlenecks decrease genetic diversity in natural populations of Dryopteris cristata. *Heredity*, **87**, 344-355.Leblanc, M., Tweed, S., Van Dijk, A., and Timbal, B. (2012). A review of historic and future hydrological changes in the Murray-Darling Basin. *Global and Planetary Change,* **80**, 226-246.

Leck, M.A., and Brock, M.A. (2000). Ecological and evolutionary trends in wetlands: evidence from seeds and seed banks in New South Wales, Australia and New Jersey, USA. *Plant Species Biology,* **15**, 97-112.

Leimu, R., and Fischer, M. (2008). A meta-analysis of local adaptation in plants. *PloS one,* **3**, e4010.

Lemly, A.D., Kingsford, R.T., and Thompson, J.R. (2000). Irrigated agriculture and wildlife conservation: conflict on a global scale. *Environmental Management,* **25**, 485-512.

Lenssen, J., Menting, F., van der Putten, W., and Blom, K. (1999). Control of plant species richness and zonation of functional groups along a freshwater flooding gradient. *Oikos*, 523-534.

Lesica, P., and Allendorf, F.W. (1995). When are peripheral populations valuable for conservation? *Conservation Biology,* **9**, 753-760.

Lytle, D.A., and Poff, N.L. (2004). Adaptation to natural flow regimes. *Trends in Ecology & Evolution,* **19**, 94-100.

Mac Nally, R., Cunningham, S.C., Baker, P.J., Horner, G.J., and Thomson, J.R. (2011). Dynamics of Murray-Darling floodplain forests under multiple stressors: The past, present, and future of an Australian icon. *Water Resources Research,* **47**, W00G05.

Mader, E., Van Vierssen, W., and Schwenk, K. (1998). Clonal diversity in the submerged macrophyte *Potamogeton pectinatus* L. inferred from nuclear and cytoplasmic variation. *Aquatic Botany,* **62**, 147-160.

Magee, J. (1991). Late Quaternary lacustrine, groundwater, aeolian and pedogenic gypsum in the Prungle Lakes, southeastern Australia. *Palaeogeography, Palaeoclimatology, Palaeoecology,* **84**, 3-42.

Maheshwari, B., Walker, K., and McMahon, T. (1995). Effects of regulation on the flow regime of the River Murray, Australia. *River Research and Applications,* **10**, 15-38.

Mandák, B., Bímová, K., and Plačková, I. (2006). Genetic structure of experimental populations and reproductive fitness in a heterocarpic plant *Atriplex tatarica* (Chenopodiaceae). *American Journal of Botany,* **93**, 1640-1649.

McGinness, H. (2017). 'Waterbird breeding and movements: Knowledge for water managers'. Available at https://research.csiro.au/ewkrwaterbirds/research-results/ [accessed 25/05/2018].

McGinness, H.M., Arthur, A.D., and Reid, J.R. (2010). Woodland bird declines in the Murray–Darling Basin: are there links with floodplain change? *The Rangeland Journal,* **32**, 315-327.

McGinness, H.M., Arthur, A.D., Davies, M., and McIntyre, S. (2013). Floodplain woodland structure and condition: the relative influence of flood history and surrounding irrigation land use intensity in contrasting regions of a dryland river. *Ecohydrology,* **6**, 201-213.

McMahon, T., and Finlayson, B. (2003). Droughts and anti-droughts: the low flow hydrology of Australian rivers. *Freshwater Biology,* **48**, 1147-1160.

Meffe, G.K., and Vrijenhoek, R.C. (1988). Conservation genetics in the management of desert fishes. *Conservation Biology,* **2**, 157-169.

Megonigal, J.P., and Day, F.P. (1992). Effects of flooding on root and shoot production of bald cypress in large experimental enclosures. *Ecology,* **73**, 1182-1193.

Menges, E.S., and Waller, D.M. (1983). Plant strategies in relation to elevation and light in floodplain herbs. *The American Naturalist,* **122**, 454-473.

Meredith, S., and Beesley, L. (2009). Watering floodplain wetlands in the Murray-Darling Basin to benefit native fish: a discussion with managers. (Department of Sustainability and Environment, Victoria.)

Merritt, D.M., Nilsson, C., and Jansson, R. (2010a). Consequences of propagule dispersal and river fragmentation for riparian plant community diversity and turnover. *Ecological Monographs,* **80**, 609-626.

Merritt, D.M., and Poff, N.L.R. (2010). Shifting dominance of riparian Populus and Tamarix along gradients of flow alteration in western North American rivers. *Ecological Applications,* **20**, 135-152.

Merritt, D.M., Scott, M.L., LeROY, P., Auble, G.T., and Lytle, D.A. (2010b). Theory, methods and tools for determining environmental flows for riparian vegetation: riparian vegetation-flow response guilds. *Freshwater Biology,* **55**, 206-225.

Middleton, B.A. (2003). Soil seed banks and the potential restoration of forested wetlands after farming. *Journal of Applied Ecology,* **40**, 1025-1034.

Millennium Ecosystem Assessment (2005). 'Ecosystems and human well-being.' (Island press Washington, DC::

Mommer, L., Lenssen, J.P., Huber, H., Visser, E.J., and De Kroon, H. (2006). Ecophysiological determinants of plant performance under flooding: a comparative study of seven plant families. *Journal of Ecology,* **94**, 1117-1129.

Mony, C., Mercier, E., Bonis, A., and Bouzille, J.-B. (2010). Reproductive strategies may explain plant tolerance to inundation: a mesocosm experiment using six marsh species. *Aquatic Botany,* **92**, 99-104.

Moxham, C., Duncan, M., and Moloney, P. (2018). Tree health and regeneration response of Black Box (Eucalyptus largiflorens) to recent flooding. *Ecological Management & Restoration,* **19**, 58-65.

Murdoch, A.J., and Ellis, R.H. (2000). Dormancy, viability and longevity. In 'Seeds: The Ecology of Regeneration in Plant Communities'. In. (Ed. M. Fenner) pp. 183-214. (CABI Publishing: Wallingford, UK).)

Murray, B.F., Reid, M.A., Capon, S.J., Thoms, M., and Wu, S.B. (2019). Gene flow and genetic structure in *Acacia stenophylla* (Fabaceae): Effects of hydrological connectivity. *Journal of Biogeography,* **46**, 1138-1151.

Murray-Darling Basin Authority (MDBA) (2011). The Living Murray Story: One of Australia's largest river restoration projects. (MDBA Publication No. 157/11, Canberra, ACT, Australia.)

Murray-Darling Basin Authority (MDBA) (2012). Assessment of environmental water requirements for the proposed Basin Plan: Great Cumbung Swamp. (MDBA, Canberra.)

Naiman, R.J., and Decamps, H. (1997). The ecology of interfaces: riparian zones. *Annual Review of Ecology and Systematics,* **28**, 621-658.

Nei, M. (1972). Genetic distance between populations. *The American Naturalist,* **106**, 283-292.

Newall, P., Lloyd, L., Gell, P., and Walker, K. (2009). Riverland Ramsar site ecological character description, Report (Project No: LE0739) prepared for the Department for Environment and Heritage, Lloyd Environmental Pty Ltd, Adelaide, SA, Australia.

Nicholls, N. (1991). The El Nino/southern oscillation and Australian vegetation. *Vegetatio,* **91**, 23-36.

Nicol, J.M., and Ganf, G.G. (2000). Water regimes, seedling recruitment and establishment in three wetland plant species. *Marine and Freshwater Research,* **51**, 305-309.

Nicol, J.M., Ganf, G.G., and Pelton, G.A. (2003). Seed banks of a southern Australian wetland: the influence of water regime on the final floristic composition. *Plant Ecology,* **168**, 191-205.

Nielsen, D.L., Podnar, K., Watts, R., and Wilson, A. (2013). Empirical evidence linking increased hydrologic stability with decreased biotic diversity within wetlands. *Hydrobiologia,* **708**, 81-96.

Nielsen, D.L., Campbell, C.J., Durant, R., Littler, R., Rees, G.N., and Watson, G.O. (2014). Seed bank and wetland vegetation dynamics in response to environmental watering Final Report. *MDFRC Publication*

Nilsson, C., Ekblad, A., Gardfjell, M., and Carlberg, B. (1991a). Long-term effects of river regulation on river margin vegetation. *Journal of Applied Ecology,* **28**, 963-987.

Nilsson, C., Gardfjell, M., and Grelsson, G. (1991b). Importance of hydrochory in structuring plant communities along rivers. *Canadian Journal of Botany,* **69**, 2631-2633.

Nilsson, C., Reidy, C.A., Dynesius, M., and Revenga, C. (2005). Fragmentation and flow regulation of the world's large river systems. *Science,* **308**, 405-408.

Nilsson, C., Brown, R.L., Jansson, R., and Merritt, D.M. (2010). The role of hydrochory in structuring riparian and wetland vegetation. *Biological Reviews,* **85**, 837-858.

Noreen, A.; Niissalo, M.; Lum, S.; Webb, E., (2016) Persistence of long-distance, insect-mediated pollen movement for a tropical canopy tree species in remnant forest patches in an urban landscape. *Heredity*, **117**, 472-480.

O'Brien, P., and Burne, R. (1994). The Great Cumbung Swamp--terminus of the low-gradient Lachlan River, Eastern Australia. *AGSO Journal of Australian Geology and Geophysics,* **15**, 223-234.

O'Donnell, J., Fryirs, K.A., and Leishman, M.R. (2016). Seed banks as a source of vegetation regeneration to support the recovery of degraded rivers: a comparison of river reaches of varying condition. *Science of the Total Environment,* **542**, 591-602.

O'Dowd, D.J., and Gill, A.M. (1986). Seed dispersal syndromes in Australian Acacia. In Seed dispersal. (Ed. D. Murray) pp. 87-121. (Academic Press: Sydney.)

Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'hara, R., Simpson, G.L., Solymos, P., Stevens, M.H.H., and Wagner, H. (2013). Package 'vegan'. *Community ecology package, version,* **2**

Olden, J.D., and Poff, N. (2003). Redundancy and the choice of hydrologic indices for characterizing streamflow regimes. *River Research and Applications,* **19**, 101-121.

Overton, I., and Doody, T. (2008). Ecosystem changes on the River Murray floodplain over the last 100 years and predictions of climate change, in: *From Headwaters to the Ocean - Hydrological Changes and Watershed Management* Eds. Taniguchi, Burnett, Fukishima, Haigh, Umezawa, pp. 599-604 (Taylor and Francis: London.)

Page, K.J. (1994). Late Quaternary stratigraphy and chronology of the Riverine Plain, southeastern Australia. *Wollongong PhD thesis, University of Wollongong*

Parsons, M., and Thoms, M. (2013). Patterns of vegetation greenness during flood, rain and dry resource states in a large, unconfined floodplain landscape. *Journal of Arid Environments,* **88**, 24-38.

Pavlova, A., Beheregaray, L.B., Coleman, R., Gilligan, D., Harrisson, K.A., Ingram, B.A., Kearns, J., Lamb, A.M., Lintermans, M., and Lyon, J. (2017). Severe consequences of habitat fragmentation on genetic diversity of an endangered Australian freshwater fish: a call for assisted gene flow. *Evolutionary Applications,* **10**, 531-550.

Peel, M.C., Finlayson, B.L., and McMahon, T.A. (2007). Updated world map of the Köppen-Geiger climate classification. *Hydrology and Earth System Sciences Discussions Discussions,* **4**, 439-473.

Pembleton, L.W., Cogan, N.O., and Forster, J.W. (2013). St AMPP: an R package for calculation of genetic differentiation and structure of mixed-ploidy level populations. *Molecular Ecology Resources,* **13**, 946-952.

Peterson, B.K., Weber, J.N., Kay, E.H., Fisher, H.S., and Hoekstra, H.E. (2012). Double digest RADseq: an inexpensive method for de novo SNP discovery and genotyping in model and non-model species. *PloS one,* **7**, e37135.

Pettit, N., and Froend, R. (2001). Availability of seed for recruitment of riparian vegetation: a comparison of a tropical and a temperate river ecosystem in Australia. *Australian Journal of Botany,* **49**, 515-528.

PlantNET (2017a). 'Royal Botanic Gardens and Domain Trust, Sydney'. Available at
http://plantnet.rbgsyd.nsw.gov.au/cgi-
bin/NSWfl.pl?page=nswfl&lvl=sp&name=Chenopodium~nitrariaceum [accessed
14/03/2017].

