



FINAL REPORT 2018

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Part 1 - Summary Details

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Part 3 – Final Report

(The points below are to be used as a guideline when completing your final report)

Background

River red gums are iconic in all cotton catchments and provide a number of valuable ecosystem services to growers, such as carbon sequestration, erosion mitigation and biodiversity conservation. There has been a decline in the health of these trees in recent decades, and it is likely that this decline is affecting the provision of ecosystem services. In addition, dead and dying trees contradict the environmentally conscious image the cotton industry aspires to. Cotton growers in the Namoi, Gwydir and Border Rivers catchments voiced their concern about the health of red gum trees and low recruitment rates and sought direction on how these factors could be addressed.

A literature review by Reid et al. (2007) provided some insight into the potential causes of river red gum dieback in the Namoi and Gwydir Valleys. The literature indicated water stress (declining groundwater levels, altered flood regimes and reduced groundwater recharge) was the most likely cause of river red gum dieback, but there were also links between grazing intensity and ecosystem health. Growers have pointed out a potential link between the abundance of lippia and ecosystem health in the Namoi Catchment. Climate change may also be implicated in river red gum dieback. With so many interacting variables operating simultaneously, there is still some ambiguity surrounding the causes of red gum dieback and low seedling recruitment, and it is unclear how growers can reverse the current trend of ecosystem decline.

Riverine ecosystems are the lifeblood of the cotton industry. They are also pivotal to the maintenance of much biodiversity in semi-arid catchments, being especially species rich and having high complementarity values (i.e. species that are not found in other parts of the landscape) compared to other vegetation types. Given the concentration of cotton production on major inland river systems, the industry has a duty of care to maintain the health of these ecosystems and protect them for future generations. By demonstrating concern for iconic national symbols in its backyard and restoring healthy riparian ecosystems, the cotton industry can build on its reputation for proactive environmental stewardship and responsible environmental management. This will have flow-on benefits for growers and marketers hoping to supply cotton to environmentally conscious manufacturers and consumers.

The average Australian cotton farm occupies approximately nine kilometres of river frontage and riparian lands. While the area of cotton production in any catchment is small, cotton farmers collectively are responsible for the management of many thousands of kilometres of riparian lands that have disproportionate importance for biodiversity conservation and ecosystem function in the semi-arid and subhumid landscapes where cotton is grown. Management of riparian zones on cotton farms forms part of a growers' social license as the downstream impacts of a grower's actions

has implications for the health and vitality of downstream sites and the river itself, and therefore determines the value of the system for the wider community.

River red gums are a national icon and fringe most river systems across the cotton-growing extent in eastern Australia. They attain grand proportions during their life-span of up to 1000 years, and capture the hearts and minds of growers who proudly point out their favourite big old tree on their farm. In addition to their aesthetic and cultural heritage values, well-connected riparian ecosystems dominated by river red gums enhance biodiversity conservation by facilitating the movement of wildlife and plant propagules through agricultural landscapes, and provide a range of ecosystem services to cotton growers and the community. However, tree health has deteriorated in many cases to the point that trees and riparian ecosystems are no longer able to provide ecosystem services, and may in fact be providing a range of disservices. For example, dead and dying trees are net C sources to the atmosphere as they respire more than they sequester, and weedy riparian zones are a source of insect pests in cotton.

Riparian areas present a complex range of management dilemmas for growers. Weeds (e.g. lippia), livestock grazing and water management influence the health of river red gum trees and the ecosystems they form. Healthy river systems are the lifeblood of the industry, and cotton growers need good information and accurate knowledge to manage riparian ecosystems responsibly. While there are areas of concern, there are also many examples of healthy riparian areas on cotton farms, and these act as exemplars for other growers to aspire to.

Objectives

1. List the project objectives and the extent to which these have been achieved, with reference to the Milestones and Performance indicators.

This project had the following objectives:

1. *Initiate project, and*
2. *Research plan/project design finalised*

This project commenced on 1st July 2015. The project team held a planning meeting in the second week of July to develop a clear direction and plan for the research. Conversations were had following that meeting with a number of past and present researchers doing research in similar or related fields to determine research directions and approaches. Experts approached included Neil Pettit (tree water use) and Michael Renton (data analysis, UWA), David Norton (forest science, Canterbury Uni), Bryce Kelly (groundwater, UNSW), David Curtis (dieback in northern NSW, UNE), Chris Nadolny (dieback in northern NSW, OEH), David Carr (dieback in northern NSW, Stringybark Ecological), Mark Silburn and Andrew Biggs (water relations, QDNRM), Tania Doody (tree water use, CSIRO), Alec Downey (Plan water relations, ICT International), Gary Taylor (entomology, Uni of Adelaide), and Nick Schultz and Megan Good (dieback in Victoria and South Australia, Federation Uni).

Shortly after commencing the project, we were able to access satellite imagery (SPOT5), multiple dates of NDVI data to assess tree condition through time, as well as a range of GIS data through a licence agreement with the Northwest LLS. We also acquired historical groundwater and river water data as well as weather data from online databases, and some preliminary management data from landholders. We visited landholders in the Boggabri region who agreed to allow access to their farms for this project and obtained details from NWLLS on lease arrangements on several travelling stock route sites. We concentrated on the Boggabri-Narrabri-Wee Waa area as this region was one of the worst-affected in the northern MDB in terms of dieback.

Shortly after the project commenced, a paper was published in the Journal of Arid Environments (Fu and Burgher 2015) investigating the relationship between vegetation condition (using NDVI) and water availability (weather [rainfall and temperature], surface and ground water) in the Namoi catchment. This paper confirmed our initial impressions following data exploration, that water availability (and temperature as it relates to water availability) is a primary driver of vegetation

condition. As such, we moved on to investigate secondary drivers of vegetation condition in both field and glasshouse-based experiments (see objective 4).

3. Finalise publications on biodiversity on cotton farms from previous FRP

Two papers have been finalised using the bird survey dataset collected in 2014 on cotton farms across the region bound by Emerald in the north and Darlington Point in the south. The first paper (currently undergoing a second round of reviews), titled: *A baseline survey of birds in native vegetation on cotton farms in inland eastern Australia*, was submitted to Wildlife Research and describes the broad patterns of bird diversity across the industry. We recorded 185 bird species in remnant and planted native vegetation on cotton farms. Species richness of bird communities declined from north to south. Bird community composition was similar in the three southern zones (Southern NSW, Macquarie and Border Rivers [Namoi, Gwydir, McIntyre and Balonne]), differing somewhat in the north (Central Queensland). The most frequent species recorded were large (>60 g), readily detected landbirds common in agricultural districts, but 26 of the 53 extant species of conservation concern in the study region were also recorded, including 16 species of declining woodland bird. Bird composition, abundance, richness, diversity and composition differed among the nine native vegetation types, with maximal and minimal bird abundance and diversity metrics recorded in river red gum-dominated riparian vegetation and grassland, respectively. Each remnant vegetation community had a generally distinct bird assemblage, indicating that all vegetation types contribute to regional biodiversity in cotton-growing zones in inland eastern Australia. Appropriate on-farm management of all remnant and planted native vegetation will assist regional biodiversity conservation. The paper recommended that in order for the Australian cotton industry to meet its stated environmental responsibilities, growers should be encouraged to prioritise the conservation management of remnant, riparian and planted native vegetation on cotton farms and the monitoring of bird species as an indicator of regional biodiversity response.

A second paper, titled: *The stability of bird assemblages across time and the reliability of snapshot surveys*, investigates the utility of snapshot biodiversity surveys and the conclusions drawn from them. Maron *et al.* (2005) questioned the utility of snapshot surveys and conclusions drawn from them after conducting a repeat survey of 26 remnant buloke (*Casuarina luehmannii*) sites in western Victoria, 7 years after the first survey; they found 'the distribution of most species did not differ significantly from that expected if species had redistributed at random among sites'. We think this is the exception rather than the rule for Australian landbird communities for three general reasons: passerine species dominate Australian landbird communities and tend to remain faithful to a site once a breeding territory has been established following natal dispersal; most species are sedentary and migratory, not nomadic; most Australian passerines are long-lived, and so surveys conducted within decadal timeframes may sample the same individuals. We tested these competing hypotheses and repeated bird surveys at 56 sites in six vegetation types in the Namoi Valley, 7 years after the first survey (conducted during Rhiannon's PhD). Bird communities in our study exhibited fairly high levels of turnover between surveys, but many species were significantly more likely to be found at the same sites in the second period as the first, and Mantel tests demonstrated that assemblage composition at sites were much more similar than expected under a random null hypothesis. For the reasons noted, we believe these results should be the general expectation when monitoring landbird communities over extended timeframes. The significance of this study for the cotton industry is that sites of high conservation value in one survey period are expected to have a similarly high value in subsequent surveys, barring a significant change in land management practices or major disturbances. This paper has implications for design of monitoring programs for birds on cotton farms as part of the industry's sustainability reporting.

Additional papers will flow from this dataset as time permits. Currently we have a visiting student from Mexico working to determine the importance of different habitat feature for bird species and guilds. This work will be reported in a subsequent CRDC project and will inform current research by Queensland University of Technology into biodiversity monitoring on cotton farms.

4. Identify stressors related to river red gum dieback at sites

a. Water stress

Sap flow meters and psychrometers were purchased from ICT International in 2015, and deployed on trees in floodplain and riparian ecosystems near Boggabri soon after. However, we discovered water stress is difficult to confirm in a three year project in a tree species that is so adaptable and able to exploit various water sources as they become available. However, other studies have illustrated water stress, and the prevalence of branchfall and canopy thinning in river red gums across the study areas certainly confirms water stress at various times during their life. Water stress also makes trees more susceptible to other stresses such as insect attack, particularly as trees recover from water stress, putting on large amounts of nutrient-rich, soft, green leaf.

The boom–bust nature of Australian inland floodplain rivers means that river red gums have experienced periods of drought and floods during their evolutionary history of 2.5 to 5 million years (Crisp et al. 2004) and have developed a range of strategies to deal with the highly variable river flows characterising semi-arid floodplain environments. The distribution of river red gums in arid and semi-arid regions makes these trees highly dependent on groundwater and overland flows (i.e. flooding) to supplement low rainfall conditions (Beadle 1981). Groundwater is the preferred water source for river red gums as it is more reliable than surface water in terms of availability (Bacon et al. 1993; Thorburn and Walker 1994). However, studies on the Chowilla floodplain in South Australia found that river red gums are opportunistic users of water, and may be able to switch between water sources when alternate water sources (i.e. soil water after rainfall) are available (Mensforth et al. 1994).

River red gums have strong roots and high root-to-shoot ratios to allow them to penetrate the soil profile in search of suitable soil conditions (i.e. moist soil during droughts and well-aerated soil during floods) and persist in tight, heavy clay soils (Jacobs 1955). Multiple tap roots of river red gums can extend to a depth of more than 10 metres (Bacon et al. 1993; Davies 1953) and their surface root system can extend at least 20 metres radially from the tree (Dexter 1967). This extensive root system allows river red gums to forage for water and nutrients over a large area. During floods, river red gums can develop aerial (aerenchymatous or adventitious) roots to cope with short-term inundation (Heinrich 1990; Jacobs 1955; Thorburn and Walker 1994; West 2007). They also have very large water-conducting vessels, some of the largest in the world at 2 mm diameter, that allow them to move large amounts of water quickly, and take advantage of water when available. Large diameter water conducting vessels may be more susceptible to cavitation.

There is much speculation around how much water is required to sustain river red gum trees with different studies producing different results depending on tree density and starting condition of the trees. However, it is likely that large amounts of water are required, with studies showing that river red gums can transpire more than 2000 mm yr⁻¹ (Marshall et al. 1997; Thorburn et al. 1993). However, river red gums adjust their water use in response to heat and water availability (Heinrich 1990; Thorburn and Walker 1994). Studies have shown that river red gums with access to abundant or reliable water sources have lower water use efficiencies than plants relying on variable water sources (Thorburn and Walker 1994). That is, trees with more water available to them for even a short period of time will use that water to promote growth and reproduction, and increase canopy condition in readiness for times of water shortage. Periods of extreme or sustained water shortage promote leaf shedding and branch fall to reduce canopy water use (Roberts and Marston 2000). Canopy density may be restored through the production of epicormic growth once more favourable conditions return. In marginal environments or during prolonged low water conditions, river red gums often have smaller leaves to reduce evapotranspiration (Stone and Bacon 1994) and can reduce active sapwood areas to further reduce water requirements (Doody et al. 2015).

In the Namoi and Gwydir catchments, dieback does not impact a particular age class of trees. Trees on the floodplain away from the river channel or permanent creeks are generally the worst-affected trees, but trees on the river banks can be affected by dieback too, and many show a thinning in their crown. In many cases, the oldest trees in an area are not the worst-affected. Trees 50–250 cm DBH

are affected by dieback to varying degrees, whereas trees > 300 cm DBH are generally in good health. On the Namoi and Gwydir floodplains, river red gum dieback is characterised by a contraction of the canopy, loss of leaves and canopy thinning, branch death and epicormic growth during periods of recovery. Old epicormic growth can be observed in many instances, while the former canopy architecture persists as dead branches throughout the new crown. Some tree recovery can be observed after rainfall or floods as shown by our water use data (Figure 1).

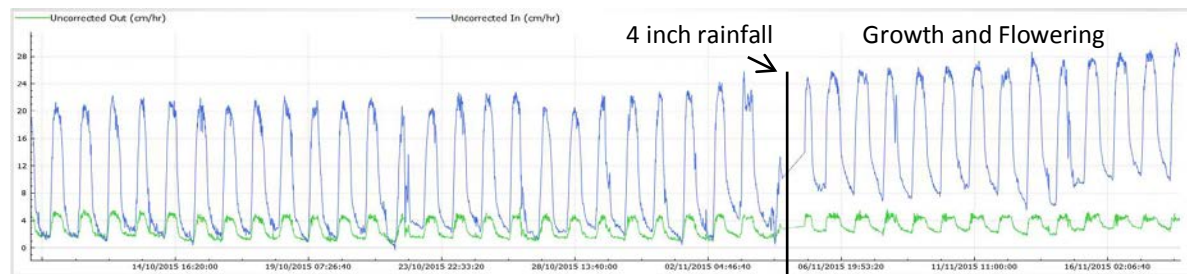


Figure 1: water use of a floodplain river red gum tree at Boggabri during very dry conditions and after rainfall. The tree maintained high water use throughout dry conditions (illustrating primary groundwater use), but increased water use following rainfall, indicating secondary soil water use.

Riparian trees also showed evidence of use of rainfall and soil moisture (Figure 2), despite ample access to river water (they had roots in the river) and ground water (aquifers are generally close to the surface near the river). The behaviour of these trees, i.e. switching between water sources and taking advantage of any water source available to them, suggests river red gums have multiple strategies available to them to access water. However, they are also very inefficient water users, using very large amounts of water when available to carry out important growth and reproductive processes, even respiring large amounts of water overnight (Figure 1).

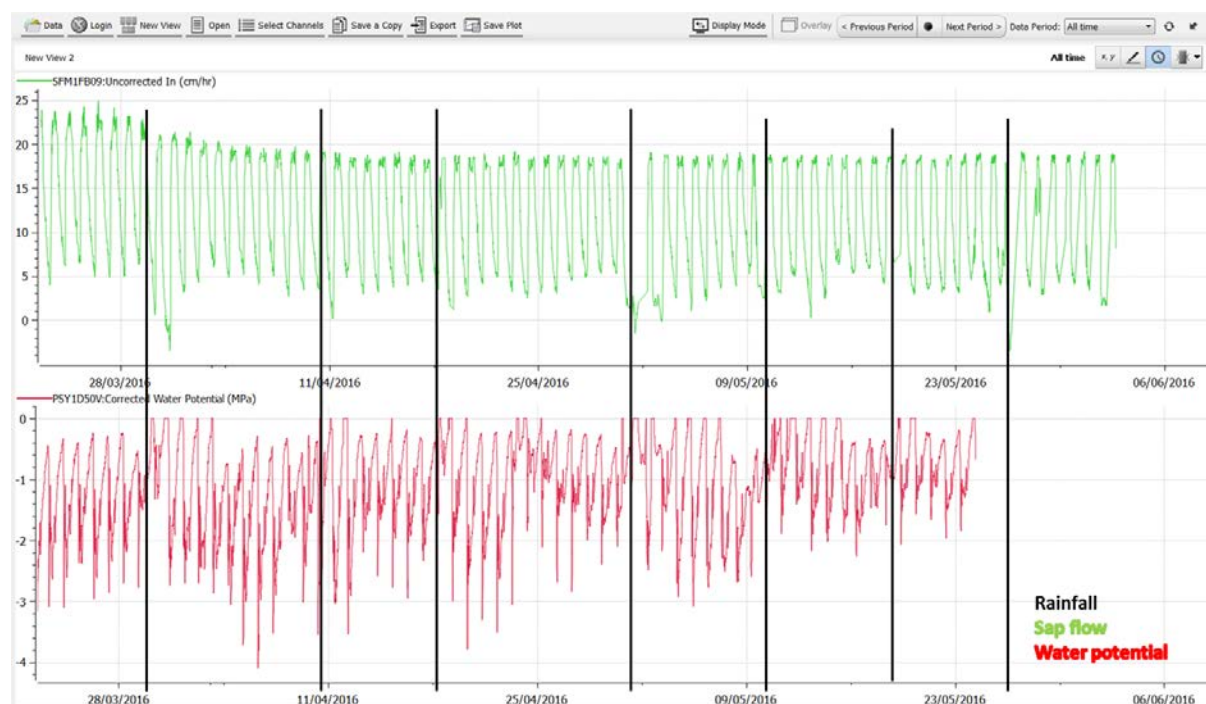


Figure 2: Water use by a riparian river red gum tree near Boggabri illustrating changes in water use in response to regular rainfall events.

b. Psyllid outbreak

During initial site visits in late winter and early spring 2015, we observed a widespread dieback event in the Namoi east of Narrabri. Road-based surveys documented the extent of the dieback, which stretched from the Moore Creek area north of Tamworth, along the Peel, Mooki and Namoi Rivers, and Coxes Creek, to sites 7 km east of Narrabri. Interestingly, the dieback was affecting river red gums of all ages, with trees on the floodplain in worse condition compared to those in the riparian

zone. Trees in plantings incorporating a mix of understorey species (e.g. bottlebrushes, wattles, etc.) seemed to be relatively unaffected. Samples of leaf material were bagged and examined on return to the lab. Upon examination, we discovered several psyllid species (later identified as *Creiis periculosa*, *Cardiaspina fiscella* and *Anoeconeossa communis* by Gary Taylor at the University of Adelaide) were present in large numbers in varying samples. *Creiis periculosa* and *Anoeconeossa communis* were present in samples collected in the Gunnedah-Boggabri-Narrabri region, while *Cardiaspina fiscella* was most abundant in samples from the Tamworth region. There was evidence of parasitism by a small (unidentified) wasp, but parasitism rates were lower than expected and not doing much to control the outbreak. The current dieback was specific to river red gums and several landholders suggested it was not the first time an outbreak has happened in the area.

Unfortunately we became aware of the psyllid outbreak too late to be able to conduct scientifically-rigorous investigations, but sought out literature on psyllids and dieback and discovered psyllid-related dieback is a natural phenomenon affecting various eucalypt species across Australia. Psyllids are responsible for dieback of grey box (*Eucalyptus molluccana*) in the Cumberland Plains region of Western Sydney (Hall et al. 2015); Sydney blue gum (*E. saligna*) on the NSW coast in a syndrome known as Bell Miner Associated dieback (Stone 1996); flooded gum (*E. rudis*) and wandoo (*E. wandoo*) in Western Australia (Kimber 1981); pink gum (*E. fasciculosa*) in South Australia (Morgan 1984); Blakely's red gum (*E. blakelyi*) in where (Clarke 1964), and river red gum in Victoria (Collett 2001). The trigger for psyllid outbreaks appears to be weather-related with warm, wet winters followed by a period of spring or summer water stress leading to increased psyllid numbers and palatability of host leaves (Collett 2001). Water-stressed trees (such as those occurring on the floodplain) are more susceptible to attack and are generally impacted more so than trees with high water availability (White 1969).

Low populations of psyllid-specific predators and/or parasitoids also enable outbreaks (Conservation and Environment 1992). Parasitism rates in the Namoi in 2015 appeared to be higher in plantings with an understorey of flowering shrubs. In addition to parasitic wasps that are attracted to flowering shrubs, the shrubs may also attract small insectivorous birds that feed on the psyllid. High predator/parasitoid abundance could explain the lower level of damage in planted sites incorporating flowering shrubs. As such, a potential management intervention that may be employed by growers to control psyllids and minimise the risk of outbreaks may be planting of mixed shrub species to provide habitat for natural enemies. Careful use of broad spectrum insecticides in crops, particularly adjacent to sensitive native vegetation may also assist in maintaining populations of natural enemies in the landscape (Gagic et al. 2018), facilitating early control of psyllid populations and minimising the extent and severity of outbreaks. The use of insecticides to control psyllid populations is not recommended in these sensitive natural areas.

c. Cockatoos

Following the psyllid damage, cockatoos were observed taking advantage of bare tree canopies, creating new perches by actively removing new growth from around tree hollows and from branches of the trees as they recover, thereby maintaining open tree canopies and opportunities to detect potential predators. Large flocks of sulphur-crested cockatoos were observed moving through sites with large amounts of leaf and small branches removed by the birds, leaving a carpet of branchlets covering the ground under affected trees. This action appears to emphasise the complexity of the dieback problem and the wide variety of factors contributing to this phenomenon with a hierarchy of factors identified as contributing to dieback.

d. Spray drift

Evidence of spray drift was noted at various locations throughout the study region, particularly along crop margins. However, we could not find evidence of widespread spray drift affecting river red gums in the study region. A glasshouse experiment was planned to investigate susceptibility of river red gums to various herbicide and defoliant chemicals. However, use of these chemicals in a glasshouse environment was considered too risky and the experiment was abandoned.

e. Low recruitment rates

Dieback-affected sites were often dominated by large old trees, with very little recruitment occurring at many sites. A range of causes for this low recruitment were suggested, including trampling by livestock, low seed production or seed quality, competition with weeds such as lippia. A search of the literature suggested that river red gums can live for up to 1000 (Colloff 2014), and while many of the trees suffering dieback are not that old. However, as trees senesce, they create and gap or opportunity in the ecosystem for recruitment, where seedlings are present. This is not happening in many situations, and we went on to investigate two factors – low seed quality and germinability, and competition with lippia.

5. *Lab and glasshouse experiments initiated to complement field restoration component*

During the initial stages of the project, we noted that Lippia (*Phyla canescens*) is commonly present at sites where river red gums are affected by dieback. Restoration of sites can be passive (e.g. unassisted regeneration) or active (e.g. management assisted regeneration). Passive regeneration, where possible, is considerably more cost-effective than active restoration. As such, we chose to investigate the impact of lippia on seed germination and seedling growth under contrasting moisture availability regimes. An advanced draft of a paper reporting the outcomes of this study will be submitted for publication in the near future.

Lippia is an invasive weed of riparian zones and floodplains in the Murray-Darling Basin (MDB) of south-eastern Australia. Originally introduced as an ornamental garden species, like many other invasive species, lippia has invaded natural areas to the detriment of ecosystem composition, structure, function and livestock grazing value. Lippia is often found at sites experiencing low levels of tree recruitment, but is lippia contributing to this low recruitment or is it just an innocent by-stander? We investigated the impact of increasing density of lippia on river red gum (*Eucalyptus camaldulensis*) recruitment in a glasshouse study under two levels of moisture availability. Germination and height of seedlings was recorded to determine whether lippia was competing with river red gum seedlings, and to compare competition under moisture limiting (dry) or ideal (wet) conditions. We found contrasting results in terms of the number of germinations between the wet and dry treatments, as competition with increasing levels of lippia cover was the dominant process in the wet treatment, but was evident in the dry treatment. There was a rapid decrease in seedling height as lippia was introduced, and further smaller decreases from there on with increasing lippia cover in both the wet and the dry treatments. There were no differences in soil moisture content with increasing lippia cover in either moisture treatment, so we believe that competition for moisture was not the limiting factor. Competition for light and space is likely to have occurred. This study confirms that the presence of lippia is likely to be a factor resulting in low levels of river red gum recruitment at sites across the Murray-Darling Basin, and that a method of control is required to allow natural recruitment to occur.