PlantNET, (2017b). *Duma florulenta* (Meisn.) T.M.Schust. In 'New South Wales Flora
Online'. In Royal Botanic Gardens and Domain Trust: Sydney, NSW, Australia.
Available at http://plantnet.rbgsyd.nsw.gov.au/cgi
bin/NSWfl.pl?page=nswfl&lvl=sp&name=Duma~florulenta

Poff, N.L., and Ward, J.V. (1989). Implications of streamflow variability and predictability
for lotic community structure: a regional analysis of streamflow patterns. *Canadian
journal of fisheries and aquatic sciences,* **46**, 1805-1818.

Poff, N.L. (1997). Landscape filters and species traits: towards mechanistic understanding
and prediction in stream ecology. *Journal of the North American Benthological
Society*, 391-409.

Poff, N.L., Allan, J.D., Bain, M.B., Karr, J.R., Prestegaard, K.L., Richter, B.D., Sparks, R.E.,
and Stromberg, J.C. (1997). The natural flow regime. *BioScience,* **47**, 769-784.

Poff, N.L., Olden, J.D., Merritt, D.M., and Pepin, D.M. (2007). Homogenization of regional
river dynamics by dams and global biodiversity implications. *Proceedings of the
National Academy of Sciences,* **104**, 5732-5737.

Poff, N.L., Richter, B.D., Arthington, A.H., Bunn, S.E., Naiman, R.J., Kendy, E., Acreman,
M., Apse, C., Bledsoe, B.P., and Freeman, M.C. (2010). The ecological limits of
hydrologic alteration (ELOHA): a new framework for developing regional
environmental flow standards. *Freshwater Biology,* **55**, 147-170.

Poff, N.L., and Zimmerman, J.K. (2010). Ecological responses to altered flow regimes: a
literature review to inform the science and management of environmental flows.
Freshwater Biology, **55**, 194-205.

Poiani, K.A., and Dixon, P.M. (1995). Seed banks of Carolina bays: potential contributions
from surrounding landscape vegetation. *American Midland Naturalist*, **134**, 140-154.

Porter, J.L., Kingsford, R.T., and Brock, M.A. (2007). Seed banks in arid wetlands with contrasting flooding, salinity and turbidity regimes. *Plant Ecology,* **188**, 215-234.

Postel, S., and Richter, B. (2012). 'Rivers for life: managing water for people and nature.' (Island Press: Washington DC).

Prati, D., and Schmid, B. (2000). Genetic differentiation of life-history traits within populations of the clonal plant *Ranunculus reptans*. *Oikos,* **90**, 442-456.

Puckridge, J., Sheldon, F., Walker, K., and Boulton, A. (1998). Flow variability and the ecology of large rivers. *Marine and Freshwater Research,* **49**, 55-72.

R Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available at http://www.R-project.org/

Raman, H., Raman, R., Kilian, A., Detering, F., Carling, J., Coombes, N., Diffey, S., Kadkol, G., Edwards, D., and McCully, M. (2014). Genome-wide delineation of natural variation for pod shatter resistance in Brassica napus. *PloS one,* **9**, e101673.

Rea, N., and Ganf, G.G. (1994). The role of sexual reproduction and water regime in shaping the distribution patterns of clonal emergent aquatic plants. *Marine and Freshwater Research,* **45**, 1469-1479.

Reid, M., Reid, M., and Thoms, M. (2016). Ecological significance of hydrological connectivity for wetland plant communities on a dryland floodplain river, MacIntyre River, Australia. *Aquatic Sciences,* **78**, 139-158.

Richter, B.D., Baumgartner, J.V., Powell, J., and Braun, D.P. (1996). A method for assessing hydrologic alteration within ecosystems. *Conservation Biology,* **10**, 1163-1174.

Roberts, J., Young, B., and Marston, F. (2000). 'Estimating the water requirements for plants of floodplain wetlands: a guide.' (Land and Water Resources Research and Development Corporation: Canberra.)

Roberts, J., Chan, C., Henderson, B., and Overton, I. (2009). Floodplain trees In 'Ecological Outcomes of Flow Regimes in the Murray-Darling Basin'. Report prepared for the National Water Commission by CSIRO Water for a Healthy Country Flagship. (Eds

I.C. Overton, M.J. Colloff, T.M. Doody, B. Henderson, & S.M. Cuddy) pp. 162-196. (CSIRO: Canberra.)

Roberts, J., and Marston, F. (2011). 'Water Regime for Wetland and Floodplain Plants: A Source Book for the Murray-Darling Basin.' (National Water Commission: Canberra, ACT, Australia.)

Roberts, J., Colloff, M., and Doody, T. (2016). Riverine vegetation of inland south-eastern Australia. In Vegetation of Australian Riverine Landscapes: Biology, Ecology and Management. (Eds S. Capon, C. James, & M. Reid) pp. 177-199. (CSIRO: Melbourne.)

Robertson, A., Bacon, P., and Heagney, G. (2001). The responses of floodplain primary production to flood frequency and timing. *Journal of Applied Ecology,* **38**, 126-136.

Rodríguez-González, P.M., Stella, J.C., Campelo, F., Ferreira, M.T., and Albuquerque, A. (2010). Subsidy or stress? Tree structure and growth in wetland forests along a hydrological gradient in Southern Europe. *Forest ecology and management,* **259**, 2015-2025.

Rogers, K., and Ralph, T.J. (2011). 'Floodplain wetland biota in the Murray-Darling Basin: water and habitat requirements.' (CSIRO: Victoria.)

Rosado, J., Morais, M., and Tockner, K. (2015). Mass dispersal of terrestrial organisms during first flush events in a temporary stream. *River Research and Applications,* **31**, 912-917.

Roshier, D., Robertson, A., and Kingsford, R. (2002). Responses of waterbirds to flooding in an arid region of Australia and implications for conservation. *Biological Conservation,* **106**, 399-411.

Sabo, J.L., Sponseller, R., Dixon, M., Gade, K., Harms, T., Heffernan, J., Jani, A., Katz, G., Soykan, C., and Watts, J. (2005). Riparian zones increase regional species richness by harboring different, not more, species. *Ecology,* **86**, 56-62.

Sankey, J.B., Ralston, B.E., Grams, P.E., Schmidt, J.C., and Cagney, L.E. (2015). Riparian vegetation, Colorado River, and climate: Five decades of spatiotemporal dynamics in

the Grand Canyon with river regulation. *Journal of Geophysical Research: Biogeosciences,* **120**, 1532-1547.

Sauter, M. (2013). Root responses to flooding. *Current Opinion in Plant Biology,* **16**, 282-286.

Schleuning, M., Becker, T., Vadillo, G.P., Hahn, T., Matthies, D., and Durka, W. (2011). River dynamics shape clonal diversity and genetic structure of an Amazonian understorey herb. *Journal of Ecology,* **99**, 373-382.

Scown, M.W., Thoms, M.C., and De Jager, N.R. (2015). Floodplain complexity and surface metrics: Influences of scale and geomorphology. *Geomorphology,* **245**, 102-116.

Sculthorpe, C.D. (1967). 'Biology of aquatic vascular plants.' (Edward Arnold: London.)

Seabloom, E.W., van der Valk, A.G., and Moloney, K.A. (1998). The role of water depth and soil temperature in determining initial composition of prairie wetland coenoclines. *Plant Ecology,* **138**, 203-216.

Seddon, J., and Briggs, S. (1998). Lakes and lakebed cropping in the Western Division of New South Wales. *The Rangeland Journal,* **20**, 237-254.

Selwood, K.E., Thomson, J.R., Clarke, R.H., McGeoch, M.A., and Mac Nally, R. (2015). Resistance and resilience of terrestrial birds in drying climates: do floodplains provide drought refugia? *Global ecology and biogeography,* **24**, 838-848.

SEWPaC (2011). Environmental Water Delivery: Lachlan River. Prepared for Commonwealth Environmental Water, Department of Sustainability, Environment, Water, Population and Communities. Commonwealth Environmental Water Holder for the Australian Government.)

Shilpakar, R.L., and Thoms, M. (2009). The character and behaviour of floodplain vegetation landscapes. *Hydrological Sciences,* **20**, 42-52.

Slatkin, M. (1977). Gene flow and genetic drift in a species subject to frequent local extinctions. *Theoretical population biology,* **12**, 253-262.

Southwood, T. (1988). Tactics, strategies and templets. *Oikos*, **52**, 3-18.

Southwood, T.R. (1977). Habitat, the templet for ecological strategies? *Journal of animal ecology,* **46**, 337-365.

Sparks, R.E. (1995). Need for ecosystem management of large rivers and their floodplains. *BioScience,* **45**, 168-182.

Stanford, J.A., and Ward, J. (1993). An ecosystem perspective of alluvial rivers: connectivity and the hyporheic corridor. *Journal of the North American Benthological Society,* **12**, 48-60.

Stanford, J.A., Ward, J., Liss, W.J., Frissell, C.A., Williams, R.N., Lichatowich, J.A., and Coutant, C.C. (1996). A general protocol for restoration of regulated rivers. *Regulated Rivers: Research & Management,* **12**, 391-413.

Stevens, L.E., Schmidt, J.C., Ayers, T.J., and Brown, B.T. (1995). Flow regulation, geomorphology, and Colorado River marsh development in the Grand Canyon, Arizona. *Ecological Applications,* **5**, 1025-1039.

Stow, A., Sunnucks, P., Briscoe, D., and Gardner, M. (2001). The impact of habitat fragmentation on dispersal of Cunningham's skink (*Egernia cunninghami*): evidence from allelic and genotypic analyses of microsatellites. *Molecular Ecology,* **10**, 867-878.

Striker, G.G. (2012). 'Flooding stress on plants: anatomical, morphological and physiological responses.' (InTech: https://www.intechopen.com/books/botany/flooding-stress-on-plants-anatomical-morphological-and-physiological-responses.)

Templeton, A.R., and Levin, D.A. (1979). Evolutionary consequences of seed pools. *The American Naturalist,* **114**, 232-249.

Templeton, A.R., Shaw, K., Routman, E., and Davis, S.K. (1990). The genetic consequences of habitat fragmentation. *Annals of the Missouri Botanical Garden*, **77**, 13-27.

Tero, N., Aspi, J., Siikamäki, P., Jäkäläniemi, A., and Tuomi, J. (2003). Genetic structure and gene flow in a metapopulation of an endangered plant species, *Silene tatarica*. *Molecular Ecology,* **12**, 2073-2085.

Thapa, R., Thoms, M., and Parsons, M. (2016). An adaptive cycle hypothesis of semi-arid floodplain vegetation productivity in dry and wet resource states. *Ecohydrology,* **9**, 39-51.

Thomas, R., Lu, Y., Cox, S., and Hunter, S. (2012). Inundation response project, executive summary, NSW Rivers Environmental Restoration Program Subprogram II. Office of Environment and Heritage NSW, Sydney.)

Thoms, M., and Sheldon, F. (2000). Water resource development and hydrological change in a large dryland river: the Barwon–Darling River, Australia. *Journal of Hydrology,* **228**, 10-21.

Thoms, M.C. (2003). Floodplain–river ecosystems: lateral connections and the implications of human interference. *Geomorphology,* **56**, 335-349.

Tilman, D., Fargione, J., Wolff, B., D'Antonio, C., Dobson, A., Howarth, R., Schindler, D., Schlesinger, W.H., Simberloff, D., and Swackhamer, D. (2001). Forecasting agriculturally driven global environmental change. *Science,* **292**, 281-284.

Tockner, K., Pennetzdorfer, D., Reiner, N., Schiemer, F., and Ward, J. (1999). Hydrological connectivity, and the exchange of organic matter and nutrients in a dynamic river–floodplain system (Danube, Austria). *Freshwater Biology,* **41**, 521-535.

Tockner, K., and Stanford, J.A. (2002). Riverine flood plains: present state and future trends. *Environmental Conservation,* **29**, 308-330.

Townsend, P.A., and Walsh, S.J. (1998). Modeling floodplain inundation using an integrated GIS with radar and optical remote sensing. *Geomorphology,* **21**, 295-312.

Van der Valk, A., and Davis, C. (1976). Changes in the composition, structure, and production of plant communities along a perturbed wetland coenocline. *Vegetatio,* **32**, 87-96.

Van der Valk, A. (1981). Succession in wetlands: a Gleasonian approach. *Ecology*, **62**, 688-696.

van Dijk, A.I., Beck, H.E., Crosbie, R.S., de Jeu, R.A., Liu, Y.Y., Podger, G.M., Timbal, B., and Viney, N.R. (2013). The Millennium Drought in southeast Australia (2001–

2009): Natural and human causes and implications for water resources, ecosystems, economy, and society. *Water Resources Research,* **49**, 1040-1057.

Van Looy, K., Tonkin, J.D., Floury, M., Leigh, C., Soininen, J., Larsen, S., Heino, J., LeRoy Poff, N., Delong, M., and Jähnig, S.C. (2018). The three Rs of river ecosystem resilience: Resources, recruitment, and refugia. *River Research and Applications*

Voesenek, L., Colmer, T., Pierik, R., Millenaar, F., and Peeters, A. (2006). How plants cope with complete submergence. *New Phytologist,* **170**, 213-226.

Vörösmarty, C.J., McIntyre, P.B., Gessner, M.O., Dudgeon, D., Prusevich, A., Green, P., Glidden, S., Bunn, S.E., Sullivan, C.A., and Liermann, C.R. (2010). Global threats to human water security and river biodiversity. *Nature,* **467**, 555.

Walker, K. (1992). 22: The River Murray, Australia: A Semiarid Lowland River. *The Rivers Handbook, Blackwell Scientific Publications, Oxford*

Walker, K.F., Sheldon, F., and Puckridge, J.T. (1995). A perspective on dryland river ecosystems. *Regulated Rivers: Research & Management,* **11**, 85-104.

Walker, K.F., Puckridge, J., and Blanch, S.J. (1997). Irrigation development on Cooper Creek, central Australia Ð prospects for a regulated economy in a boom-and-bust ecology. *Aquatic Conservation: Marine and Freshwater Ecosystems,* **7**, 63-73.

Ward, D., Shrestha, M.K., and Golan-Goldhirsh, A. (2011). Evolution and ecology meet molecular genetics: adaptive phenotypic plasticity in two isolated Negev desert populations of *Acacia raddiana* at either end of a rainfall gradient. *Annals of Botany,* **109**, 247-255.

Ward, J. (1989). The four-dimensional nature of lotic ecosystems. *Journal of the North American Benthological Society,* **8**, 2-8.

Ward, J., Tockner, K., and Schiemer, F. (1999). Biodiversity of floodplain river ecosystems: ecotones and connectivity. *Regulated Rivers: Research & Management,* **15**, 125-139.

Ward, J.V., and Stanford, J. (1995). Ecological connectivity in alluvial river ecosystems and its disruption by flow regulation. *Regulated Rivers: Research & Management,* **11**, 105-119.

Wassens, S., Hall, A., Osborne, W., and Watts, R.J. (2010). Habitat characteristics predict occupancy patterns of the endangered amphibian Litoria raniformis in flow-regulated flood plain wetlands. *Austral Ecology,* **35**, 944-955.

Wei, X., Meng, H., and Jiang, M. (2013). Landscape genetic structure of a streamside tree species *Euptelea pleiospermum* (Eupteleaceae): contrasting roles of river valley and mountain ridge. *PloS one,* **8**, e66928.

Weir, B.S., and Cockerham, C.C. (1984). Estimating F-statistics for the analysis of population structure. *Evolution,* **38**, 1358-1370.

Welcomme, R.L. (1979). 'Fisheries ecology of floodplain rivers.' (Longman: London.)

Welling, C.H., Pederson, R.L., and Van der Valk, A.G. (1988). Recruitment from the seed bank and the development of zonation of emergent vegetation during a drawdown in a prairie wetland. *The Journal of Ecology*, **76**, 483-496.

Wen, L., Ling, J., Saintilan, N., and Rogers, K. (2009). An investigation of the hydrological requirements of River Red Gum (Eucalyptus camaldulensis) Forest, using Classification and Regression Tree modelling. *Ecohydrology,* **2**, 143-155.

Wilcock, P.R., Kondolf, G.M., Matthews, W., and Barta, A.F. (1996). Specification of Sediment Maintenance Flows for a Large Gravel-Bed River. *Water Resources Research,* **32**, 2911-2921.

Willi, Y., Fracassetti, M., Zoller, S., and Van Buskirk, J. (2018). Accumulation of mutational load at the edges of a species range. *Molecular Biology and Evolution,* **35**, 781-791.

Williams, L., Reich, P., Capon, S.J., and Raulings, E. (2008). Soil seed banks of degraded riparian zones in southeastern Australia and their potential contribution to the restoration of understorey vegetation. *River Research and Applications,* **24**, 1002-1017.

Willson, M.F., and Traveset, A. (2000). The ecology of seed dispersal. In Seeds: the ecology of regeneration in plant communities. (Ed. M. Fenner) pp. 85-110. (CABI Publishing: University of Southhampton, UK.)

Wilson, G.G. (2009). Managing environmental flows in an agricultural landscape: the Lower Gwydir floodplain. Final report to the Australian Government Department of Environment, Water, Heritage and the Arts. University of New England and Cotton Catchment Communities Cooperative Research Centre, Armidale, NSW.)

Wright, S. (1943). Isolation by distance. *Genetics,* **28**, 114.

Yan, J., Zhu, M., Liu, W., Xu, Q., Zhu, C., Li, J., and Sang, T. (2016). Genetic variation and bidirectional gene flow in the riparian plant *Miscanthus lutarioriparius*, across its endemic range: implications for adaptive potential. *Gcb Bioenergy,* **8**, 764-776.

Young, A., Boyle, T., and Brown, T. (1996). The population genetic consequences of habitat fragmentation for plants. *Trends in Ecology & Evolution,* **11**, 413-418.

Young, A.G., Clarke, G.M., and Cowlishaw, G. (2000). 'Genetics, demography and viability of fragmented populations.' (Cambridge University Press: Cambridge, UK.)

Young, W.J. (2001). 'Rivers as ecological systems: the Murray-Darling Basin.' (CSIRO: Canberra.)

Zhu, Z., and Woodcock, C.E. (2012). Object-based cloud and cloud shadow detection in Landsat imagery. *Remote Sensing of Environment,* **118**, 83-94.

9. Appendices

Appendix A Floodplain-river connection

Table A 1 Connection metrics for all 19 floodplain wetland sites used in this study, in the lower Lachlan Catchment under 'without development' (W) and current (C) flow conditions for the Lachlan River, and the change in the number of connections (*N*), change in median frequency (*D*), and Change in median dry spell (*T*) for all 19 sites. All connect duration and dry spell duration metrics are in number of days.

Sites	Number of connections		Connection duration (mean)		Connection duration (median)		Connection duration (max)		Dry spell (mean)		Dry spell (median)		Dry spell (max)		Flood timing (median month)		Change		
	W	C	W	C	W	C	W	C	W	C	W	C	W	C	W	C	*N*	*D*	*T*
Temporary floodplain lakes																			
Whealbah Billabong X338172, Y6274467	251	160	30.6	32.4	19	19	279	265	143.1	239.5	44	41.5	1453	3589	Sept.	Sept.	0.64	1.00	0.94
Booligal Swamp X307255, Y6261297	259	171	26.0	24.8	15	11	276	257	142.3	229.7	41	56	1457	3598	Sept.	Oct.	0.66	0.73	1.37
Lake Ita X249792, Y6207216	641	231	23.9	30.7	5	9	330	279	44.2	157.9	17	33	617	1764	Oct.	Oct.	0.36	1.80	1.94
Lake Marool X226857, Y6205171	574	198	24.7	33.7	6	12	313	276	51.4	186.3	13	27	648	1766	Oct.	Oct.	0.34	2.00	2.08
Clear Lake X229650, Y6202274	651	245	24.8	30.5	6	8	332	283	42.3	147.4	16	26	509	1764	Oct.	Oct.	0.38	1.33	1.63
Lignum Lake X224289, Y6207707	504	177	24.5	34.0	6	12	295	268	62.2	212.0	15	58	654	1768	Oct.	Oct.	0.35	2.00	3.87
Intermittent river red gum swamps																			
Moon Moon Swamp X325858, Y6263409	460	179	23.3	34.0	8	18	288	270	71.6	209.2	12.5	54	663	1776	Sept.	Sept.	0.39	2.25	4.32
Whealbah Billabong X337918, Y6274914	202	137	28.8	29.8	18	16	272	257	186.8	287.3	40	145	1473	3607	Sept.	Sept.	0.68	0.89	3.63

Site																	N	D	T
Clear Lake X229604, Y6202303	176	91	38.3	40.7	20	17	273	247	209.0	435.1	53	249	1748	3635	Oct.	Oct.	0.52	0.87	4.70
Hazelwood X358200, Y6290031	315	209	21.3	23.7	13	13	218	263	117.2	184.7	28	32	1455	1819	Sept.	Sept.	0.66	1.00	1.14
The Ville X271453, Y6212184	482	175	24.4	33.0	6	12	293	266	66.2	215.7	16	58	655	1768	Oct.	Oct.	0.36	2.00	3.63
Intermittent black box swamps																			
Hunthawang X358200, Y6290031	49	41	21.0	24.6	16	16	91	144	854.2	1017.4	395	366	4154	6816	Aug.	Sept.	0.84	1.00	0.93
Hazelwood X358200, Y6290031	76	36	19.8	28.0	13	18	143	143	548.5	1154.8	128	208	4043	6816	Nov.	Sept.	0.47	1.40	1.62
Lake Ita X249792, Y6207216	128	67	30.1	33.4	19	16	255	238	309.2	610.3	101	307	3632	4348	Oct.	Oct.	0.52	0.84	3.05
Booligal Swamp X307195, Y6261295	200	140	18.4	15.5	9	3	210	207	199.3	294.8	62	200	1783	3738	Sept.	Oct.	0.70	0.33	3.23
Terminal wetlands																			
Lake Bullogal X270817, Y6224432	64	36	52.00	51.0	30	37	241	221	621.7	1132.5	203	320	3638	6017	Oct.	Oct.	0.56	1.24	1.58
Lake Tarwong X242787, Y6243706	48	27	43.2	54.4	25	39	238	219	850.3	1509.6	362	1082	3654	6027	Oct.	Sept.	0.56	1.59	2.99
Willandra @ Kilfera X230204, Y6333062	36	26	34.4	46.6	18	39	179	218	1148.6	1575.2	380	562	5836	6042	Sept.	Sept.	0.72	2.17	1.48
Middle Creek @ Cobb HW X286377, Y6297602	57	37	33.0	46.7	20	32	185	229	721.6	1105.5	402	657	3644	5832	Sept.	Oct.	0.65	1.60	1.63
Total	272	125	28.6	34.1	14	18	248	239	336.3	573.9	123	236	2196	3815			0.46	1.28	1.93
Temporary floodplain lakes	480	197	25.8	31.0	10	12	304	271	80.9	195.5	24	40	889	2375			0.41	1.25	1.65
Intermittent river red gum swamps	327	158	27.2	32.3	13	15	269	261	130.2	266.4	30	108	1199	2521			0.48	1.18	3.60
Intermittent black box swamps	113	71	22.3	25.4	14	13	175	183	477.8	769.3	171	270	3403	5430			0.63	0.93	1.58
Terminal wetlands	51	32	40.7	49.7	23	37	211	222	835.6	1330.7	337	655	4193	5980			0.61	1.59	1.95

N Change in the number of connections;

D Change in median duration of connections;