6. *Investigate the impacts of tree health on seed production, viability, germination and establishment*

An advanced draft of a paper reporting the outcomes of this study will be submitted for publication in the near future. Tree dieback occurs in a variety of ecosystems around the globe, with moisture stress and insect herbivory cited as the leading causes. Climate change will exacerbate moisture stress and herbivory in many parts of the globe, driving further tree dieback and accelerated loss of biodiversity and ecosystem services. The impacts of tree dieback on extant trees are obvious, but the implications of dieback for regeneration and growth of offspring are unknown. Studies have shown seed production by dieback-affected trees is significantly lower than that produced by healthy trees, but very few studies go on to examine impacts on seed weight and germinability, and seedling growth. We collected seed from trees representing a range of canopy health classes, landscape positions and water availabilities occurring at collection sites. Seed weight decreased with increasing leaf damage and canopy density, and decreasing water availability to the parent tree. Total germination behaved similarly, with germination decreasing with increasing leaf damage and canopy density, and decreasing with water availability to the parent tree and seed weight. The mean time taken for seeds to germinate increased with increasing seed weight. All seedling growth

measurements were related to seed size, with larger seeds producing faster growing seedlings. This study illustrates how dieback and water availability compromises the ability of trees to regenerate naturally through impacts on seed quality. At sites where dieback is impacting extant trees, human intervention may be required to increase regeneration and future tree cover using externally sourced seed from healthy trees.

7. Determine growth rates of river red gums in different condition states

In 2016, we applied for a Science and Innovation Award for Young People in Agriculture, Fisheries and Forestry. Unfortunately this application was unsuccessful. The proposal submitted was to measure carbon sequestration in riparian lands, with a large component of the budget allocated to the purchase of dendrometers to measure tree growth.

Also in 2016, we applied for AINSE funds to investigate the drivers of river red gum dieback using ITRAX-XRF, a sophisticated method to measure tree growth through time by identifying growth rings using very fine-scale changes in wood density and matching this with concentrations of a range of elements known to correlate with early and late season tree growth. The beauty of the ITRAX-XRF system is that live trees can be studied with minimal impact by taking a core through the tree for analysis, as opposed to traditional dendrochronology methods that rely on a cross-section of the tree trunk to be taken for analysis, necessitating the tree to be fallen. Traditional methods of dendrochronology do not provide reliable data for most Australian tree species as tree growth is possible year-round and growth rings are not necessarily correlated with yearly growth. In the case of river red gums, growth can be correlated with flood history during the period of European occupation of Australia, but again, traditional dendrochronology is unreliable in delineating growth rings in this species. Unfortunately a suitable coring device for river red gum was not available commercial given the density of the wood. Commercial devices have been developed for softwoods in America, but these were deemed unsuitable for river red gum. Prototypes of coring devices were developed by the Science Engineering Workshop at UNE and tested on a range of tree species on the Tablelands, before the device deemed most appropriate was taken to the field to take samples of river red gums near Narrabri. Unfortunately our most suitable device failed when used to take samples from river red gums due to the amount of water being pumped through those trees, even during drought conditions (the trees were accessing ground water). As such, we were unable to obtain samples and were therefore unsuccessful in conducting this work.

8. Restoration experiments initiated at a demonstration site;

9. Restoration field trial monitoring and data collection, and

10. Publication of field trial results in scientific literature

We obtained funds from Northwest Local Land Services to establish a restoration trial at a 3 ha site at Wallah (Maules Creek Road), east of Narrabri. The aim of the trial is to investigate different methods of planting trees (tubestock and direct seeding) and test the impact of a range of seed amendments, including mycorrhizal inoculant and a primer treatment to encourage faster germination of seed. The trial will also investigate the impact of provenance on the germination, survival and growth of trees in the field using seed from six sites in the Namoi and Gwydir regions. The site will determine cost-effective methods to revegetate riparian lands across the cotton industry. Obviously it is cheaper to plant seed directly at the site, rather than using tubestock, so if we can increase the success rate of direct seeding trials, there is scope to drastically reduce the cost of revegetation on cotton farms for multiple uses.

Site preparation, including slashing, weed control, ripping and mounding of planting lines has been carried out and seedlings to be planted at the site are currently being maintained in a glasshouse at UNE. Unfortunately seasonal conditions (i.e. lack of soil moisture) have prevented us from planting this site to date. In order to progress plans for this site, we have settled on a planting date of March 2019 and have facilities in place to water trees if required, with assistance from Field's Environmental Solutions (currently undertaking revegetation works at coal mines in the local area), local landholders and NWLLS.

Methods

2. Detail the methodology and justify the methodology used. Include any discoveries in methods that may benefit other related research.

First bird paper

Study region and cotton-growing zones

Birds in native vegetation were sampled on cotton farms in four cotton-growing zones in inland eastern Australia: (1) Central Qld (i.e. the Fitzroy River basin); (2) Border Rivers, comprising the Condamine–Culgoa River basin (including the Balonne River) in southern Qld and the Border Rivers (including the MacIntyre, Gwydir and Namoi River catchments in north-western NSW); (3) Macquarie, consisting of the Macquarie River basin in central NSW, and (4) Southern NSW zone, consisting of the Lachlan and Murrumbidgee catchments. A distance of 1256 km separated the northern sites near Emerald in Central Qld and the southern sites near Darlington Point, southern NSW.

The native vegetation of the study region consists of *Eucalyptus*-dominated riparian forests and floodplain woodlands, *Casuarina*-dominated floodplain forests, *Acacia*-dominated floodplain low forest, low open woodland and tall open shrubland, planted woody native vegetation for agroforestry or environmental values, and native and derived grasslands. Not all vegetation types are found across the whole study region, and the abundance of vegetation communities in each zone varied.

Eight principal native vegetation types and well as stands of native tree and shrub plantings (revegetation) were found on cotton farms: River red gum (*Eucalyptus camaldulensis*) forests and woodlands; Coolibah (*Eucalyptus coolabah*); Black box (*Eucalyptus largiflorens*); Poplar box (*Eucalyptus populnea*) woodlands; Myall (*Acacia pendula*) shrublands; Belah (*Casuarina cristata*) forests; Brigalow low-forest; Grasslands, and Planted native woody vegetation ranging in age from 15 to 25 years.

Field sampling design and antecedent rainfall

Birds were surveyed at a total of 197 sites across the four cotton-growing zones between 25 September and 29 November 2014. Cotton farms were chosen in each zone in order to sample the maximum geographical spread of farms. Within each farm, locations were chosen to sample each type of native vegetation present. Where examples of a vegetation type were scarce in a zone, two sites of the same vegetation type were sampled on the one farm. Owing to the variable occurrence of each vegetation type across the study region, between 13 and 46 sites of seven of the native vegetation types were sampled (Table 2), but only two stands of brigalow were encountered. Conditions were generally dry across the study region, with rainfall in the 12 months preceding the surveys below average across much of inland eastern Australia (Commonwealth of Australia 2015d).

Birds were censused in a 2-ha quadrat in native remnant or planted vegetation at each site on two separate but usually consecutive mornings by different observers (Julian Reid and Stuart Green). Censuses were conducted between sunrise and 1100 hours and lasted 20 mins. Weather conditions were mostly cloudless or partly cloudy and still or with a light breeze. Windy conditions affected about one in five censuses, but very windy conditions and rain were avoided. The air temperature ranged between 13°C and 29°C, averaging 20°C. All birds seen or heard in the 2-ha quadrat during the census were recorded along with their activity (e.g. flying through the plot, perched in a tree, etc.). Birds that were interacting with the site (e.g. birds that were perched, on the ground, foraging, including raptors, aerial insectivours, etc.) were regarded as 'in' the quadrat. Other birds that were flying through or over were recorded as 'out', along with additional species seen or heard outside the quadrat during or immediately before or after the census. Species recorded as 'out' were excluded from analyses, but are reported in Results. The dimensions and orientation of quadrats depended on the configuration of the remnant vegetation at the site but were generally 200 × 100 m. All sites of the same vegetation type were a minimum of 200 m apart and generally >500 m apart to minimise the likelihood of recording the same individuals at nearby sites. Avian nomenclature follows Christidis and Boles (2008).

Across all sites, species richness (S), abundance (total number of individual birds) and Shannon–Wiener diversity (H') were calculated by cotton-growing zone and vegetation type for all species recorded in censuses as well as just for landbirds. Evenness of bird communities by zone and vegetation type were calculated ($H'/\ln(S)$) just for landbirds and for a reduced set of sites ($n = 185$) with two or more species present (formulae from Pielou 1966). Significant differences in abundance, species richness, diversity and evenness of landbird assemblages between regions and vegetation types were identified using generalised linear models (assuming normal distribution for diversity and evenness and quasi-poisson for richness and abundance). Where the global test was significant, pairwise differences between zones and vegetation types were considered significant if reciprocal modelled 95% confidence intervals did not include the other level's estimate.

Species accumulation curves were used to compare the regional bird species pools of the four cotton-growing zones, using the Kindt exact estimator in 'vegan' (Oksanen et al. 2016). ANOSIM tests (Clarke 1993), implemented in vegan, were used to examine whether bird community composition varied significantly across zones and vegetation types using a matrix of log-transformed abundances for 133 landbird species and 186 sites, having removed all nine grassland sites with no species, the two brigalow sites and two nocturnal species. The Bray–Curtis dissimilarity matrix was converted to ranks, and the ANOSIM test statistic, R, calculated to compare mean within-group ranked dissimilarities. Positive R values indicated greater compositional similarity within site groups (zones or vegetation types) than between groups, and Monte Carlo randomisation tests were used to assess the significance of the results (Clarke 1993; Oksanen et al. 2016). ANOSIM was also used to test for significant pair-wise comparisons between zones and vegetation types ($\alpha = 0.05$ and using Holm's sequential Bonferroni correction for post-hoc pairwise tests, based on 9999 randomisations for all tests).

Regression of assemblage composition on zones and vegetation types, jointly, was carried out using Canonical Correspondence Analysis (CCA) in vegan, to allow graphical representation (ordination) of these relationships to test the significance of both factors. Only sites with three or more landbird species recorded and only species with three or more occurrences were retained in the matrix of 175 sites by 99 species, with abundances log-transformed prior to chi-square transformation (Oksanen et al. 2016). The implementation of CCA in vegan uses the methods of Legendre and Legendre (2012), with the transformed species values subjected to weighted linear regression on the constraining variables (zone and vegetation type) and the fitted values submitted to correspondence analysis performed via singular value decomposition. For the ordination plot, site scores are weighted averages of the species scores (i.e. weighted by the transformed species values), with factor centroids obtained as the arithmetic average of sites belonging to each class in constrained space. The first two constrained ordination axes were plotted showing sites and factors (vegetation types as centroids, zones as 70% confidence interval ellipses), and the following Monte Carlo randomisation tests of significance were performed with 9999 permutations: (1) the full model (zone and vegetation type); (2) the partial fit of each term, and (3) each of the constrained CCA axes. Generalized linear models, curve fitting and associated tests, and multivariate analyses were performed in R version 3.1.2 (R Core Team 2014).

Second bird paper

Study area

The Namoi catchment drains west from the Great Dividing Range in northern NSW, Australia. The expansive floodplain associated with the Namoi River straddles the Brigalow Belt South and Darling Riverine Plains bioregions (Department of Environment and Energy 2017) and is dominated by fertile black, grey and brown vertosol soils favoured for dryland and irrigated cropping, and livestock (sheep and cattle) grazing. Two large, nationally-significant vegetation remnants (the Pilliga Scrub and Nandewar Range) flank the region, providing important habitat for a range of species (Date *et al.* 2002), while connectivity through the landscape is offered by the riparian corridor associated with the Namoi River and its tributaries and distributaries (Eco Logical Australia 2009).

The distribution of native vegetation on the Namoi floodplain is correlated with flood frequency, and consists of two dominant associations, river red gum (*Eucalyptus camaldulensis*) riparian forests and woodlands with a shrubby or grassy understorey, and coolibah (*E. coolabah*) woodlands grading into black box (*E. largiflorens*) woodlands, generally with a grassy understorey, but often with varying cover of chenopod spp. away from the river in the west of the region. Myall (*Acacia pendula*) tall shrublands with a grassy understorey, often with chenopod species co-occurring, exist on rarely flooded rises across the region. Grasslands and derived grasslands (cleared of trees) also occur across the floodplain and are dominated by Mitchell grass (*Astrebla lappacea*) in the west of the region, curly windmill grass (*Enteropogon acicularis*), cup grasses (*Eriochloa* spp.) and summer grasses (*Paspalidium* spp.) in the centre, and plains grass (*Austrostipa aristiglumis*) in the east. Environmental and agroforestry plantings, often less than 2 ha in size and consisting of a range of eucalypt and *Acacia* species, also occur across the region. Sites were chosen to represent the dominant vegetation types occurring on farms across the floodplain, and comprised five black box, 18 coolibah, 11 grassland, six myall, five revegetation and 11 river red gum sites. Most habitats were widely dispersed across the region, with the exception of black box, confined to western parts. The surveyed revegetation sites all occurred in the east. The surveyed vegetation remnants were generally small (particularly in eastern and central areas), variously grazed by sheep and/or cattle or ungrazed, and many wooded sites had experienced some degree of vegetation thinning (ringbarking or timber collection).