T Change in median dry spell
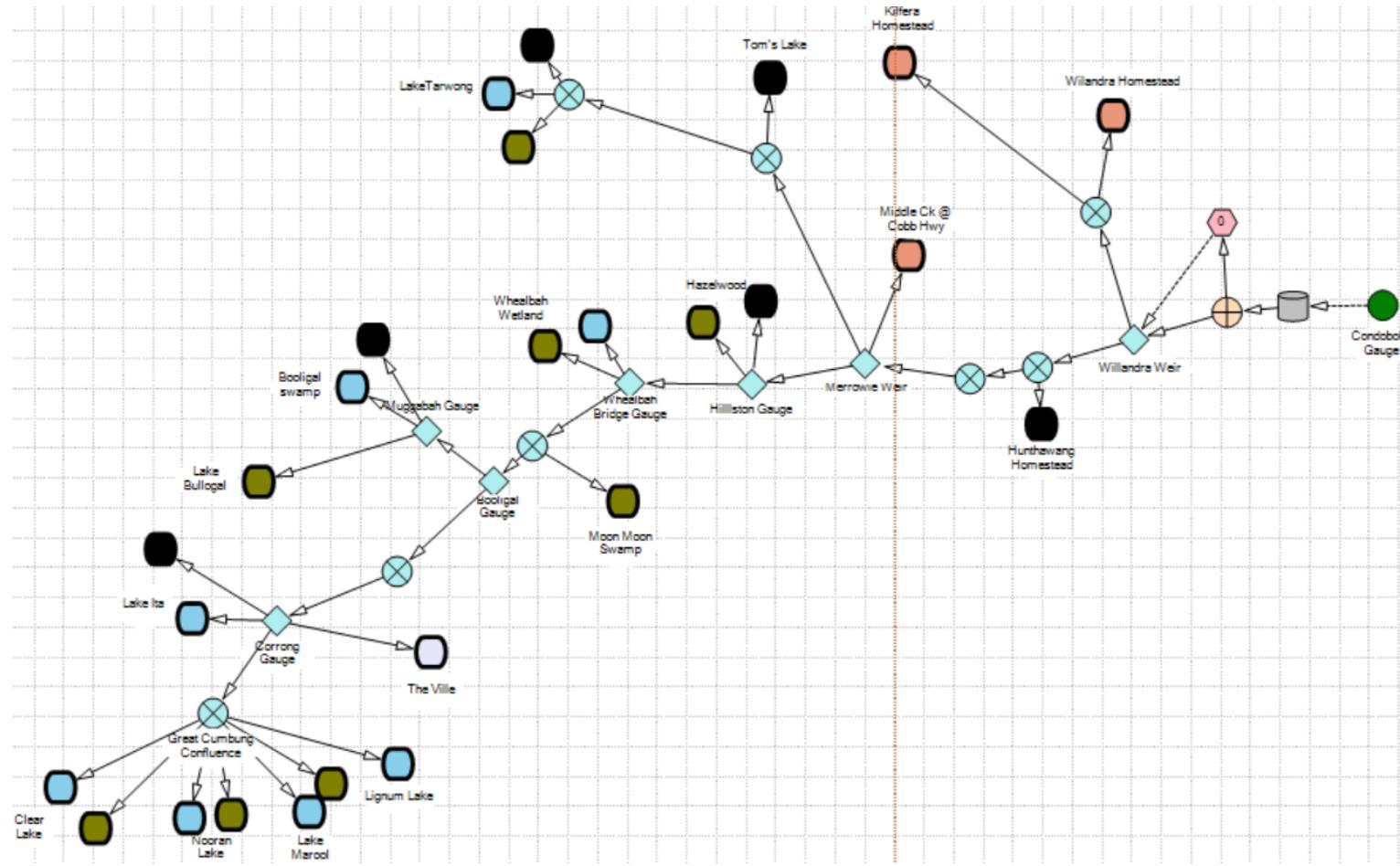
Figure A 1 Diagrammatic model used in the water modelling program Model Manager to calculate connection metrics. Blue shapes represent temporary floodplain lakes, green shapes represent intermittent river red gum swamps, black shapes represent intermittent black box swamps, and red shapes represent lignum shrublands.

Appendix B Seed bank study
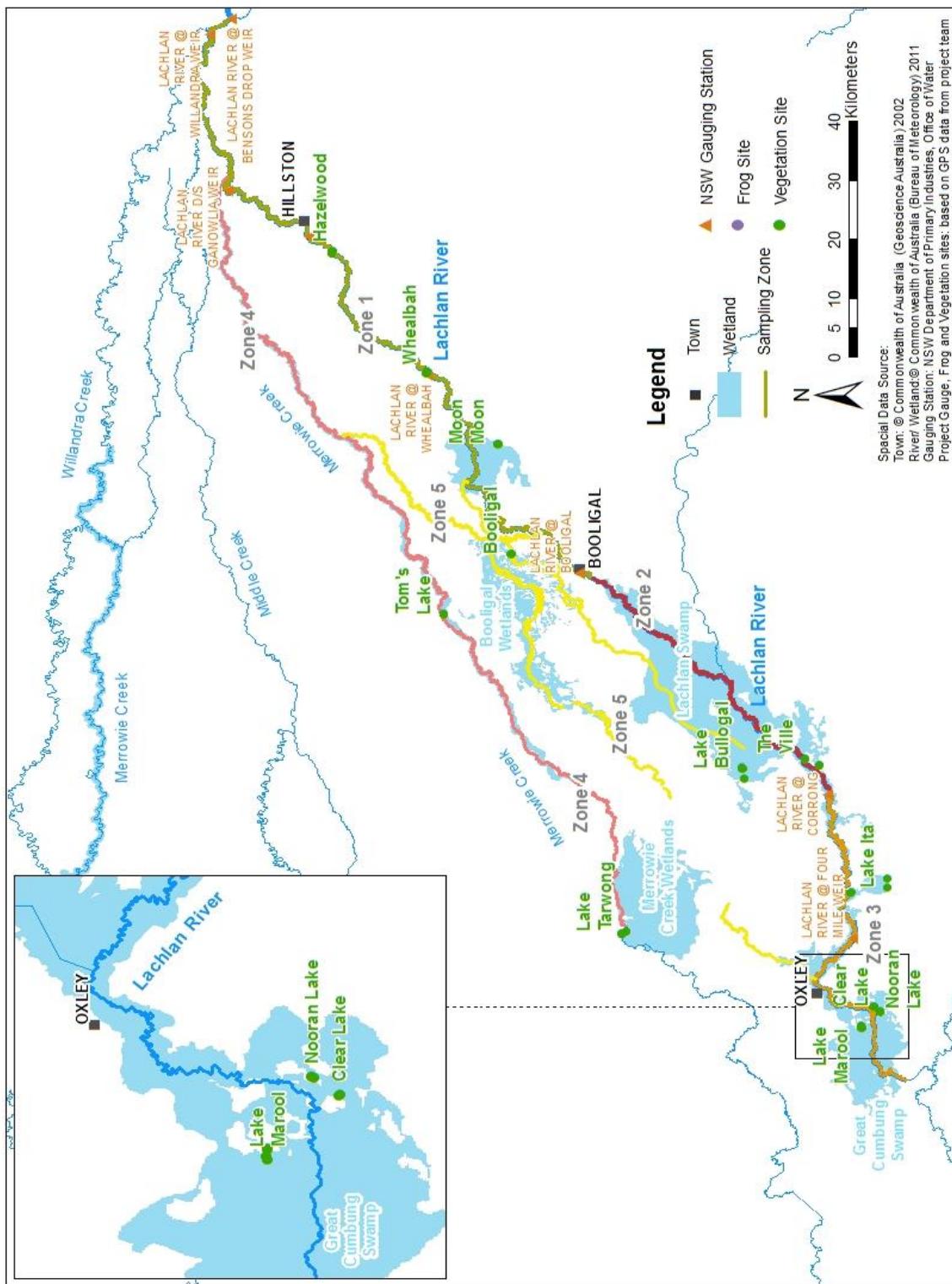
Figure B 1 The lower Lachlan River system, showing the vegetation monitoring sites from which vegetation data were used.

Table B 1 Likelihood of a species germinating during the seedling emergence experiment, within each of the experimental treatments, based on the total number of individuals of each species that germinated within each treatment.

Species	Rainfall	Waterlogged	Receding	Submerged
Alopecurus geniculatus	0.000	0.038	0.000	0.000
Alternanthera denticulata	0.000	0.038	0.038	0.000
Ammannia multiflora	0.000	0.000	0.077	0.000
Atriplex semibaccata	0.077	0.077	0.000	0.000
Atriplex suberecta	0.038	0.000	0.000	0.000
Brachyscome paludicola	0.000	0.038	0.000	0.000
Bulbine semibarbarta	0.000	0.038	0.000	0.000
Callitriche sonderi	0.038	0.154	0.000	0.000
Capsella bursa pastoris	0.077	0.038	0.000	0.000
Carrichtera annua	0.038	0.115	0.000	0.000
Centaurium tenuiflorum	0.000	0.038	0.000	0.000
Centipeda cunninghamii	0.077	0.615	0.346	0.000
Chara spp	0.000	0.000	0.038	0.423
Cirsium vulgare	0.038	0.000	0.000	0.000
Conyza sumatrensis	0.000	0.115	0.000	0.000
Crassula decumbens	0.154	0.154	0.000	0.000
Cyperus difformis	0.000	0.192	0.346	0.000
Cyperus sanguinolentus	0.000	0.038	0.038	0.000
Damasonium minus	0.000	0.192	0.231	0.038
Dysphania pumilio	0.038	0.154	0.038	0.000
Eclipta platyglossa	0.000	0.192	0.077	0.000
Einadia nutans	0.154	0.154	0.000	0.000
Elatine gratioloides	0.000	0.115	0.115	0.269
Eleocharis acuta	0.000	0.038	0.115	0.000
Epilobium billardierianum subsp Hydrophilum	0.000	0.115	0.154	0.000
Eucalyptus camaldulensis	0.000	0.077	0.000	0.000
Eucalyptus largiflorens	0.000	0.038	0.000	0.000
Euchiton sphaericus	0.000	0.038	0.000	0.000
Euphorbia drummondii	0.000	0.038	0.000	0.000
Fumaria capreolata	0.000	0.038	0.000	0.000
Galium murale	0.000	0.077	0.000	0.000
Glinus lotoides	0.000	0.154	0.038	0.000
Gnaphalium polycaulon	0.000	0.038	0.000	0.000
Goodenia heteromera	0.000	0.000	0.077	0.000
Heliotropium europaeum	0.000	0.000	0.038	0.000
Hordeum leporinum	0.038	0.000	0.000	0.000
Isolepis australiensis	0.038	0.154	0.077	0.000
Juncus aridicola	0.000	0.038	0.000	0.000

Juncus articulatus	0.000	0.115	0.154	0.000
Juncus flavidus	0.000	0.154	0.038	0.000
Lachnagrostis filiformis	0.038	0.154	0.077	0.000
Lemna disperma	0.000	0.000	0.038	0.038
Limosella australis	0.038	0.192	0.231	0.000
Lolium rigidum	0.038	0.000	0.000	0.000
Lythrum hyssopifolia	0.000	0.308	0.192	0.000
Marsilea drummondii	0.000	0.000	0.000	0.077
Medicago polymorpha	0.308	0.038	0.038	0.000
Melilotus indicus	0.038	0.038	0.000	0.000
Mentha australis	0.000	0.077	0.038	0.000
Mimulus gracilis	0.000	0.000	0.038	0.038
Myosurus australis	0.115	0.231	0.038	0.000
Myriophyllum crispatum	0.000	0.385	0.154	0.000
Myriophyllum variifolium	0.000	0.000	0.000	0.038
Myriophyllum veruccosum	0.000	0.077	0.423	0.654
Nitella furcata	0.000	0.000	0.000	0.346
Oxalis corniculata	0.000	0.154	0.000	0.000
Persicaria decipiens	0.000	0.038	0.000	0.000
Persicaria prostrata	0.000	0.038	0.000	0.000
plantago gaudichaudii	0.000	0.038	0.000	0.000
Poa fordeana	0.038	0.000	0.000	0.000
Polygonum plebeium	0.115	0.308	0.231	0.000
Potamogeton tricarinatus	0.000	0.000	0.038	0.154
Pseudognaphalium luteoalbum	0.038	0.231	0.000	0.000
Ranunculus pumilio var pumilio	0.077	0.154	0.077	0.000
Rhodanthe moschata	0.038	0.038	0.000	0.000
Rorippa eustylis	0.038	0.077	0.000	0.000
Rorippa laciniata	0.000	0.269	0.000	0.000
Rumex brownii	0.000	0.038	0.000	0.000
Schismus barbatus	0.038	0.000	0.000	0.000
Scleroblitum atriplicinum	0.077	0.077	0.000	0.000
Senecio cunninghamii	0.000	0.038	0.000	0.000
Senecio runcinifolius	0.154	0.115	0.000	0.000
Sisymbrium erysimoides	0.462	0.346	0.000	0.000
Spergularia rubra	0.000	0.077	0.038	0.000
Stellaria media	0.038	0.000	0.000	0.000
Stemodia florulenta	0.000	0.038	0.077	0.000
Tetragonia eremaea	0.269	0.077	0.038	0.000
Triglochin procera	0.000	0.000	0.077	0.000
Urochloa panicoides	0.000	0.038	0.000	0.000
Verbena gaudichaudii	0.000	0.192	0.077	0.000
Veronica peregrina	0.000	0.077	0.000	0.000
Wahlenbergia gracilis	0.077	0.077	0.000	0.000
Zygophyllum apiculatum	0.077	0.000	0.000	0.000

Table B 2 Likelihood of a species germinating during the seedling emergence experiment, within each of the three floodplain habitat types, based on the total number of individuals that germinated within each habitat type.