Climate

The study region has a semi-arid, subtropical climate with unreliable and sporadic rainfall. Mean annual rainfall declines from east to west across the region, varying from 592 mm near Boggabri (−30.7000°, 150.0333°; 246 m a.s.l.) to 436 mm near Walgett (−30.0167°, 148.1167°; 136 m a.s.l.), with a slight summer dominance (BOM 2018). Mean maximum summer temperatures increase across the region from east to west, ranging between 32 and 35°C, while winter mean minima are 3–5°C. Plant growth is limited by moisture across the study region, with the eastern half falling into agro-bioclimatic class E3 (Hutchinson *et al.* 2005), denoting a climate where most plant growth occurs in summer due to temperature limitation in winter, while the western half is in class E4 indicating relatively even plant growth throughout the year (i.e. mild winters). These categories align with the bioregional boundaries.

Bird sampling methods

Birds were censused by two different observers (Nick Reid and Stuart Green) in a 2-ha area of the target vegetation type for 20 minutes during one morning (sunrise to 10:00) and one afternoon sampling period (16:00 to sunset) on different days between 10 September and 17 September 2007. In 2014, birds were censused (Julian Reid and Stuart Green) during two consecutive mornings (sunrise to 11:00) between 26 October and 9 November in plots oriented as closely as possible to those surveyed in 2007. Weather conditions were noted at the commencement of each census and were generally fine and sunny (overcast on several mornings in 2007) and still or with a slight breeze during both survey periods. Very windy conditions and rain were avoided. All birds seen or heard in the 2-ha plot during the census period were recorded along with bird activity (e.g. flying through the plot, perched in a tree, etc.). Birds that were perched, on the ground or foraging were regarded as 'in' the quadrat. Birds that flew through or over, or had no obvious interaction with the site (e.g. not hunting, feeding, nesting etc.), were recorded as 'out' along with additional species seen or heard outside the quadrat during or immediately before or after the census. Birds recorded as 'out' were excluded from further analysis to standardize survey area and focus on individuals and species reliant on the vegetation. The dimensions and orientation of plots depended on the configuration of the area to be surveyed, but were generally 200 × 100 m. All quadrats of the same vegetation type were a minimum of 200 m apart and generally >500 m apart to minimize the likelihood of recording the same individuals at nearby sites.

Analysis

We surveyed 56 sites in both survey periods of which 29 were river red gum or coolibah (RRG/Cool), and the balance of sites were black box, myall, grassland or native revegetation. Analyses were performed on two datasets: (1) the complete dataset of 56 sites, and (2) the 29 RRG/Cool sites. A

few comparable analyses were performed on the other 27 sites, to investigate whether habitat type might influence our conclusions.

We followed the analytical methods of Maron *et al.* (2005) closely, and so scant details are given here except where we departed from their approach. Abundance across sites (the sum of all individuals in both censuses in a survey period), expressed as assemblage-wide density (per ha, total abundance/4), was compared for each species between the 2 years with Wilcoxon signed-rank tests, excluding those that occurred in fewer than five sites and those that were not present during one of the survey periods. Pearson correlation analysis was used to determine whether log-transformed total density at sites was correlated across survey periods. A paired *t*-test of the log-transformed site densities between years was used to investigate whether assemblage abundance in the two survey periods differed significantly. Pearson correlation was also used to determine whether log-transformed species richness of individual sites was correlated across survey periods. A paired *t*-test of log-transformed species richness at sites between years compared the number of species per site recorded in the two survey periods.

As defined by Maron *et al.* (2005: 385) we calculated the minimum turnover between survey periods (*T*, equivalent to the Jaccard index of dissimilarity on presence-absence data) for each site. This statistic and its mean were calculated for all species combined, and for the three bird-movement categories of nomadic, migratory and sedentary species, separately. Assignment of species to movement categories largely followed Maron *et al.* (2005), with minor revisions (e.g. Reid & Fleming 1992; Chan 2001; see Table S1). Paired *t*-tests on *T* were conducted between each pair of bird-movement categories. For each bird species that occurred during both survey periods, we calculated the percentage change in distribution (*D*) between the two periods using the formula of Maron *et al.* (2005: 385; again, Jaccard's index, but performed on the transpose of the data matrix). We conducted pairwise comparisons of the mean percentage change in distribution for each bird-movement category with *t*-tests, assuming unequal variances.

Like Maron *et al.* (2005: 386) we used Monte Carlo randomization tests to assess whether the distribution of a species among sites in survey period 2 was consistent with a random reallocation from survey period 1 (1000 permutations used; our test statistic was the number of matching sites). We used two-tailed tests given the possibility that a species could potentially occupy a very different suite of sites in period 2, as informed by the conclusions of Maron *et al.* (2005). We also used the discrete-probability hypergeometric distribution, to test if the number of shared sites, *k*, occupied by a species in both periods differed significantly from that expected by chance: that is, the discrete probabilities were calculated for each permissible value of *k*, given *K* (the number of sites occupied by the species in period 1), *n*₂ (the number of sites occupied by the species in period 2) and *N* = 56 (the number of sites in the complete data set). Use of the hypergeometric distribution further allowed us to determine minimum sizes of *K*, and *k* and *n*₂, for a given *N*, for which a species could have a significantly similar (cumulative probability < 0.025 for the observed number of shared sites or more) or different (observed number of shared sites or fewer) distribution among sites in periods 1 and 2. For instance, for the discrete probability of a species occupying none of the same sites in period 2 (zero shared sites) to be less than 0.025, for *N* = 56 the number of non-zero observations in both periods would be 13 in both surveys (or 10 and 16 site occurrences as the most extreme distribution for a combined 26 occurrences across both periods). Minimum values of *K* and *n*₂ to be able to obtain a significantly similar distribution across the two periods were two and two (with the same two sites occupied). Therefore, the power to detect a significant shift in site occupation across survey periods was much smaller than that to detect a significantly stable distribution pattern, given most species occurred infrequently, and only 13 species in the full data set had sufficient non-zero records to test for a significant shift.

Bray–Curtis similarity (Sørensen index) matrices were calculated for abundance and presence-absence data sets, having appended the second period's data to the first (matrix of 112 'sites' x 94 species). Between the two survey periods we calculated the mean percentage similarity between each site in period 1 and all other sites in period 2. We also calculated the mean percentage

similarity between sites within each survey period. We separately calculated the percentage similarity between the same sites across the two periods, to assess the relative strength of site self-similarity.

Analysis of similarity (ANOSIM: Clarke 1993) was used to explore differences between the bird assemblages recorded in each of the two survey periods, using both total abundance and presence/absence data. Non-metric multi-dimensional scaling (NMDS) was used to ordinate sites according to bird community composition in PRIMER (Clarke & Warwick 2006). Bird abundance data were log-transformed ($\ln[x+1]$) and site similarity calculated using Bray–Curtis dissimilarity. Vegetation type and year were overlaid on the least-stress NMDS ordination (from 50 random starts) to illustrate patterns in bird community composition in relation to these factors.

We performed two sets of analyses not undertaken by Maron *et al.* (2005). These tested explicitly whether the bird assemblages at sites in one period were more similar than expected by chance to the same sites in the other period than to other sites. Mantel correlation tests (Mantel 1967; Mac Nally 1990), using the Pearson R correlation coefficient as the test statistic (Legendre & Fortin 1989; Legendre & Legendre 1998), and similar (ordinary least-squares) regressions of dissimilarity matrices (Smouse *et al.* 1986; Goslee & Urban 2007) were undertaken on the 2007 and 2014 Bray-Curtis distances (both presence/absence and log-transformed abundances). Probabilities were evaluated using structured randomizations of the data, where the rows and columns of one matrix were jointly randomized (Goslee & Urban 2007), accounting for the lack of independence of individual Bray-Curtis values. In the case of the simple Mantel correlation test, 9999 randomizations of the 2014 distance matrix were used to compile the null distribution of the test statistic (R), and this matrix was the one randomized in subsequent multi-variable distance-matrix tests. We extended this analysis by performing partial Mantel tests accounting for the effects of pairwise geographic distances between all sites. These addressed the hypothesis that a significant similarity in assemblage composition at sites over time did not arise purely from the geographic distribution of the sites. Plots of Bray-Curtis assemblage distance vs geographic distance indicated a square-root transformation of the latter was required to linearize the relationships (see Goslee & Urban 2007). The residuals from the regression of the 2007 and 2014 bird assemblage distances on the square root of geographic distances were correlated to obtain the partial correlation coefficient (R_p). A partial Mantel correlogram (Goslee & Urban 2007) of the 2007–2014 assemblage correlations, using R_p , separated into 12 distance classes was examined to check whether fine-scale spatial autocorrelation occurred. Finally, the 2014 Bray-Curtis matrix was regressed on the 2007 and geographic distance matrices and a model triangular matrix representing the six (or two) habitats, where cells representing sites of the same habitat took zeros and all other contrasts took ones. The residual distances of these regressions were also plotted as correlograms over 12 distance classes. All Mantel tests and matrix regressions (using 9999 randomizations) and correlogram analyses (999 randomizations for each distance class) were conducted using the software 'ecodist' (Goslee & Urban 2007, 2015) on the R platform (R Core Team 2016).

Lippia paper

This experiment was conducted in a factorial randomised block design spread across one room in a glasshouse to test the effects of two moisture treatments and six lippia cover densities on the germination and growth of river red gums. Treatment combinations were applied in four replicate blocks using a total of 48 (6 cover treatments \times 2 moisture treatments \times 4 replicate blocks) black plastic recycle crates sourced from the Armidale Regional Council. Boxes were $\frac{3}{4}$ filled with a black vertosol soil collected near Boggabri on the Namoi River floodplain (30°45'03''S, 150°05'11''E) in February 2018 where both river red gums and lippia were present. On return to Armidale, the soil was pushed through a 2 cm \times 1 cm mesh to break up very large aggregates and carefully divided evenly into the boxes so as not to create soil compaction. Boxes were moved into the glasshouse where the temperature was maintained at less than 26°C for the duration of the experiment.

Once in the glasshouse, the boxes were watered regularly for three weeks to facilitate germination of a range of species and deplete the soil seed bank. All plants that germinated in the boxes that

were not planted by us were removed throughout the duration of the experiment. In March 2018, zero, one, two, three, four or five × 20 cm lengths of lippia were planted into the boxes for the 0%, 10%, 25%, 50%, 75% and 90% cover treatments, respectively. Lippia fragments were collected at the same site as the soil and stored in a fridge (4°C) until required. All boxes continued to be watered and weeded regularly until the desired cover of lippia was established. Cover was estimated in each box using a point-intersect method on an 8 × 8 cm grid, with 30 points recorded per box. Cover classes ($\pm 3\%$) were maintained through careful thinning of lippia at regular intervals throughout the experiment.

Once the desired cover of lippia was achieved, the two moisture treatments were applied: (1) 'wet' which allowed no drainage of water from the crate after sealing drainage holes with silicone gel ($n = 24$), and (2) 'dry' which had a small hole in each corner at the bottom of the box to allow water to drain ($n = 24$), although every effort was made throughout the experiment not to water the 'dry' boxes to the point of draining. Soil moisture measurements were taken using a handheld TDR moisture meter half-way through the experiment (i.e. between germination and harvest of the river red gums) to determine percent volumetric water content for every box 24 hours after watering (T_1) and again five days later before the boxes were watered (T_2).

In May 2018, 50 g (± 0.001 g) of *Eucalyptus camaldulensis* subsp. *camaldulensis* seed (including chaff) was spread evenly over each box. The seed had been collected 18 months earlier from 40 trees at four sites within 100 km of the site where the soil was sourced, and stored at room temperature. A growth cabinet-based germination test conducted one month before sowing showed approximately 63% seed germinability. Germinants were counted in a 25 × 25 cm quadrat located in the centre of each box one month after the seed was sown and again at two months after seed was sown. One week after the second germination count, seedlings were thinned back to 12 evenly spaced seedlings (three rows of four seedlings) in each box. The strongest, most mature seedling within 2 cm of each of the 12 pre-located points in each box was retained while all other seedlings were removed and discarded. Wooden skewers were inserted alongside each seedling to mark its location, with any unmarked germinants found during the remainder of the experiment carefully removed.

In October 2018, seedling height for each of the seedlings in each box was measured using a metal ruler to measure from ground level to the tallest growing point. The mean height of seedlings for each box was determined and used in statistical analyses of treatment effects. All statistical analyses were carried out using Statistix 8 (Analytical Software 2003). Block was initially included in the analyses, but was not a significant effect in any analysis and was subsequently discarded from all models. Two-way Analysis of Variance (ANOVA) was used to test the effects of moisture, lippia cover and the interaction between the two on soil moisture levels, and river red gum germination and growth (height). Post-hoc tests to confirm significantly different groups of means was carried out using a Least Significant Difference (LSD) test.

Seed germination paper

Study region

Seed was collected in mid-July 2016 from trees at each of four travelling stock reserve sites located near Narrabri in the Namoi catchment of northern New South Wales, Australia. The sites were Myall Vale (30°11'34"S, 149°32'42"E), Tarriaro (30°24'10"S, 149°54'08"E), Wallah (30°26'57"S, 149°57'00"E) and Old Narrabri Road (30°34'23"S, 150°0'15"E). The distance between the eastern and western-most sites was approximately 60 km. Riparian vegetation is well-connected in this part of the catchment (EcoLogical Australia 2009) with the greatest distance between subject trees and their nearest neighbours being approximately 50 m, allowing opportunities for outcrossing and therefore gene flow to occur between sites. Accelerated dieback of river red gums has been observed in this region since the 1970s (Reid et al. 2007). Past dieback and recovery events were evident on many trees where epicormic growth of various ages was present in the canopy. Rainfall at Narrabri averages 660 mm per year and is slightly summer-dominant (Bureau of Meteorology 2018). Temperatures vary from a mean maximum of 34°C to a mean minimum of 19°C

in January and 18°C to 3.7°C in July. Monthly rainfall in the three years leading up to seed collection was generally lower than the long-term average, with the rainfall received in the months of July to October (leading into flowering) being at least 30 mm below long-term monthly averages in 2014, 2015 and 2016. Monthly temperatures in the three years leading up to seed collection were within 5°C of the regional long-term average, and except on three occasions, were within 3°C of the regional long-term average.

The Namoi River is a heavily regulated system through the construction of Keepit and Splitrock Dams in the upper catchment, as well as a series of weirs along the length of the river. The majority of flow releases from the two dams are associated with cotton irrigation during the period October to February (i.e. spring/summer). Controlled environmental flows can occur throughout the year. River heights in the three years leading up to seed collection were below average, except during summer of 2013/14 when irrigation water was plentiful and a large area of irrigated cotton was grown in the region. Groundwater is available within the root zone of trees close to the river channel, but the depth varies over short distances and groundwater is often inaccessible to trees on the floodplain (Kelly et al. 2013). Soils dominated by river red gums in the region are deep, fertile, black or grey vertosols, often with high organic matter content (Smith et al. 2015) owing to the highly productive nature of these ecosystems (Smith et al. 2017).

During the 12 months prior to sampling, simultaneous outbreaks of three psyllid species (*Creiis periculosa*, *Cardiaspina fiscella* and *Anoeconeossa communis*) in different parts of the region caused severe leaf damage and canopy loss of river red gums, with greater concentrations of the insects observed in the two eastern-most sites (Wallah and Old Narrabri Road). Unseasonably moist conditions were experienced in June at sites to the east of the study region (i.e. at Gunnedah, BOM), and this was the likely catalyst for psyllid outbreaks. Isolated trees occurring on the floodplain appeared to be impacted more than trees occurring in the riparian zone, with water stress likely contributing to this observation (White 1969). At the time of seed collection, canopy cover was returning, with epicormic growth stimulated by above-average rainfall in November 2015 and January 2016 present on most trees, but persisting low soil moisture conditions were hampering tree recovery.

Ten river red gum trees were chosen for seed collections at each of the four sites. Trees within sites were chosen to represent a range of canopy health classes, landscape positions (floodplain vs riparian) and water availabilities occurring at the site. All trees had a DBH of at least 80 cm, and were therefore considered to be old growth (remnant) trees. At each tree, a variety of tree health and landscape context variables were measured, the GPS location was recorded and photos of each tree were taken. Approximately 0.5 kg of seed capsules were collected for germination and growth studies and stored in paper bags to allow capsules to dry and release seed. Samples were stored at room temperature in a laboratory for one week prior to separating seed from the capsules by pouring the sample through a 2 mm sieve and shaking vigorously. Three replicates of 100 seeds were extracted from each sample and weighed using a five-decimal place balance to determine average seed weight for each tree.

Germination study

Twenty-five seeds were sown onto white cotton make-up pads in glass petri dishes laid out in a randomised block design including three replicate dishes in each of three growth cabinets ($n = 9$ petri dishes for each tree). Growth cabinets were set at 12 hours light/dark and 25°C/15°C temperature regime. Replicates were rotated within each growth cabinet (top to bottom and front to back) to eliminate any effect of replicate that may arise due to differences in temperature or light distribution within each growth cabinet. Initial analyses indicated that replicate had no significant impact on germination, and was therefore excluded from further analyses. Petri dishes were kept moist with tap water throughout the duration of the experiment (20 days) after an initial application of 20 mm of water at the start of the experiment. Seeds were recorded as germinated when the first signs were visible of a root emerging from the seed. Cumulative germination was recorded every day for the first 10 days, and every second day for a further 10 days.

Growth study

Seed collected from each tree was planted into pots in a randomised block design consisting of six blocks to account for differences in air movement and temperature in one room of the glasshouse. Pots were constructed from white PVC plastic plumbing pipe (9 cm diameter, 40 cm deep) with a cap on the bottom incorporating large drainage holes and fly screen mesh to prevent soil loss. Searles premium potting mix (containing re-wetting granules and water crystals, controlled release fertiliser, zeolite and added trace elements and minerals, and peat moss plus organic compost; Searles Garden Products 2017) was used for the experiment. Seeds were sown on the surface of the potting mix and anchored by a thin layer of river sand. Seedlings were thinned soon after acquiring their first set of true leaves with one individual typical of the size and vigour of individuals remaining in each pot. No supplementary fertilizer was applied and the soil was kept moist throughout the experiment with daily watering. Pots were rotated within blocks, and blocks were rotated twice between benches during the experiment to eliminate effects due to uneven distribution of temperature or light in the room. The experiment ran for three months, i.e. seeds were planted on 24/3/17 and harvested on 30/6/17.

At the conclusion of the experiment, the height from ground level, stem diameter near ground level, leaf and stem wet weights, and leaf and stem dry weights (following drying in a fan-forced oven at 40 °C for 10 days) of each plant were recorded. One-hundred individual wet leaves representing a variety of sizes were traced and weighed to two decimal places. Each traced leaf was then imported into Adobe Illustrator (Adobe 2018) and a silhouette of the leaf was created. Leaf area was calculated by importing the silhouette of each leaf into the Assess program (Lamari 2002). A regression relationship between leaf wet weight and leaf area allowed for calculation of total leaf area for each plant.

Data analysis

For the seed germination study, we analysed how different potential explanatory variables related to the tree producing the seed influenced three dependent variables: seed weight, mean time to germination (MGT) and proportion of germination to day 20. Canopy density, leaf damage, water availability class and distance to nearest neighbour were considered as potential explanatory variables. Water availability was considered to be an ordered numerical ranking rather than an unordered factor, with water availability decreasing with increasing class number (Table 2). We also considered the effects of sites and growth cabinets, as either random or fixed effects. All analyses were conducted in R (R Core Team 2018), with the 'nlme' package (Pinheiro et al 2018) used for mixed effects models and modelling of variance structure, and the 'GerminaR' package (Lozano-Isla et al 2017) used for calculating MGT (Czabator, 1962). We checked for correlation between the explanatory variables, and found it to be low. Seed weight and MGT were modelled as having a Gaussian error distribution using 'lm', 'lme' and 'gls' functions. Germination on day 20 (number germinated out of 25 seeds) was modelled as having a binomial error distribution using the 'glm' function.

For the seedling growth data, we analysed whether four dependent variables measured on the seedling (total leaf area, total dry leaf weight, diameter at ground level and height) differed significantly according to seed weight, as well as the canopy density, leaf damage, nearest neighbour and water availability of the tree that the seeds were collected from, using linear models. Block was also included as a potential explanatory variable to account for any possible block effects. Model simplification was conducted based on AIC. Residuals were checked visually, variance structure was modelled using the 'weights' option in the 'gls' and 'nlme' functions where indicated to account for heteroscedasticity, and modelling of variance structure retained when this improved AIC. Log-transformation of the dependent variable was applied when this reduced skewness in the residuals. For the germination responses (MGT and germination to day 20), we did a final test adding seed weight into the best final model to see whether it added explanatory power after all the parental variables had been accounted for.

Results

3. Detail and discuss the results for each objective including the statistical analysis of results.

First bird paper

Some 185 bird species were recorded in native remnant and planted vegetation on cotton farms, comprising 155 landbird and 30 waterbird species, including 26 of the 53 extant species of conservation significance known to occur in the study region (Barrett *et al.* 2003) and 16 species of declining woodland bird (Reid 1999). The species most frequently recorded at sites were large (>60 g), readily detected landbirds common in agricultural districts in eastern Australia (Table S2), such as galah (*Eolophus roseicapillus*, recorded at 83% of locations), Australian magpie (*Cracticus tibicen*, 82%), magpie-lark (*Grallina cyanoleuca*, 78%), Australian raven (*Corvus coronoides*, 76%), crested pigeon (*Ocyphaps lophotes*, 75%) and pied butcherbird (*Cracticus nigrogularis*, 75%). Bird abundance was relatively low compared to expected detection rates due to the dry conditions, with species that flock generally being recorded in the largest numbers in sites where they occurred.

Some 148 species were recorded during censuses, comprising 132 species of native landbird, three species of introduced landbird and 13 species of native waterbird (Table S2). Waterbirds were only infrequently recorded in censuses, and more so in the Central Qld zone (38% of censuses) than elsewhere (12% Border Rivers, 0% Macquarie, 10% Southern NSW). The few species and individuals of waterbird recorded during censuses had little impact on overall comparisons of avian diversity among zones since census results were dominated by landbirds. Accordingly, and as terrestrial rather than aquatic habitats were the focus of this study, the remainder of the results refer to landbirds only. The 132 species of native landbird represented 56% of the 233 species that occur in the four cotton-growing zones (Barrett *et al.* 2003), although it must be noted that our surveys concentrated on the floodplain portion of these zones and did not sample the forested slopes and uplands where many bird species reside.

Landbird mean abundance varied from 21–27 birds per census among zones, with most birds recorded in Macquarie sites and fewest in the Border Rivers, but the difference was not significant. The evenness and Shannon–Wiener diversity of landbird assemblages declined from Central Qld (0.87 and 2.31, respectively) to southern NSW (0.81 and 1.86, respectively) but the differences between zones were similarly non-significant. Species richness, however, differed between zones ($F_{(3,193)} = 2.67$, $P \leq 0.05$, dispersion parameter for quasipoisson = 3.95), with most species in Central Qld (15.3 ± 1.6 species, mean \pm 1 s.e.m.) and significantly fewer species further south in the Border Rivers, Macquarie and southern NSW zones (9.1–11.0 species per site). Species accumulation curves showed a similar pattern, with the species pool declining from north to south.

Bird community composition varied significantly among cotton-growing zones (ANOSIM $R = 0.093$, $P < 0.01$), bird composition in the Central Qld zone differing from the three southern zones, whose bird assemblages overlapped substantially. Although many bird species varied in abundance among zones, most differences were due to species being more abundant in some parts of their range than others parts, rather than species being beyond their distributional limits. Obvious biogeographical differences were apparent, however, with the red-backed fairy-wren (*Malurus melanocephalus*) and white-throated honeyeater (*Melithreptus albogularis*) only recorded in Central Qld (being out of range further south), and the yellow rosella (*Platycercus elegans flaveolus*) only recorded in southern NSW (not extending further north).

Bird abundance ($F_{(8, 188)} = 27.17$, $P \leq 0.001$, dispersion = 6.09), species richness per census ($F_{(8, 188)} = 2352$, $P \leq 0.001$, dispersion = 2.37) and Shannon–Wiener (SW) diversity all varied significantly ($F_{(8, 179)} = 26.22$, $P \leq 0.001$) among the nine vegetation types. All of these measures were maximal in river red gum sites and minimal in grassland communities. Grassland was the only vegetation type in which no birds were recorded during the two census periods (nine of 39 sites). The remaining vegetation types had intermediate bird abundance, richness and diversity, and significantly greater values than grassland. Five vegetation types approached river red gum in terms of abundance, richness or diversity, notably brigalow (in all three metrics), black box and revegetation (in abundance and SW diversity), belah (in richness and SW diversity) and poplar box (in abundance).

Coolibah and myall, on the other hand, all had lower bird abundance, species richness and SW diversity than river red gum.