Species	Intermittent black box swamps	Intermittent river red gum swamps	Temporary floodplain wetlands
Alopecurus geniculatus	0.000	0.023	0.000
Alternanthera denticulata	0.000	0.045	0.000
Ammannia multiflora	0.042	0.023	0.000
Atriplex semibaccata	0.000	0.068	0.028
Atriplex suberecta	0.000	0.000	0.028
Brachyscome paludicola	0.042	0.000	0.000
Bulbine semibarbata	0.000	0.023	0.000
Callitriche sonderi	0.042	0.023	0.083
Capsella bursa pastoris	0.042	0.045	0.000
Carrichtera annua	0.042	0.045	0.028
Centaurium tenuiflorum	0.000	0.023	0.000
Centipeda cunninghamii	0.292	0.227	0.278
Chara spp	0.083	0.159	0.083
Cirsium vulgare	0.000	0.000	0.028
Conyza sumatrensis	0.042	0.045	0.000
Crassula decumbens	0.167	0.091	0.000
Cyperus difformis	0.250	0.159	0.028
Cyperus sanguinolentus	0.000	0.045	0.000
Damasonium minus	0.208	0.159	0.000
Dysphania pumilio	0.042	0.091	0.028
Eclipta platyglossa	0.042	0.114	0.028
Einadia nutans	0.125	0.091	0.028
Elatine gratioloides	0.208	0.136	0.056
Eleocharis acuta	0.042	0.068	0.000
Epilobium billardierianum sub sp. Hydrophilum	0.000	0.114	0.056
Eucalyptus camaldulensis	0.000	0.045	0.000
Eucalyptus largiflorens	0.042	0.000	0.000
Euchiton sphaericus	0.000	0.023	0.000
Euphorbia drummondii	0.000	0.000	0.028
Fumaria capreolata	0.000	0.023	0.000
Galium murale	0.000	0.045	0.000
Glinus lotoides	0.000	0.000	0.139
Gnaphalium polycaulon	0.000	0.000	0.028
Goodenia heteromera	0.042	0.023	0.000
Heliotropium europaeum	0.000	0.000	0.028
Hordeum leporinum	0.000	0.023	0.000

Isolepis australiensis	0.125	0.091	0.000
Juncus aridicola	0.000	0.023	0.000
Juncus articulatus	0.000	0.136	0.028
Juncus flavidus	0.042	0.091	0.000
Lachnagrostis filiformis	0.083	0.114	0.000
Lemna disperma	0.000	0.045	0.000
Limosella australis	0.250	0.068	0.083
Lolium rigidum	0.000	0.023	0.000
Lythrum hyssopifolia	0.042	0.159	0.139
Marsilea drummondii	0.000	0.023	0.028
Medicago polymorpha	0.083	0.114	0.083
Melilotus indicus	0.000	0.000	0.056
Mentha australis	0.125	0.000	0.000
Mimulus gracilis	0.083	0.000	0.000
Myosurus australis	0.083	0.114	0.083
Myriophyllum crispatum	0.083	0.182	0.111
Myriophyllum variifolium	0.000	0.023	0.000
Myriophyllum veruccosum	0.292	0.227	0.361
Nitella furcata	0.083	0.023	0.167
Oxalis corniculata	0.000	0.045	0.056
Persicaria decipiens	0.000	0.023	0.000
Persicaria prostrata	0.000	0.000	0.028
plantago gaudichaudii	0.042	0.000	0.000
Poa fordeana	0.042	0.000	0.000
Polygonum plebeium	0.167	0.068	0.278
Potamogeton tricarinatus	0.042	0.091	0.000
Pseudognaphalium luteoalbum	0.125	0.045	0.056
Ranunculus pumilio var pumilio	0.083	0.136	0.000
Rhodanthe moschata	0.042	0.023	0.000
Rorippa eustylis	0.083	0.000	0.028
Rorippa laciniata	0.083	0.023	0.111
Rumex brownii	0.042	0.000	0.000
Schismus barbatus	0.000	0.023	0.000
Scleroblitum atriplicinum	0.042	0.023	0.056
Senecio cunninghamii	0.000	0.000	0.028
Senecio runcinifolius	0.083	0.023	0.111
Sisymbrium erysimoides	0.250	0.295	0.056
Spergularia rubra	0.042	0.045	0.000
Stellaria media	0.042	0.000	0.000
Stemodia florulenta	0.000	0.045	0.028
Tetragonia eremaea	0.167	0.091	0.056
Triglochin procera	0.000	0.023	0.028
Urochloa panicoides	0.000	0.023	0.000
Verbena gaudichaudii	0.125	0.045	0.056

Veronica peregrina	0.000	0.045	0.000
Wahlenbergia gracilis	0.042	0.068	0.000
Zygophyllum apiculatum	0.042	0.000	0.028

Table B 3 Species occurrence (presence/absence) observed during field surveys (in three hydrological conditions) and during the soil seed bank germination experiment (within the four experimental treatments).

Species	Extant - Dry	Extant - Partially Wet	Extant - Wet	Seedbank Waterlogged	Seedbank Rainfall	Seedbank Receding	Seedbank Submerged
Abutilon theophrasti	1	1	0	0	0	0	0
Acacia stenophylla	1	1	0	0	0	0	0
Alopecurus geniculatus	1	0	0	1	0	0	0
Alternanthera denticulata	1	1	1	1	0	1	0
Alternanthera nodiflora	1	0	0	0	0	0	0
Ammannia multiflora	0	0	0	0	0	1	0
Arabidella nasturtium	1	0	0	0	0	0	0
Asperula gemella	1	0	0	0	0	0	0
Aster subulatus	1	0	0	0	0	0	0
Atriplex leptocarpa	1	0	0	0	0	0	0
Atriplex lindleyi	1	0	0	0	0	0	0
Atriplex nummularia	1	0	0	0	0	0	0
Atriplex semibaccata	1	0	0	1	1	0	0
Atriplex suberecta	1	0	0	0	1	0	0
Atriplex vesicaria	1	0	0	0	0	0	0
Azolla filiculoides	1	1	1	0	0	0	0
Bergia trimera	1	0	0	0	0	0	0
Boerhavia dominii	1	1	0	0	0	0	0
Bolboschoenus fluviatilis	1	1	0	0	0	0	0
Brachyscome basaltica var. gracilis	1	1	0	1	0	0	0
Brachyscome goniocarpa	1	0	0	0	0	0	0
Brassica tournefortii	0	1	0	0	0	0	0
Bulbine bulbosa	0	0	1	0	0	0	0
Bulbine semibarbarta	0	0	0	1	0	0	0

Callitriche sonderi	0	1	0	1	1	0	0
Calotis scabiosifolia	1	0	0	0	0	0	0
Calotis scapigera	0	1	0	0	0	0	0
Capsella bursa pastoris	1	0	0	1	1	0	0
Cardamine hirsuta	1	0	0	0	0	0	0
Carrichtera annua	1	0	1	1	1	0	0
Centaurea melitensis	0	0	1	0	0	0	0
Centaurium tenuiflorum	0	0	0	1	0	0	0
Centipeda cunninghamii	1	1	1	1	1	1	0
Chara spp.	0	0	0	0	0	1	1
Chenopodium album	1	0	0	0	0	0	0
Chenopodium melanocarpum	1	0	0	0	0	0	0
Chenopodium murale	1	0	0	0	0	0	0
Chenopodium nitrariaceum	1	1	0	0	0	0	0
Cirsium vulgare	1	1	1	0	1	0	0
Conyza bonariensis	1	0	1	0	0	0	0
Conyza sumatrensis	1	1	0	1	0	0	0
Coronidium rutidolepis	1	0	0	0	0	0	0
Crassula decumbens	0	0	0	1	1	0	0
Cucumis myriocarpus	1	0	0	0	0	0	0
Cynodon dactylon	1	0	1	0	0	0	0
Cyperus difformis	1	1	1	1	0	1	0
Cyperus gymnocaulos	1	1	0	0	0	0	0
Cyperus sanguinolentus	0	0	0	1	0	1	0
Damasonium minus	0	0	0	1	0	1	1
Duma florulenta	1	1	0	0	0	0	0
Dysphania pumilio	1	1	1	1	1	1	0
Echium plantagineum	1	1	1	0	0	0	0
Eclipta platyglossa	1	1	0	1	0	1	0
Einadia nutans subsp. linifolia	1	1	0	0	0	0	0

Einadia nutans var. nutans	1	1	0	1	1	0	0
Elatine gratioloides	0	0	0	1	0	1	1
Eleocharis acuta	1	1	1	1	0	1	0
Eleocharis plana	0	0	1	0	0	0	0
Eleocharis pusilla	1	0	0	0	0	0	0
Enchylaena tomentosa	1	1	0	0	0	0	0
Epaltes australis	0	0	1	0	0	0	0
Epilobium billardierianum subsp. *Hydrophilum*	0	0	0	1	0	1	0
Eragrostis australasica	1	0	0	0	0	0	0
Erodium malacoides	1	0	0	0	0	0	0
Eucalyptus camaldulensis	1	1	1	1	0	0	0
Eucalyptus largiflorens	1	1	1	1	0	0	0
Euchiton sphaericus	1	0	0	1	0	0	0
Euphorbia drummondii	1	1	0	1	0	0	0
Euphorbia planiticola	0	1	0	0	0	0	0
Fumaria capreolata	1	0	0	1	0	0	0
Galium gaudichaudii	1	0	0	0	0	0	0
Galium murale	0	0	0	1	0	0	0
Geococcus pusillus	1	0	0	0	0	0	0
Geranium solanderi	1	0	0	0	0	0	0
Glinus lotoides	1	1	0	1	0	1	0
Gnaphalium polycaulon	0	0	0	1	0	0	0
Goodenia cycloptera	1	0	1	0	0	0	0
Goodenia heteromera	0	0	0	0	0	1	0
Haloragis glauca f. glauca	1	1	1	0	0	0	0
Heliotropium curassavicum	1	0	1	0	0	0	0
Heliotropium europaeum	1	1	1	0	0	1	0
Hordeum leporinum	1	1	1	0	1	0	0
Hydrocotyle trachycarpa	1	0	0	0	0	0	0

Isolepis australiensis	0	1	1	1	1	1	0
Juncus aridicola	0	0	0	1	0	0	0
Juncus articulatus	0	0	0	1	0	1	0
Juncus flavidus	1	0	0	1	0	1	0
Lachnagrostis filiformis	1	0	1	1	1	1	0
Lactuca saligna	1	0	1	0	0	0	0
Lactuca serriola	1	0	0	0	0	0	0
Lemna minor	0	1	1	0	0	1	1
Lepidium fasciculatum	1	0	0	0	0	0	0
Limosella australis	0	0	1	1	1	1	0
Lolium rigidum	1	1	0	0	1	0	0
Lycium ferocissimum	1	0	0	0	0	0	0
Lythrum hyssopifolia	1	1	1	1	0	1	0
Maireana brevifolia	1	0	0	0	0	0	0
Maireana decalvans	1	0	0	0	0	0	0
Malva parviflora	1	0	1	0	0	0	0
Malva preissiana	1	0	0	0	0	0	0
Marrubium vulgare	1	0	0	0	0	0	0
Marsilea drummondii	1	1	1	0	0	0	1
Medicago polymorpha	1	1	1	1	1	1	0
Medicago praecox	1	1	0	0	0	0	0
Melilotus indicus	1	0	0	1	1	0	0
Mentha australis	1	1	1	1	0	1	0
Mimulus gracilis	0	0	0	0	0	1	1
Modiola caroliniana	1	0	0	0	0	0	0
Myosurus australis	1	0	0	1	1	1	0
Myriophyllum crispatum	0	0	0	1	0	1	0
Myriophyllum variifolium	0	0	0	0	0	0	1
Myriophyllum verrucosum	1	0	1	1	0	1	1
Nitella furcata	0	0	0	0	0	0	1

Nitraria billardierei	1	0	0	0	0	0	0
Onopordum acanthium	1	0	0	0	0	0	0
Oxalis corniculata	1	1	1	1	0	0	0
Paspalidium jubiflorum	1	1	0	0	0	0	0
Paspalum distichum	1	1	0	0	0	0	0
Persicaria decipiens	1	0	0	1	0	0	0
Persicaria lapathifolia	1	0	1	0	0	0	0
Persicaria prostrata	1	0	0	1	0	0	0
Phalaris minor	0	0	1	0	0	0	0
Phalaris paradoxa	1	0	0	0	0	0	0
Phyla nodiflora	1	1	0	0	0	0	0
Phyllanthus lacunarius	0	0	1	0	0	0	0
plantago gaudichaudii	0	0	0	1	0	0	0
Poa fordeana	1	0	0	0	1	0	0
Polygonum aviculare	1	1	1	0	0	0	0
Polygonum plebeium	1	0	1	1	1	1	0
Polypogon monspeliensis	1	0	1	0	0	0	0
Potamogeton tricarinatus	0	0	0	0	0	1	1
Pratia concolor	1	1	1	0	0	0	0
Pseudognaphalium luteoalbum	0	1	1	1	1	0	0
Pseudoraphis spinescens	1	0	0	0	0	0	0
Radyera farragei	1	0	0	0	0	0	0
Ranunculus pumilio	0	1	0	1	1	1	0
Ranunculus undosus	1	1	0	0	0	0	0
Raphanus raphanistrum	0	1	0	0	0	0	0
Rapistrum rugosum	1	0	0	0	0	0	0
Rhagodia spinescens	1	0	0	0	0	0	0
Rhodanthe corymbiflora	1	0	0	0	0	0	0
Rhodanthe moschata	0	0	0	1	1	0	0
Rorippa eustylis	1	1	1	1	1	0	0