Bird community composition differed with vegetation type (ANOSIM $R = 0.298$, $P = 0.001$). Post-hoc tests revealed that river red gum differed in bird composition from all other vegetation types, and about half of the remaining vegetation types differed in composition from each other ($P < 0.05$). Australian magpies occurred frequently (>20% occurrence) in all vegetation types, and several other species occurred in most habitats (e.g. yellow-throated miner, crested pigeon, pied butcherbird and galah; Table 4), each vegetation type was generally characterised by at least two and generally several bird species that were distinct to it or at most to one or two other habitat types. Bird species recorded frequently only in river red gum included peaceful dove (*Geopelia striata*), whistling kite (*Haliastur sphenurus*), brown treecreeper (*Climacteris picumnus*), noisy friarbird (*Philemon corniculatus*), white-breasted woodswallow (*Artamus leucorhynchus*), restless flycatcher (*Myiagra inquieta*) and fairy martin (*Petrochelidon ariel*). At the other extreme, bird species characterising depauperate grassland communities included white-winged fairy-wren (*Malurus leucopterus*), crested pigeon, Australasian pipit (*Anthus novaeseelandiae*) and nankeen kestrel (*Falco cenchroides*). The constrained ordination that accounted for the joint effects of geographic zone and vegetation type shows the dominant gradients in bird assemblage composition across the study region. The first axis represented the major faunal transition between central Queensland and the three zones to the south and explained a slightly higher amount of variance than the second. The second axis represented the dominant landform and vegetation sequence within zones, with riparian river red gum sites at one end of this gradient and grasslands at the other. The two factors explained 17.5% of the variance of the chi-square transformation data table, and the overall model, the first seven constrained axes, and the partial effects of each factor were all highly significant ($P < 0.001$).

Second bird paper

Rainfall

In the 12 months preceding the 2007 surveys, rainfall in the eastern and western half of the study area was below the long-term average by 113 and 38 mm, respectively, while cumulative rainfall for the two preceding years was 6 mm above average at Boggabri in the east but 114 mm below average at Walgett in the west. In the 12 months preceding the 2014 surveys, rainfall was 133 and 199 mm below average in the east and west, respectively. This was the second dry year in a row in the region, with rainfall being 271 mm and 391 mm below average in Boggabri and Walgett, respectively, over the two preceding years.

Species richness

We recorded 94 landbird species across all sites and both surveys, 82 species in period 1 and 81 species in period 2, and 86 species just in the RRG/Cool sites (78 and 70 species in periods 1 and 2, respectively). Mean species richness per site (± 1 SE) was similar in both periods for all sites (11.1 ± 0.77 and 10.3 ± 0.83 , respectively) as well as just for the RRG/Cool sites (13.6 ± 1.07 and 12.7 ± 0.92 , respectively). Paired *t*-tests of the difference in species richness per site between years were not significant for either dataset. Species richness per site, however, was positively correlated between the two periods (all sites: $r = 0.70$, $P < 0.001$, $n = 56$; RRG/Cool sites: $r = 0.78$, $P < 0.001$, $n = 29$).

Abundance

Although 10–14% more birds were observed in total in 2007 than 2014 (2700 vs 2435 individuals across all sites and 1913 vs 1641 birds in RRG/Cool sites, respectively), paired *t*-tests indicated that bird density per site did not differ significantly between the two periods (all sites: 12.1 ± 1.15 vs 10.9 ± 1.12 birds ha^{-1} ; $t_{55} = 1.373$, ln-transformed, $P = 0.18$; RRG/Cool: 16.5 ± 1.66 vs 14.1 ± 1.45 birds ha^{-1} ; $t_{28} = 1.540$, ln-transformed, $P = 0.13$). Across all sites, 50 species (53%) were more abundant in 2007 and 38 (40%) were more abundant in 2014, with only four species varying significantly in density between the two surveys: Galah and Cockatiel were more abundant in September 2007 while Crested Pigeon and Sacred Kingfisher were in greater numbers in October–November 2014. Considering only the RRG/Cool sites, 52 (60%) species were more abundant in 2007 versus 32 (37%) species in 2014, with just seven species varying significantly in abundance: in

addition to the Galah, Cockatiel and Sacred Kingfisher already noted, Grey Shrike-thrush was more abundant in 2007 whereas Noisy Friarbird, Pied Butcherbird and Willie Wagtail were in greater numbers in 2014. Bird density per site was correlated between the two periods, varying consistently among sites in both datasets (all sites: $r = 0.47$, $P < 0.001$, $n = 56$; RRG/Cool: $r = 0.45$, $P < 0.05$, $n = 29$).

Turnover

Mean minimum species turnover per site was 69% considering all 56 sites, and 63% for just the RRG/cool sites. For both datasets, turnover of sedentary species was significantly lower than for migratory and nomadic species (Tables 2, 3), which did not vary in turnover. Species turnover varied significantly among habitats, with river red gum (60%) and coolibah (64%) having lower turnover than other habitats (74–79%; ANOVA, $F_{5, 50} = 3.320$, $P = 0.011$).

Species distribution

For species that were recorded in both surveys, the mean difference in distribution between surveys among all 56 sites and among the RRG/Cool sites was 70% and 66%, respectively. This means that of all the sites that a species was recorded in either survey, on average it occurred in 30% of all 56 sites during both surveys and in 70% in only a single survey. The mean difference in distribution was significantly greater for migratory and nomadic species with intermediate and high values of D , respectively, than for sedentary species with lower values of D . Migratory species had significantly lower values of D than nomadic species across all sites. One species, Eastern Yellow Robin, had a D -value of 0%, occurring in the same RRG/Cool site in both years. However, a further eight species – Rock Dove, White-bellied Sea-Eagle, Little Corella, White-winged Fairy-wren, White-plumed Honeyeater, Zebra Finch, Horsfield's Bronze-Cuckoo and White-throated Gerygone – showed the maximum matching possible given varying numbers of site records in the two surveys. Little Corella and White-winged Fairy-wren were observed at four sites in 2014 and these sites had all been occupied in 2007, and this was the case for the 14 sites occupied by White-plumed Honeyeater in 2007 (16 site occurrences in 2014). In the five remaining cases, the species was only detected at one site in either 2007 or 2014. By comparison, Grey Fantail had the most strikingly different distribution between the two surveys, being detected at 11 sites in 2007 and none in 2014. Of those species detected at one or more sites in both surveys, Cockatiel had the most improbable discrepancy in distribution across years, being observed at 24 sites in 2007, four in 2014 and with only one site in common.

Randomization tests showed that of the 69 species recorded across the 56 sites in both surveys, 24 species had a significantly smaller difference in distribution, D , among sites than expected if their redistribution had been random. None had a significantly higher D than expected by chance. Of the 24 species, 20 were sedentary species (of 43 sedentary species), three were migratory (of 14), and one was nomadic (of 12 nomadic species). Considering just RRG/Cool sites, 17 of the 62 species recorded in both years in these habitats occurred in more of the same sites than expected due to chance alone (upper 2.5% tail). These species were largely a subset of the 24 species above, with one difference: the sedentary Magpie-lark was site-faithful in RRG/Cool sites but not across all sites.

We also used the hypergeometric distribution to identify species exhibiting significant distributional overlap between years. Across all sites, 26 species showed a greater degree of co-occurrence at sites across the two periods than expected by chance alone at $P \leq 0.05$ (23 species at $P < 0.025$). Again, no species exhibited the reverse pattern of significantly avoiding previously occupied sites in the second survey, leaving 43 species that were no more likely to be recorded during survey period 2 in sites in which they had occurred during the first survey than expected by chance. The hypergeometric test confirmed the identity of 23 of the 24 species above as being improbably likely to occur at the same sites in 2014 where they were detected in the first period. At RRG/Cool sites, 20 species were more likely to occupy the same sites in both surveys than due to chance alone ($P \leq 0.05$; 17 species at $P \leq 0.025$), including the 17 species identified using Monte-Carlo randomization tests.

Assemblage structure: bird abundance

The composition of bird assemblages based on abundance data at all sites differed slightly but significantly between years (ANOSIM, $R_A = 0.036$, $P = 0.019$), while that at the RRG/Cool sites did not ($R_A = 0.004$, $P = 0.342$). When we excluded four migratory species that were either on passage through the region in 2007 (Grey Fantail, Cockatiel) or had taken up residence in the region by the time of the 2014 survey (Dollarbird and Sacred Kingfisher), the ANOSIM result for all 56 sites was not significant ($R_A = 0.012$, $P = 0.159$), meaning that the bird assemblages did not differ significantly between years once these four migratory species were removed. For all sites, the average Bray–Curtis dissimilarity between survey periods was 87.5, and the within-survey-period dissimilarities for periods 1 and 2 were 86.4 and 86.6, respectively. By contrast, the mean dissimilarity between bird assemblages for the same sites in survey periods 1 and 2 was only 64.5. Similarly for the RRG/Cool sites, the average Bray–Curtis dissimilarity between survey periods was 80.7; the within-group dissimilarities for survey periods 1 and 2 were 80.9 and 79.2, respectively, whereas the mean dissimilarity in bird assemblages at each site between survey periods was only 53.3. In other words, the strongest signal in both datasets was the site self-similarity across time (35–47%) compared to otherwise low, matrix-wide, between-survey similarities of 13–19%.

Assemblage structure: presence/absence

Bird assemblage composition across all sites based on species incidence also differed significantly between years ($R = 0.034$, $P = 0.020$) but the RRG/Cool sites did not ($R = 0.024$, $P = 0.146$). Again, when the four migratory species above were excluded, the ANOSIM for all sites was not significant ($R_A = 0.011$, $P = 0.169$), meaning that in the absence of the four migratory species, the bird assemblages were similar in the two survey periods. For the full dataset, average Bray–Curtis dissimilarity between years was 78.8; within-year dissimilarities for 2007 and 2014 were 77.2 and 77.7, respectively, while mean dissimilarity between site bird assemblages across the two years was only 54.7. The same pattern was seen for RRG/Cool sites, with an average dissimilarity between survey periods of 71.9; within-group dissimilarities for survey periods 1 and 2 of 70.3 and 70.2, respectively, but a mean dissimilarity between bird assemblages for each site across time of only 46.6. Hence, the strongest signal in the incidence data was again the site self-similarity across time (45–53%) compared to otherwise low similarities among sites within and between times (21–28%).

Mantel tests, matrix regression and ordination

Mantel tests of the correlation between the bird assemblages in 2007 and 2014 across all sites and just for the RRG/Cool sites were highly significant for both bird abundance and presence-absence data sets (Table 6). Partial Mantel tests accounting for geographic distance between the sites were also highly significant, with all $R_p > 0.16$. The partial Mantel correlograms of the residuals showed no evidence of fine-scale spatial autocorrelation, i.e. residuals were not significantly positively correlated with distance over the first distance class (there were no significant distance classes in the 2007 datasets; there were one or more significant negative lags at the third or greater distance classes in the 2014 datasets reflecting nonlinear fits in the 2014 assemblage-geographic distance relationships).

With the Bray–Curtis distance matrices of 2007 assemblages fitted first in the six regression models (three habitat combinations by abundance and presence-absence), the partial effects of habitat were highly significant in models using all sites ($P < 0.001$), less significant ($P < 0.01$) in models combining black box, grassland, myall and revegetation, and not significant in either model of RRG/Cool. The partial effects of geographic distance were fairly weak but significant ($P < 0.05$) in all models. While significantly correlated in all models, the strength of the relationship between the two assemblage matrices varied depending on the combination of habitats tested. The effects of the 2007 assemblages on those observed in 2014 was significant in all cases, and strongest for the RRG/Cool combination (model $r^2 > 0.35$), compared with models for all habitats (model $r^2 > 0.19$) and the combination of black box, grassland, myall and revegetation (model $r^2 > 0.13$); also, the abundance models had stronger relationships than presence-absence models, accounting for an additional 20% variance in the RRG/Cool model. The ordination of the full dataset in two dimensions showed that, although the 2007 sites tended to plot above (i.e. more positive on the y-axis than) the 2014 sites, the sites in both survey periods were largely intermingled, meaning that similar bird assemblages were observed in both years. This fact, combined with the significant Mantel and

partial Mantel tests and matrix regressions, and earlier results of species distribution, demonstrated a pronounced degree of site self-similarity in our dataset, despite the high levels generally of minimum turnover (T), change in species' distribution (D) and compositional (Bray–Curtis) dissimilarity.