Species							
Rorippa laciniata	0	0	0	1	0	0	0
Rorippa palustris	0	0	1	0	0	0	0
Rumex brownii	0	0	0	1	0	0	0
Rumex crystallinus	1	0	0	0	0	0	0
Rumex tenax	1	0	0	0	0	0	0
Salsola kali var. kali	1	0	0	0	0	0	0
Schenkia australis	0	0	1	0	0	0	0
Schismus barbatus	1	0	0	0	1	0	0
Scleroblitum atriplicinum	1	0	0	1	1	0	0
Sclerolaena birchii	1	0	0	0	0	0	0
Sclerolaena brachyptera	1	0	0	0	0	0	0
Sclerolaena muricata	1	1	0	0	0	0	0
Sclerolaena parviflora	1	0	0	0	0	0	0
Sclerolaena tricuspis	1	0	0	0	0	0	0
Senecio cunninghamii var. cunninghamii	1	0	1	1	0	0	0
Senecio runcinifolius	1	1	1	1	1	0	0
Sida corrugata	1	0	0	0	0	0	0
Sida fibulifera	1	0	0	0	0	0	0
Sisymbrium erysimoides	1	1	1	1	1	0	0
Sisymbrium irio	1	0	0	0	0	0	0
Solanum esuriale	1	1	0	0	0	0	0
Solanum nigrum	1	0	0	0	0	0	0
Sonchus oleraceus	1	1	0	0	0	0	0
Spergularia rubra	0	0	0	1	0	1	0
Stellaria media	1	0	0	0	1	0	0
Stemodia florulenta	1	0	1	1	0	1	0
Tetragonia eremaea	1	1	0	1	1	1	0
Triglochin dubia	0	1	0	0	0	1	0
Urochloa panicoides	0	0	0	1	0	0	0

Urtica urens	1	0	0	0	0	0	0
Verbena gaudichaudii	0	0	0	1	0	1	0
Verbena officinalis	1	1	1	0	0	0	0
Verbena supina	1	0	0	0	0	0	0
Veronica peregrina	0	0	0	1	0	0	0
Vulpia bromoides	1	0	0	0	0	0	0
Wahlenbergia gracilis	0	0	0	1	1	0	0
Xanthium occidentale	1	1	0	0	0	0	0
Xanthium spinosum	1	1	0	0	0	0	0
Zygophyllum apiculatum	1	1	0	0	1	0	0

Table B 4 Species list from the soil seed bank germination experiment, native or introduced, their life cycle, family, and their water plant functional group (WPFG).

Species	Native/Introduced	Life cycle	Family	WPFG
Alopecurus geniculatus	Introduced	Perennial	Poaceae	Tda
Alternanthera denticulata	Native	Annual	Amaranthaceae	Tda
Ammannia multiflora	Native	Annual	Lythraceae	Tda
Atriplex semibaccata	Native	Perennial	Chenopodiaceae	Tdr
Atriplex suberecta	Native	Annual	Chenopodiaceae	Tdr
Brachyscome paludicola	Native	Perennial	Asteraceae	Tda
Bulbine semibarbata	Native	Annual	Asphodelaceae	Tda
Callitriche sonderi	Native	Annual	Callitrichaceae	NA
Capsella bursa-pastoris	Introduced	Annual	Brassicaceae	Tdr
Carrichtera annua	Introduced	Annual	Brassicaceae	Tdr
Centaurium tenuiflorum	Introduced	Annual	Gentianaceae	Tdr
Centipeda cunninghamii	Native	Perennial	Asteraceae	Tda
Chara spp.	Native	annual/ perennial	Characeae	Sr
Cirsium vulgare	Introduced	Biennial	Asteraceae	Tdr
Conyza sumatrensis	Introduced	Annual	Asteraceae	Tdr
Crassula decumbens	Native	annual	Crassulaceae	Tda
Cyperus difformis	Native	annual	Cyperaceae	ATe
Cyperus sanguinolentus	Native	annual/ perennial	Cyperaceae	ATe
Damasonium minus	Native	annual/ perennial	Alismataceae	ARp
Dysphania pumilio	Native	annual	Chenopodiaceae	Tdr
Eclipta platyglossa	Native	Annual/biannual	Asteraceae	Tda
Einadia nutans	Naitve	Perennial	Chenopodiaceae	Tdr
Elatine gratioloides	Native	Annual	Elatinaceae	ARp
Eleocharis acuta	Naitve	Perennial	Cyperaceae	ATe

Epilobium billardierianum subsp. Hydrophilum	Native	annual/ perennial	Onagraceae	
Eucalyptus camaldulensis	Native	Perennial	Myrtaceae	ATw
Eucalyptus largiflorens	Native	Perennial	Myrtaceae	Tda
Euchiton sphaericus	Native	annual	Asteraceae	Tdr
Euphorbia drummondii	Native	Perennial	Euphorbiaceae	Tdr
Fumaria capreolata	Introduced	Annual	Papaveraceae	NA
Galium murale	Introduced	Annual	Rubiaceae	Tdr
Glinus lotoides	Native	Annual	Molluginaceae	Tdr
Gnaphalium polycaulon	Introduced	Annual	Asteraceae	Tdr
Goodenia heteromera	Native	annual/perennial	Goodeniaceae	Tdr
Heliotropium europaeum	Introduced	Annual	Boraginaceae	Tdr
Hordeum leporinum	Introduced	Annual	Poaceae	Tdr
Isolepis australiensis	Native	Annual	Cyperaceae	NA
Juncus aridicola	Native	Perennial	Juncaceae	ATe
Juncus articulatus	Introduced	Perennial	Juncaceae	ATe
Juncus flavidus	Native	Perennial	Juncaceae	ATe
Lachnagrostis filiformis	Native	annual/ perennial	Poaceae	Tda
Lemna disperma	Native	Herb	Araceae	ARf
Limosella australis	Native	Perennial	Scrophulariaceae	ATl
Lolium rigidum	Introduced	Annual	Poaceae	Tdr
Lythrum hyssopifolia	Native	Annual	Lythraceae	Tda
Marsilea drummondii	Native	Perennial	Marsileaceae	ARf
Medicago polymorpha	Introduced	Annual	Fabaceae	Tdr
Melilotus indicus	Introduced	Annual	Fabaceae	Tdr
Mentha australis	Native	Perennial	Lamiaceae	Tda
Mimulus gracilis	Native	annual/ perennial	Phrymaceae	Tda
Myosurus australis	Native	Annual	Ranunculaceae	Tda
Myriophyllum crispatum	Native	Perennial	Haloragaceae	ARp
Myriophyllum variifolium	Native	Perennial	Haloragaceae	ARp

Myriophyllum veruccosum	Native	Perennial	Haloragaceae	ARp
Nitella furcata	Native		Characeae	Sr
Oxalis corniculata	Introduced	Perennial	Oxalidaceae	Tdr
Persicaria decipiens	Native	Annual	Polygonaceae	ATe
Persicaria prostrata	Native	Perennial	Polygonaceae	Tda
plantago gaudichaudii	Native	Perennial	Plantaginaceae	Tdr
Poa fordeana	Native	Perennial	Poaceae	Se
Polygonum plebeium	Native	Annual	Plantaginaceae	Tda
Potamogeton tricarinatus	Native	Perennial	Potamogetonaceae	ARp
Pseudognaphalium luteoalbum	Native	Annual	Asteraceae	Tdr
Ranunculus pumilio var. pumilio	Native	Annual	Ranunculaceae	ATe
Rhodanthe moschata	Native	Annual	Asteraceae	Tdr
Rorippa eustylis	Native	Annual	Brassicaceae	Tdr
Rorippa laciniata	Native	Perennial	Brassicaceae	Tdr
Rumex brownii	Native	Perennial	Polygonaceae	Tda
Schismus barbatus	Introduced	Annual	Poaceae	NA
Scleroblitum atriplicinum	Native	Annual	Chenopodiaceae	Tdr
Senecio cunninghamii	Native	Perennial	Asteraceae	Tdr
Senecio runcinifolius	Native	Perennial	Asteraceae	Tdr
Sisymbrium erysimoides	Introduced	Annual	Brassicaceae	NA
Spergularia rubra	Introduced	Annual/biannual	Caryophyllaceae	Tdr
Stellaria media	Introduced	Annual	Caryophyllaceae	Tdr
Stemodia florulenta	Native	Perennial	Plantaginaceae	Tda
Tetragonia eremaea	Native	Annual/biannual	Aizoaceae	Tdr
Triglochin procera	Native	Perennial	Juncaginaceae	Se
Urochloa panicoides	Introduced	Annual	Poaceae	Tdr
Verbena gaudichaudii	Native	Perennial	Verbenaceae	Tdr
Veronica peregrina	Introduced	Annual	Plantaginaceae	Tda
Wahlenbergia gracilis	Native	Perennial	Campanulaceae	Tda

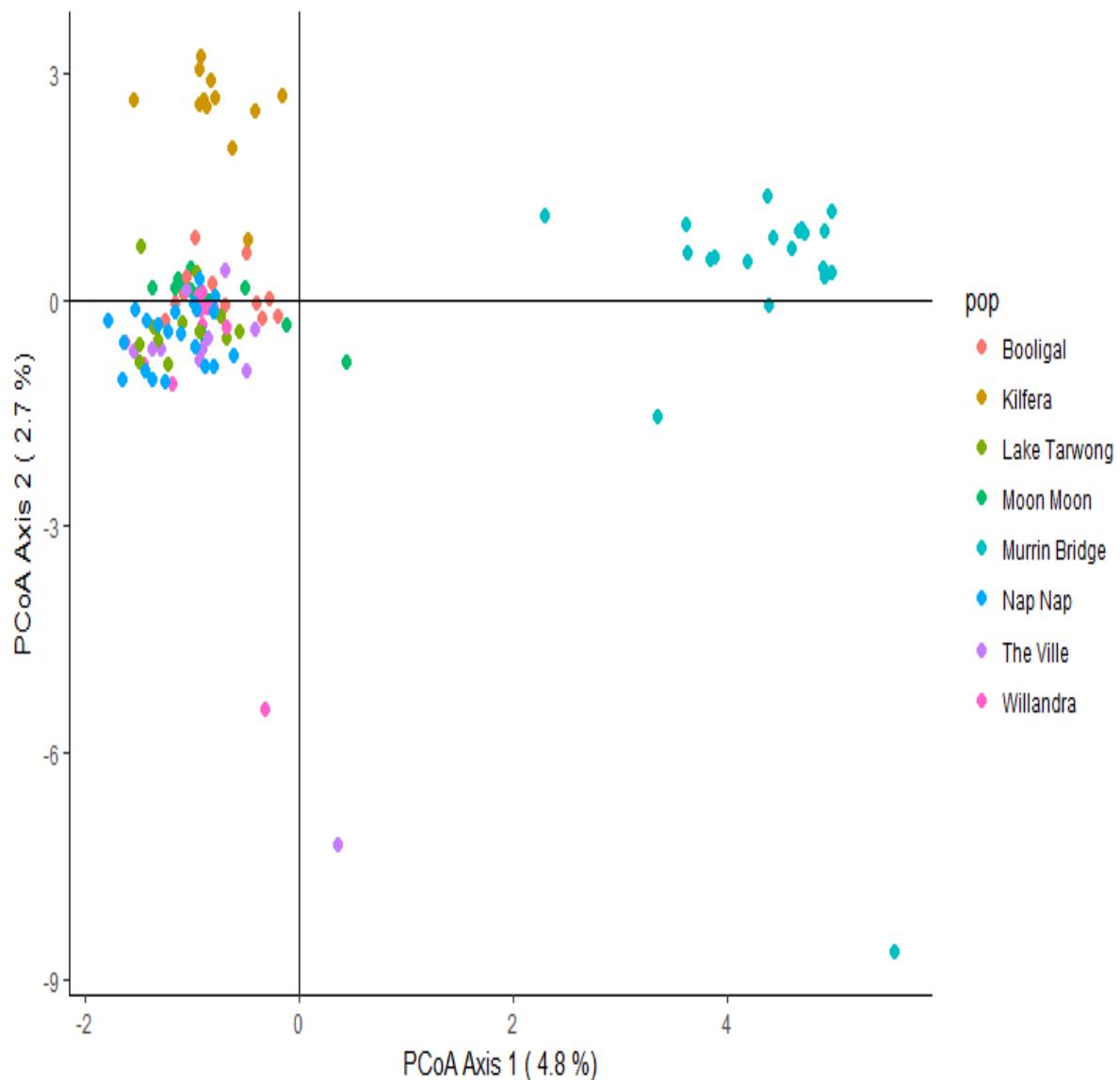| *Zygophyllum apiculatum* | Native | Annual | Zygophyllaceae | Tdr |

Appendix C Results of genetic analysis

Figure C 1 PCoA plot of 112 tangled lignum genotypes (with individuals from Middle Creek and Hunthawang removed) across eight sites in the mid and lower Lachlan River Catchment, and lower Murrumbidgee River Catchment.
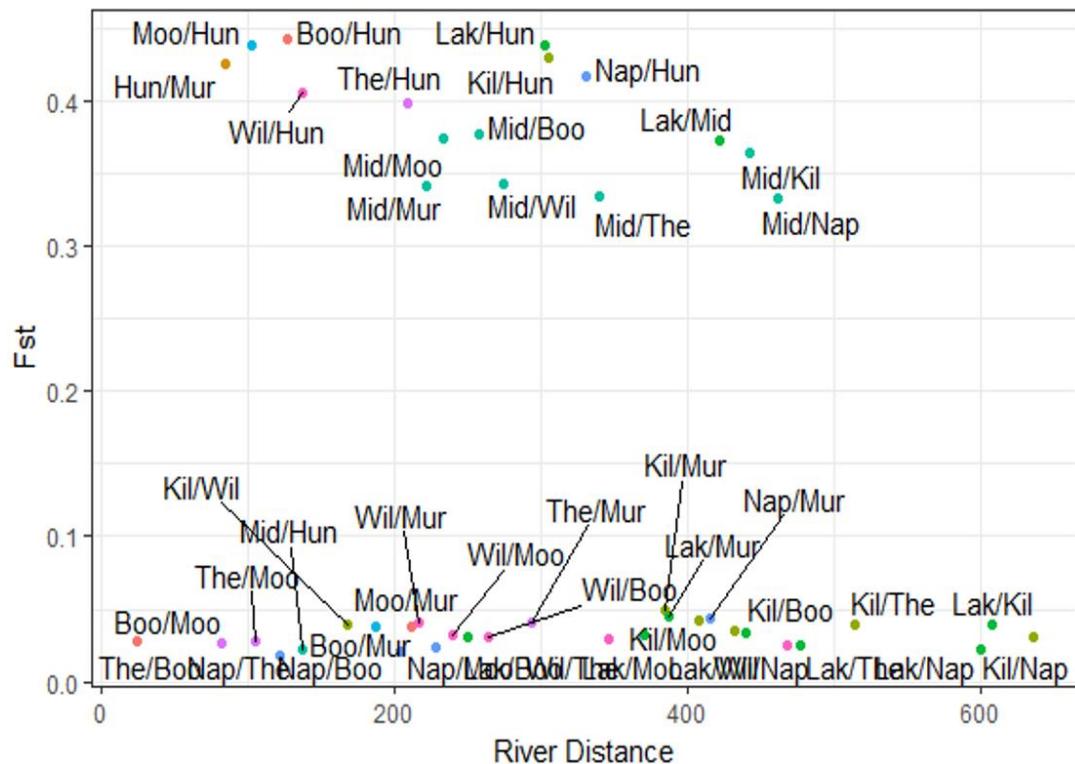
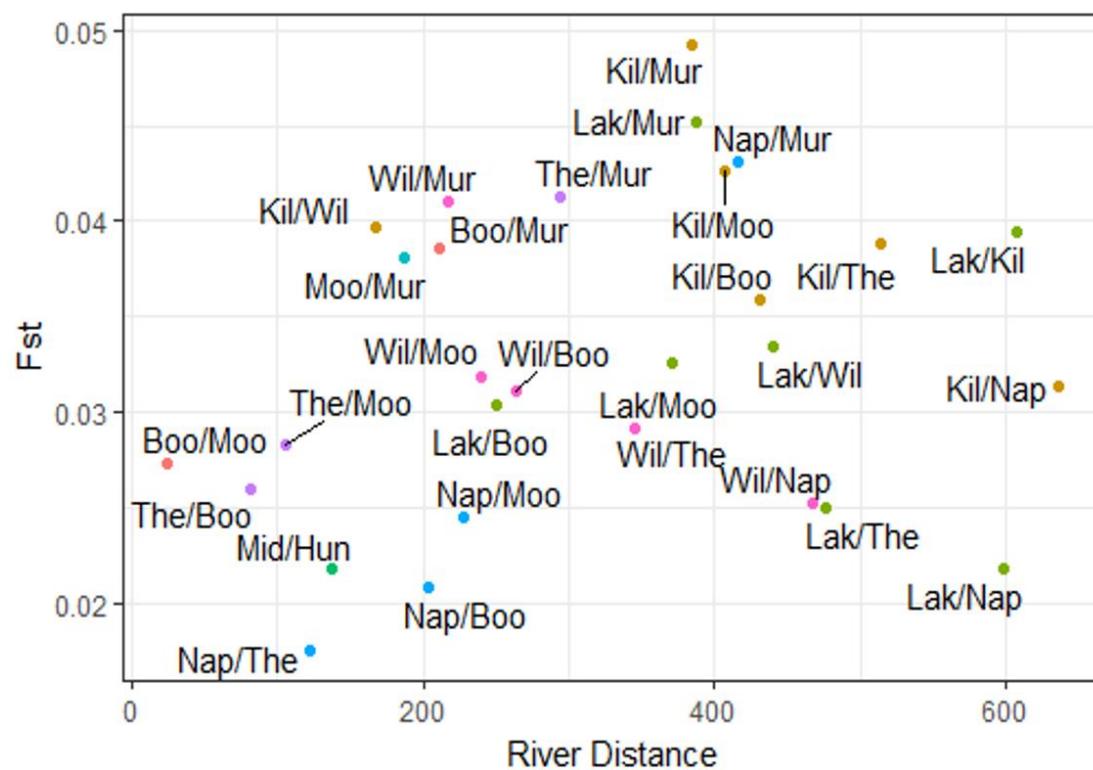**Figure C 2 pairwise F$_{ST}$ and river distance in tangled lignum between each site.**

Figure C 3 Pairwise F$_{ST}$ and river distance in tangled lignum between sites, filtered at F$_{ST}$ >0.1.
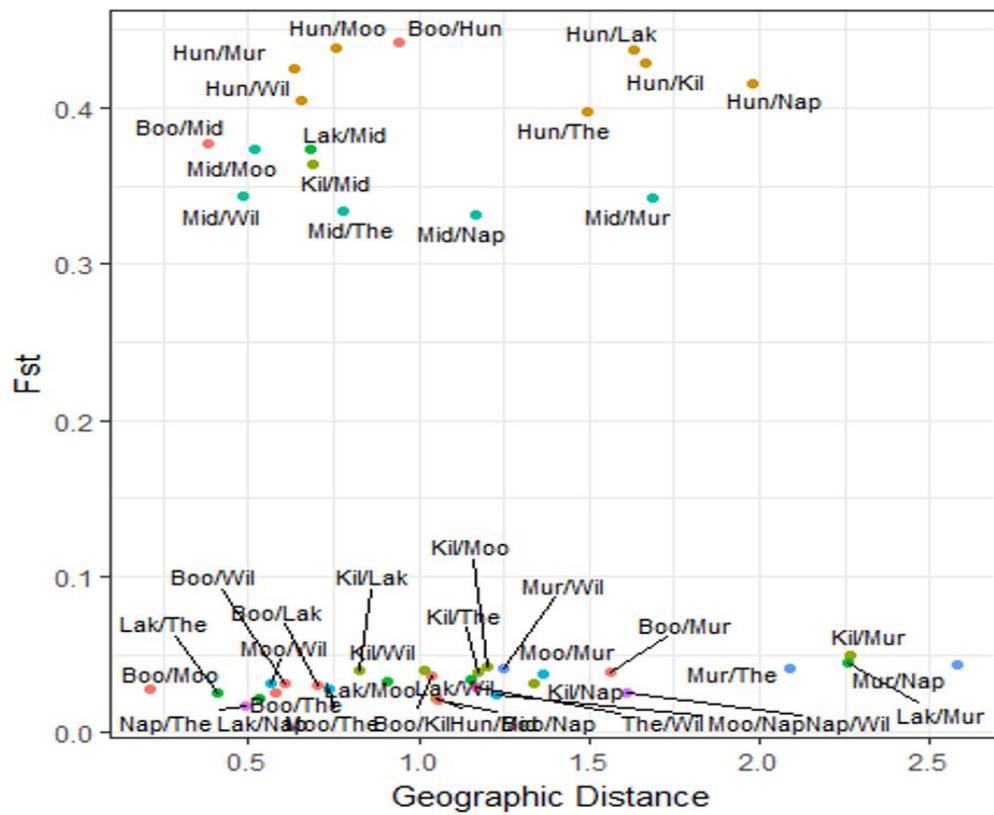
Figure C 4 Pairwise F$_{ST}$ and geographic distance (lat/long) in tangled lignum between each site.
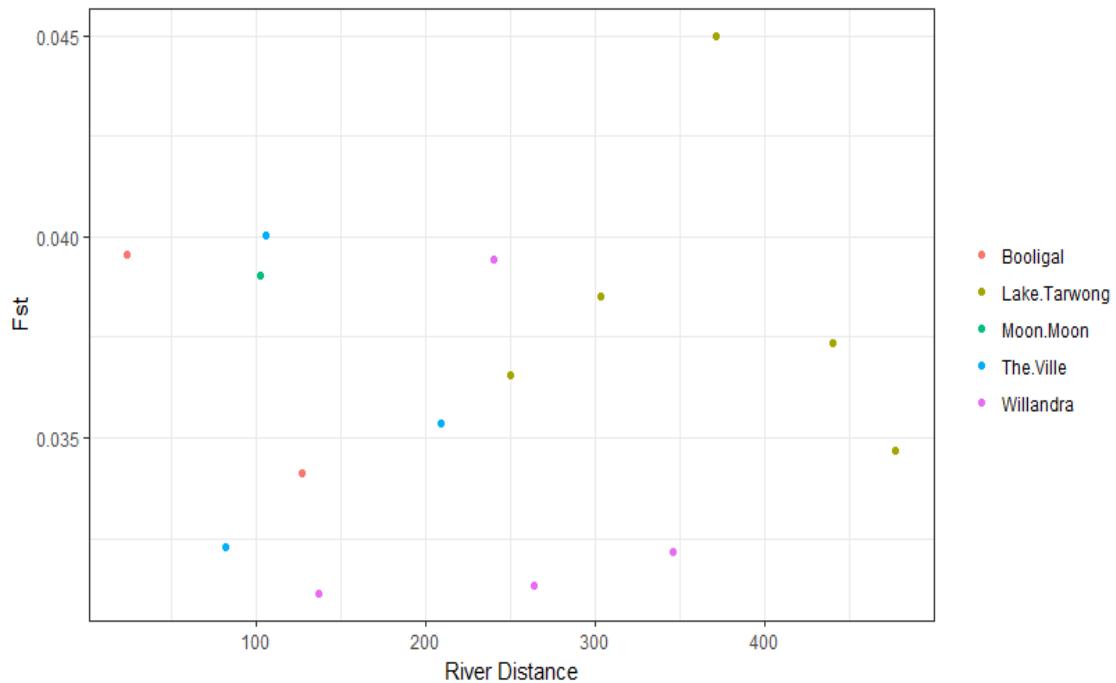
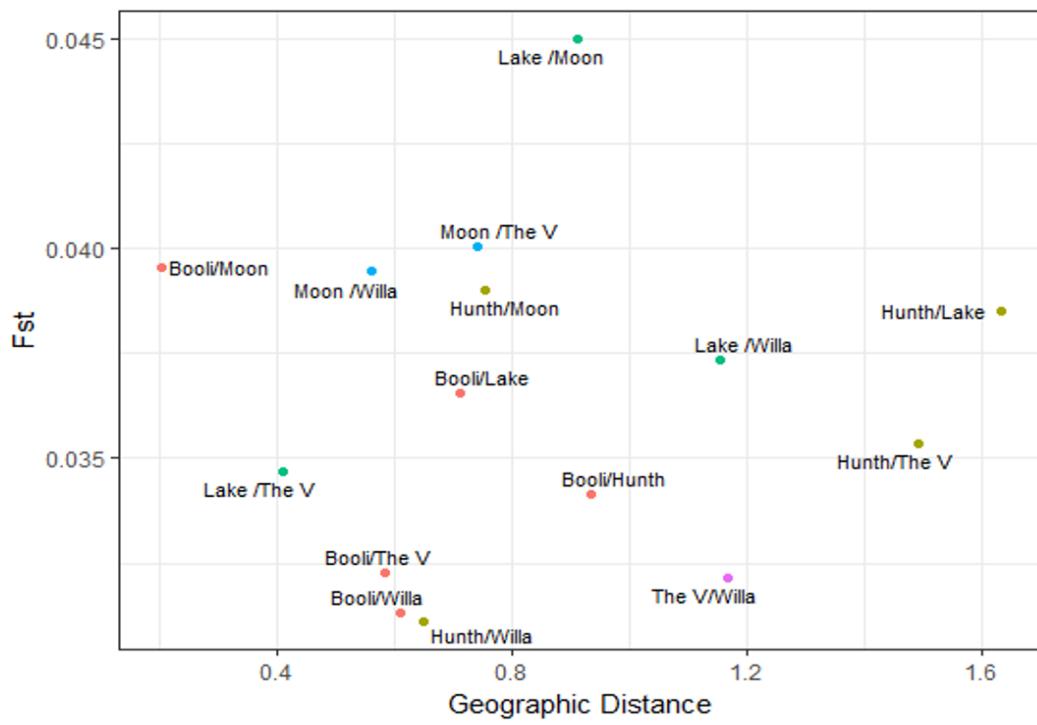**Figure C 5 Pairwise F$_{ST}$ and river distance (km) in river cooba between each site.**