Lippia paper

Soil moisture

Mean volumetric soil moisture content varied between 42.23 ± 0.31 (T_1) and 37.80 ± 0.35 (T_2) in the wet treatments, and 30.08 ± 0.55 (T_1) and 19.73 ± 0.91 (T_2) in the dry treatments. At T_1 , there were significant differences in moisture content due to both moisture treatment ($F_{1,36} = 515.19$, $P > 0.0001$) and lippia cover ($F_{5,36} = 4.04$, $P = 0.0052$), but the interaction was not significant. In this instance, the 0% lippia cover treatment showed significantly lower moisture content compared to all other cover treatments, and the dry treatment showed significantly lower moisture content compared to the wet treatment. At T_2 , the effect of lippia cover was no longer significant and again, the interaction between lippia cover and moisture treatment was not significant. The significant moisture treatment ($F_{1,36} = 373.13$, $P > 0.0001$) again indicated that the wet treatment had higher moisture content than the dry treatment. When the loss of moisture between T_1 and T_2 was examined, there was a significant difference due to moisture treatment only, with the difference between T_1 and T_2 being greater in the wet rather than the dry treatment ($F_{1,36} = 68.26$, $P > 0.0001$).

River red gum germination

The mean number of river red gum germinants differed due to both moisture availability and lippia cover as illustrated by a significant moisture*cover interaction ($F_{5,36} = 2.48$, $P = 0.05$, Fig. 1). In the wet treatment, the highest germination was recorded in the low lippia cover treatments, i.e. 0%, 10%, 25% and 50% lippia cover returned the highest germination counts. In the dry treatment, the highest germination was recorded at intermediate levels of lippia cover, with the 25%, 50% and 75% lippia cover treatments recording similarly high germination. Significantly lower germination was recorded in the wet treatments in the 75% and 90% lippia cover classes, and in the dry treatment in the 0%, 10% and 90% lippia cover classes.

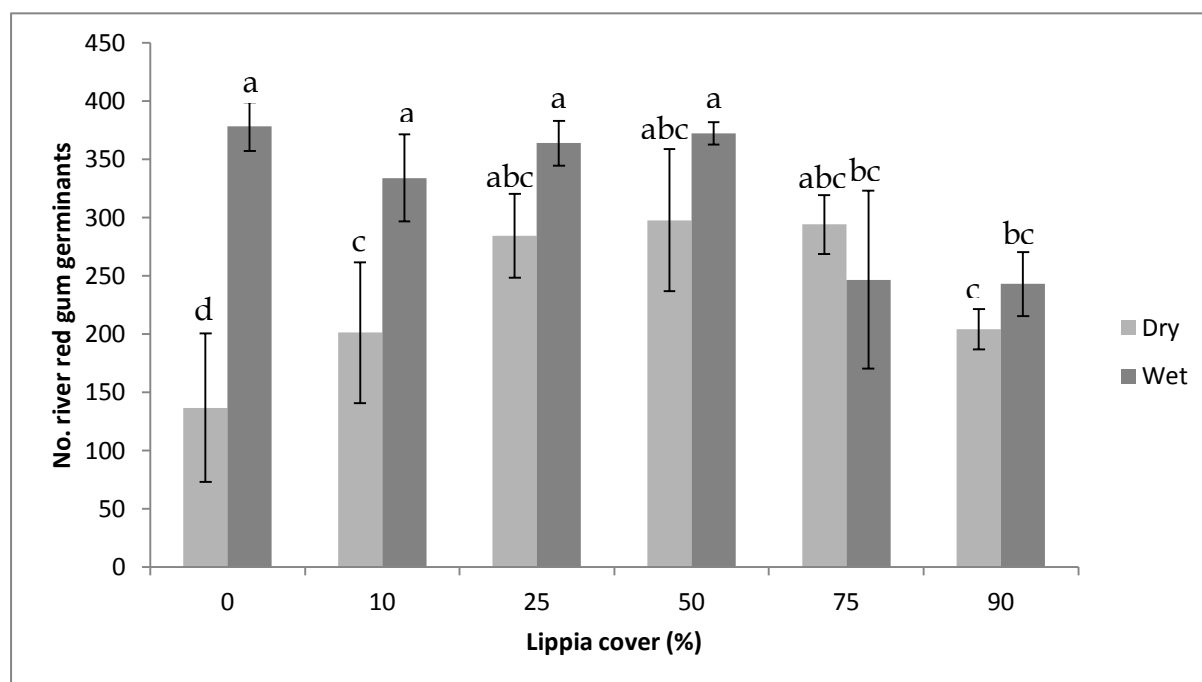


Fig. 1: Mean number of river red gum germinants (± 1 SEM) in each lippia cover and moisture treatment combination. Lower case letters denote significantly different groups of means (LSD).

River red gum height

The mean height of river red gum seedlings differed due to both moisture availability and lippia cover as shown by a significant moisture*cover interaction ($F_{5,36} = 3.56$, $P = 0.01$, Fig. 2). Seedlings in

the wet treatment were consistently taller than those in the comparable dry treatment in almost all of the lippia cover classes, except when there was no lippia cover and the wet and dry treatments produced seedlings of similar height. Seedling height also differed significantly between lippia cover classes, with a big reduction in seedling height between the 0% and 10% lippia treatments and a steady decline in mean height with increasing lippia cover in both the wet and dry treatments.

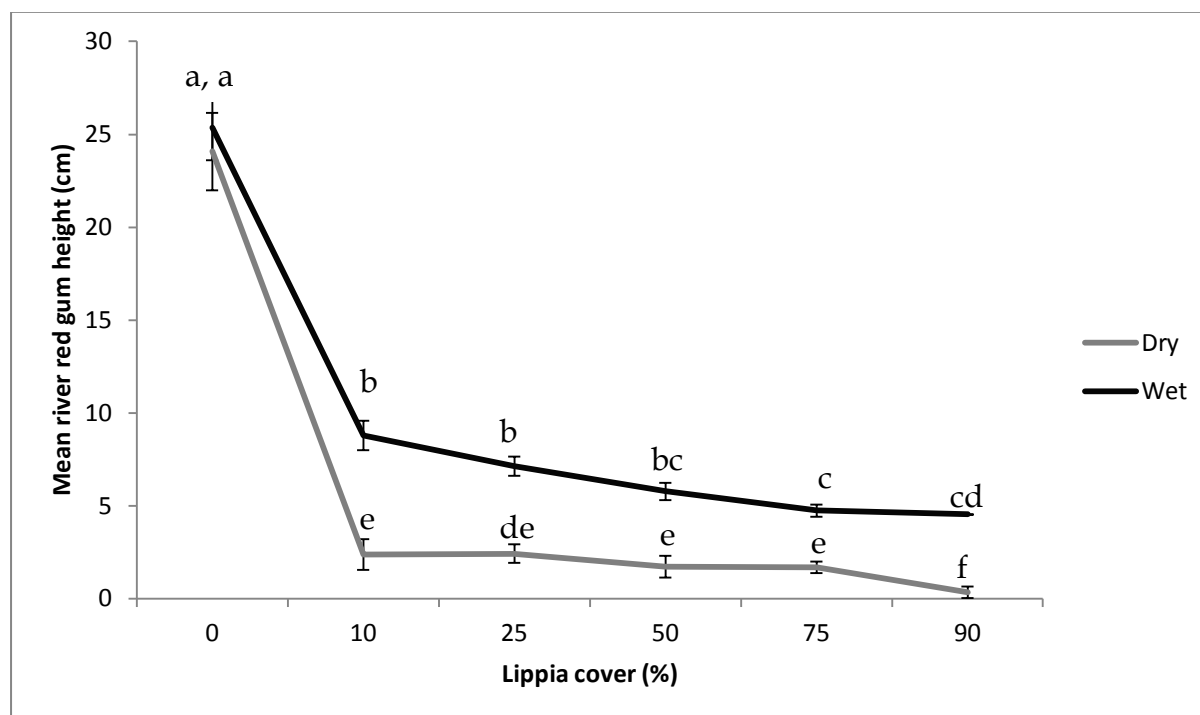


Fig. 2: Mean river red gum seedling height (± 1 SEM) in each lippia cover and moisture treatment combination.

Seed germination paper

Seed weight

Seed weight decreased as leaf damage and canopy density increased and water availability to the parent tree decreased (Table 1). There was also evidence of differences in seed weight among sites.

Table 1: Regression coefficient estimates and associated p-values from marginal t-tests for explanatory variables remaining in simplified models for seed weight, mean germination time and germinant proportion on day 20. n/a means variable did not remain in simplified model.

	Seed weight		Mean germination time		Day 20 germination	
	Estimate	Pr(> t)	Value	p-value	Estimate	Pr(> z)
(Intercept)	41.197	<0.0001	1.385	<0.0001	3.935	<0.0001
Canopy density	-0.063	0.029	n/a	n/a	-0.029	<0.0001
Leaf damage	-0.128	<0.0001	n/a	n/a	-0.008	<0.0001
Water availability	-2.953	<0.0001	0.071	0.016	-0.288	<0.0001
Water availability^2	n/a	n/a	-0.010	0.088	n/a	n/a
Site B	2.102	0.115	0.077	<0.0001	0.342	<0.0001
Site C	2.610	0.047	0.060	<0.0001	0.183	0.018
Site D	-2.235	0.055	0.045	0.002	0.499	<0.0001
Growth cabinet THREE	n/a	n/a	0.020	0.088	-0.124	0.040
Growth cabinet TWO	n/a	n/a	0.082	<0.0001	-0.043	0.483

Seed germination

Mean germination time (MGT) increased non-linearly with decreasing water availability to the parent tree, reaching a plateau for the lowest levels of water availability (Table 3). There was also evidence of differences among sites and growth cabinets. Adding seed weight to the final model of

parental drivers significantly improved the model ($p < 0.0001$) and water availability became insignificant, indicating that the effect of water availability to the parent tree on MGT was probably acting largely via changes in seed weight.

Germination to day 20 decreased as canopy density and leaf damage increased and as water availability to the parent tree decreased (Table 1). There was also evidence of differences among sites and growth cabinets. Adding seed weight to the final model of parental drivers significantly improved the model ($p < 0.0001$) but the other parental drivers remained significant, indicating that the effect of water availability to the parent tree on germination to day 20 was probably acting via other mechanisms as well as changes in seed weight.

Seedling vigour

Leaf area increased as seed weight increased ($p < 0.0001$), as did leaf weight ($p = 0.008$), seedling stem diameter ($p < 0.0001$), and seedling height ($p < 0.0001$), and there was no evidence of any further effects due to other parental factors (Figure 3).

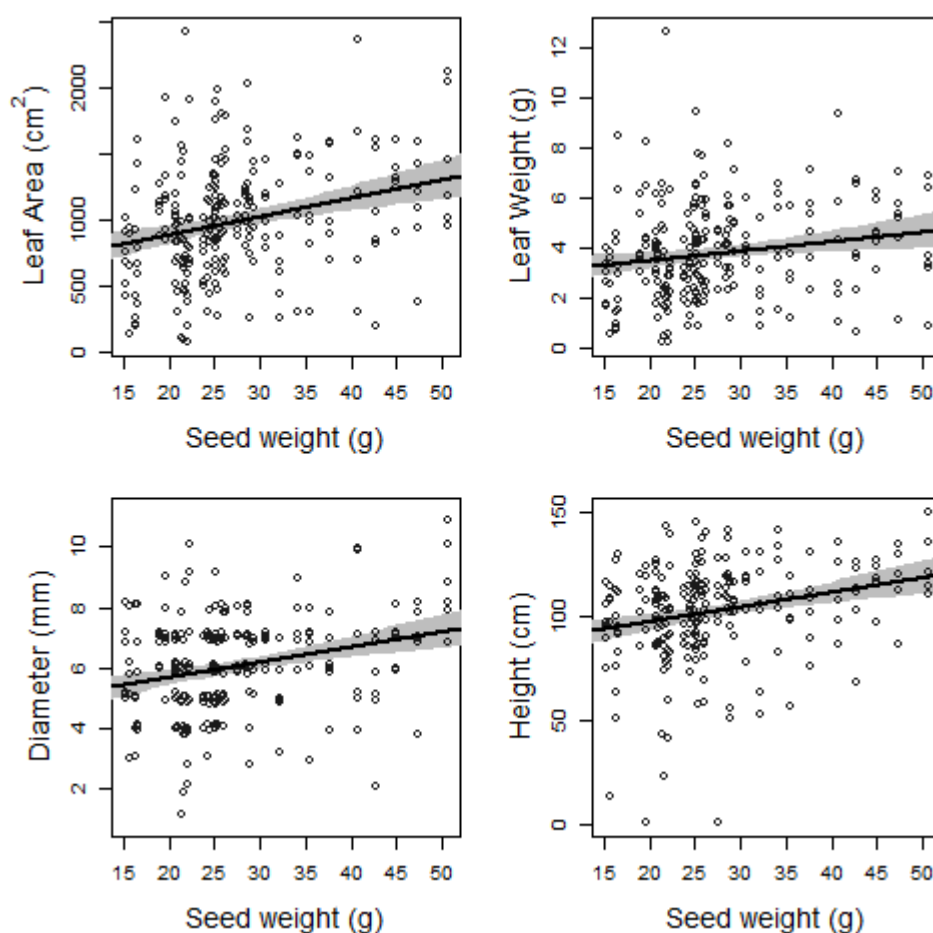


Figure 3: Total leaf area, weight, diameter and height plotted against seed weight for all seedlings. Each point represents observed measures for one seedling. The solid line and the shaded ribbon show the predicted mean and the 95% confidence interval in the mean according to the fitted linear model.