221

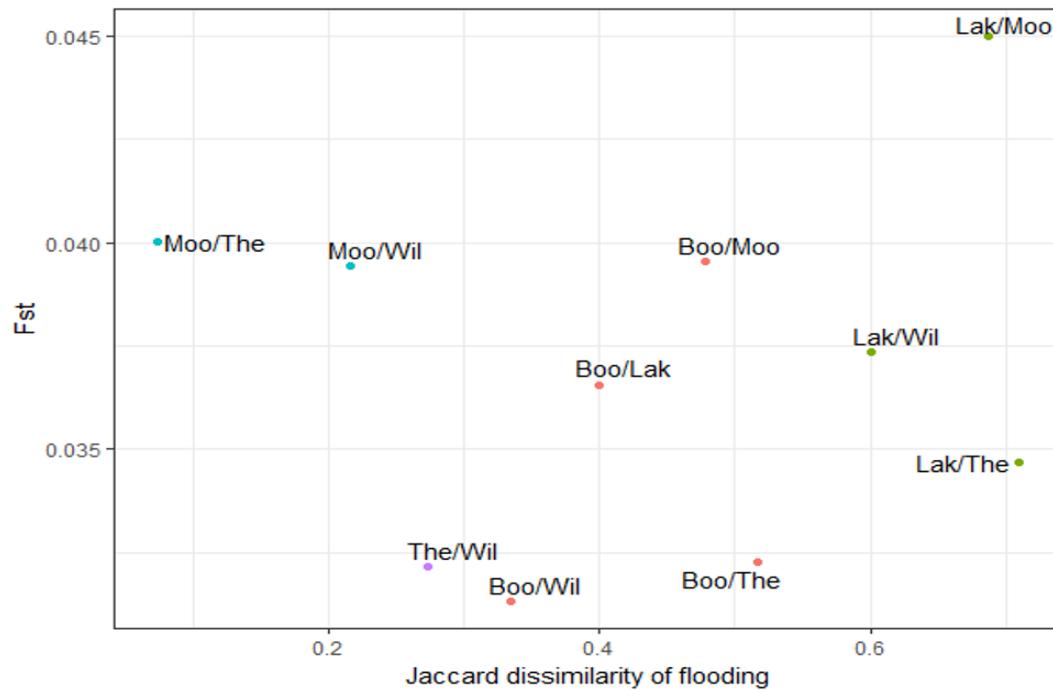**Figure C 6 Pairwise F$_{ST}$ and geographic distance (lat/long) in river cooba between each site.**

Figure C 7 Pairwise F$_{ST}$ and Jaccard dissimilarity of flooding for river cooba. Hunthawang was excluded, as some of the plants sampled were growing over the main channel of the Lachlan River, hence would not require flooding to disperse seeds via hydrochory.
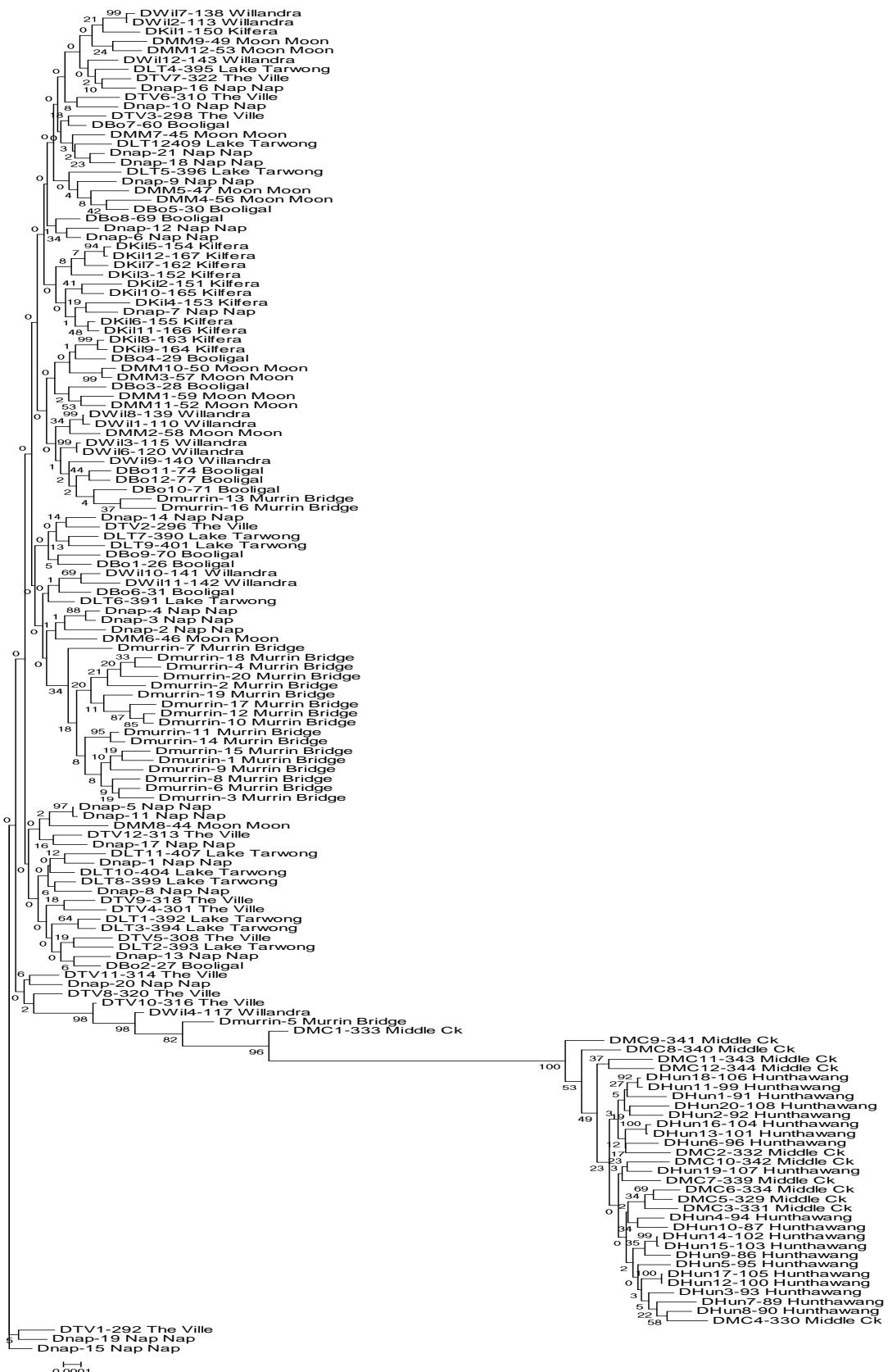
Figure C 8 Phylogenetic tree of (144) tangled lignum genotypes collected from 10 sites on the mid and lower Lachlan River and Murrumbidgee River.
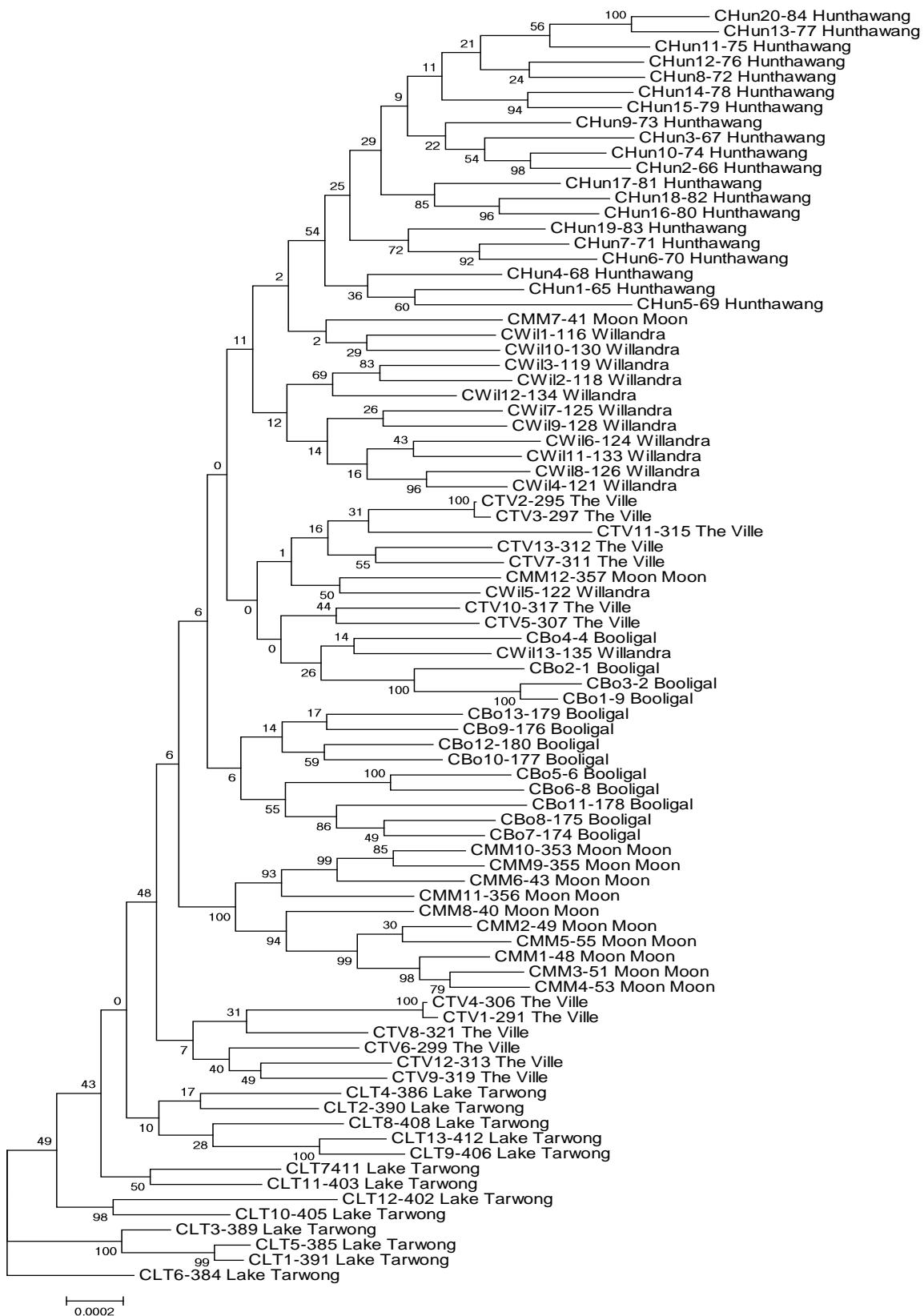
Figure C 9 Phylogenetic tree of (86) river cooba genotypes collected from six sites on the Lower Lachlan River.