Outcomes

4. Describe how the project's outputs will contribute to the planned outcomes identified in the project application. Describe the planned outcomes achieved to date.

This project sought to investigate factors contributing to dieback and low recruitment rates of river red gums in cotton-growing regions, and determine management actions that growers can take to increase riparian ecosystem health, and reverse the current trend of declining ecosystem health. We identified a range of factors contributing to tree dieback on cotton farms, including water stress,

insect (psyllid) herbivory, cockatoo-mediated defoliation of trees and low recruitment due to low seed quality and competition with lippia. A secondary objective of the project was to report on avian biodiversity on cotton farms, highlighting the value of riparian zones for biodiversity conservation and thereby confirming the importance of riparian zone management to improve ecosystem health.

This project highlighted a number of factors contributing to tree ill-health and low recruitment rates on cotton farms of the northern Murray-Darling Basin. Knowledge of these factors is vital to guide management of these ecosystems to improve tree health and biodiversity conservation value. A key output of this project is the production of a tree dieback identification and management guide that is now available through the Cottoninfo website (www.cottoninfo.com.au/publications/tree-dieback-guide). A key focus for management should be to increase tree cover by encouraging natural regeneration, or planting trees at highly degraded sites, to provide important ecosystem services and biodiversity conservation values and demonstrate commitment to sustainable environmental management on cotton farms.

First bird paper

The Australian cotton industry in eastern Australia spans four bioregions (Brigalow Belt North and South, Darling Riverine Plains and Riverina bioregions: Commonwealth of Australia 2015) and is partly located within a nationally recognised biodiversity hotspot (Commonwealth of Australia 2017). Twenty-six bird species of conservation significance were recorded on cotton farms in this study, including 16 species of declining woodland bird (Reid 1999), which are of particular concern due to the habitat loss, alteration and fragmentation associated with agricultural development (Robinson and Traill 1996; Ford *et al.* 2001; Ford 2011). Managing and restoring habitat for these species will benefit a range of species, both rare and common, and should allow them to persist in these landscapes. In addition to maintaining and restoring remnant vegetation, a program of revegetation incorporating structurally-complex habitat (planting both trees and shrubs) and increased connectivity through cotton-growing landscapes could significantly benefit these species (Lentini *et al.* 2011; Lindenmayer *et al.* 2012a,b).

The cotton industry is situated predominantly along inland rivers where many bird species are concentrated, particularly in semi-arid regions. Riparian communities dominated by river red gum in cotton growing areas are a clear priority for conservation and management in the first instance, owing to high species richness and bird abundance and the regional distinctiveness of the bird community. Some 19 species were found only in river red gum sites (recorded at 1–2% of sites; e.g. leaden flycatcher *Myiagra rubecula*, spotted pardalote *Pardalotus punctatus*, forest kingfisher *Todiramphus macleayii* and six species of waterbird), while the Vulnerable brown tree creeper and Declining restless flycatcher were found in greatest abundance in this vegetation type.

While riparian vegetation is a priority, sympathetic conservation management of a range of vegetation communities is required to maximise bird diversity at landscape and regional scale, with several of the surveyed vegetation types providing the sole regional habitat for various species. In addition, different habitat types in space and time are required by different species (Lambeck and Saunders 1993). A total of 12 species, including the black-chinned honeyeater and hooded robin (both listed as Vulnerable) and crested bellbird (Declining) were recorded only in coolibah woodlands (in small numbers), while five species, including the chestnut-breasted manikin *Lonchura castaneothorax*, ground cuckoo-shrike *Coracina maxima* and brown quail *Coturnix ypsilophora*, were found exclusively in grasslands. The presence of mistletoes in myall and belah favoured the occurrence of nectarivorous and frugivorous bird species, including the nationally Vulnerable painted honeyeater *Grantiella picta*.

While conservation management of remnant and regenerating native vegetation is important where it remains, we also found that mixed-species native plantings of shrubs and trees supplemented the value of remnant vegetation as bird habitat, and were utilised by large numbers of birds.

Revegetation was second only to river red gum in the number of birds found at sites, with a similar Shannon–Wiener diversity. Three species (Australian king parrot, southern whiteface and southern boobook) were only recorded in revegetation. This result validates the efforts of landholders

undertaking revegetation on of their properties, demonstrating value for biodiversity conservation and return on investment as found by many prior studies (e.g. Barrett *et al.* 2008; Cunningham *et al.* 2008; Munro *et al.* 2011; Lindenmayer *et al.* 2012a).

How should the cotton industry respond?

This study confirms the value of native vegetation and revegetation for avian biodiversity in cotton-growing landscapes, and suggests that intensive irrigated crop production and conservation can go hand in hand. Cotton farmers have the opportunity to demonstrate environmental stewardship by protecting and managing remnant vegetation in riverine and floodplain ecosystems for biodiversity and ecosystem, service values, re-establishing native vegetation where appropriate, monitoring native birds and enhancing the conservation outlook for declining species in the agriculturally and ecologically productive floodplain landscapes occupied by the industry. Cotton farms in Australia vary in size and ownership, from <1000 ha to more than 50 000 ha, with a mixture of both family-owned and large corporate holdings. A recent survey of cotton growers indicated that the average farm consists of 68% irrigated and dryland cropping, 26% grazing and 5% ungrazed native vegetation (Roth Rural 2013), with the area of native vegetation declining from north to south. A subsequent survey indicated that 68% of cotton farms have riparian areas, with an average length of 7.5 km of creek or river frontage (Roth Rural 2014). Approximately 55% of cotton growers have livestock on their farm at least some of the time, but 18% of growers completely exclude stock from riparian zones and a further 54% of growers exclude stock from riparian zones most of the time (Roth Rural 2014). How much native vegetation is required to maintain biodiversity in agricultural landscapes and how should it be managed?

Smith *et al.* (2013) suggested a farm should be made up of no more than 30% high-intensity production (e.g. cropping and sown, fertilised pastures), with the remaining landscape made up of at least 10% native vegetation managed for conservation, 20% native vegetation managed for production (such as native forestry or grazing), and no more than 40% moderate intensity production (e.g. some tree clearing to increase grazing production), in order to maintain biodiversity and healthy, functioning environments. It will be up to the cotton industry and individual growers to decide whether these guidelines are achievable, given the real-estate value of irrigated floodplain and cropland in inland eastern Australia, and to demonstrate alternative means of achieving regional biodiversity conservation objectives where the 30:10:20:40 guidelines of Smith *et al.* (2013) are not adhered to. Research in southern NSW has shown that woodland revegetation increases the number and diversity of birds on farms, regardless of the amount of remnant habitat, even in largely cleared farmscapes (Lindenmayer *et al.* 2018). Given that most cotton farms have at least some remnant vegetation, efforts should be made to encourage growers to implement revegetation projects to increase the extent and connectivity between remnant vegetation patches, no matter how little native vegetation each farm might have. The popularity of the cotton industry's *Birds on Cotton Farms* (Ford and Thompson 2006) project suggests that birds will be a successful focus for the industry to encourage sympathetic conservation management of remnant native vegetation as well as revegetation for biodiversity and other ecosystem service benefits.

Second bird paper

We repeated most of the analyses with our dataset that Maron *et al.* (2005) conducted, to compare our results as closely as possible with theirs and test whether bird assemblages in our study region demonstrated strong site self-similarity or, as they found, random redistribution of species among sites over time. Our results when compared with theirs demonstrated both similarities and differences. Some differences may be accounted for by the exceptionally dry year experienced in the Wimmera prior to the first survey of Maron *et al.* (2005: 386), *e.g.*, the significant increase in species richness and almost threefold increase in mean abundance in their second survey. The most striking difference in results between the two studies, however, was the large and highly significant degree of site self-similarity in our study – 23% (all sites) and 27% (RRG/Cool sites) greater Bray–Curtis similarity in abundance composition, compared with just 4% greater similarity in same-site comparisons in the Maron study. Using presence/absence data, the disparity was even larger in our study, with 24% (all sites) and 35% (RRG/Cool sites) greater Bray–Curtis similarity for same-site

comparisons than the mean between-survey values, compared with an 8% greater self-site similarity reported by Maron *et al.* (2005). That is, ignoring changes in abundance, which can be substantial as we noted earlier, there was a high likelihood of finding the same species at riparian (RRG/Cool) sites in the Namoi valley when surveyed 7 years later (Bray–Curtis similarity = 53%). We also observe that there was a reasonably high likelihood of finding the same species at the buloke sites of Maron (*et al.* 2005), given the 48% Bray–Curtis similarity they reported for presence/absence data, a figure slightly greater than we obtained (45%) across all sites.

Maron *et al.* (2005) reported that community composition varied significantly between their two survey periods using abundance (but not presence/absence) data, and that turnover at sites (63%) and distributional change among species (68%) were high between the two surveys; also, only five species were reported to be site-faithful. Maron *et al.* (2005) attributed the randomness in species occupancy over time to the erratic and unpredictable nature of the Australian climate and the nomadism and migratory behaviour of Australian birds in response to fluctuating resource availability. We obtained very similar results for turnover (63%) and distributional change (66%) at the RRG/Cool sites (slightly higher across all sites), but in stark contrast to Maron *et al.* (2005) many species were site-faithful in our study, with 17 (Monte Carlo randomizations) or 20 (hypergeometric) species showing a significant degree of site fidelity to RRG/Cool sites. Even more species (24 and 26 respectively) displayed significant levels of site fidelity across the entire data set, but this increase would be anticipated given both the increased species pool and the greater range of habitat types – both the Monte Carlo randomization and hypergeometric tests are comparative in that the results are influenced by sites where species are not recorded, and so any tendency towards habitat preferencing by birds will increase the likelihood of detecting significant site fidelity. The fact that Maron *et al.* (2005) found significantly greater levels of among-site similarity of species' abundances within each survey period (mean of 31%) than between surveys (23%) is hardly surprising given the threefold increase in abundance in their second survey. Our ANOSIM results using abundances were not significant, once we had accounted for a few migratory species that were either in passage in 2007 or had settled by the time of the 2014 surveys conducted later in spring and, even with those migratory species included, the among-site similarity within a survey period (20% among the RRG/Cool sites) was similar to the between-survey mean similarity (19%; abundance data). More interesting is the higher level of abundance-based compositional similarity within each survey period found by Maron *et al.* (2005) within a single habitat, buloke (31%), than we found in two habitats (20%) and all six habitats (14%); the equivalent figure for just the other four habitats ($n = 27$ sites) was 19% mean similarity within survey period. The within-habitat, within-survey values we obtained with abundance data for our six habitats were: RRG - 29%; Cool - 20%; black box - 31%; grassland - 12%; myall - 17%; revegetation - 25%. These results show that table-wide faunal similarity decreases as different habitats are combined, and that habitat should be controlled for in investigations of this type. The site self-similarity percentage similarity values we obtained for each habitat, i.e. between surveys (and with the mean between-survey values, excluding the same-site comparisons, in brackets), were: RRG - 51% (27%); Cool - 44% (19%); black box - 29% (22%); grassland - 18% (11%); myall - 27% (15%); revegetation - 27% (19%). These results show that while the site self-similarity signal was always evident, it could be fairly weak (7-8% increase over the background between-survey similarity in three habitats), and as Maron *et al.* (2005) reported in buloke patches. The results for black box are incongruous in that the site self-similarity signal was slightly weaker than the between-sites, within-survey similarity, but this may have partly arisen from the close proximity of these five sites (maximum separation of 28 km between pairs of black box sites, with four sites within 5 km of each other), such that local conditions at the time of survey, particular to each period, could have had a more uniform, and homogenizing, influence on assemblages.

The Mantel tests and results of distance-matrix regression, in conjunction with the similar species pools recorded in both periods, provide clear evidence, in some habitats at least, particularly river red gum and coolabah, that there was a high degree of site self-similarity in avifaunal composition from one survey period to the next. We argue that these results refute the alternative hypothesis of species *generally* redistributing themselves at random among sites, even though some species may have done so. At RRG/Cool sites, the full regression model accounted for 55% of the variance in the

2014 abundance dissimilarity matrix, and over 50% of this variance was explained by the observed distribution and abundance of species across these 29 sites in 2007 (Table 6: Mantel $R = 0.73$). In this model, the habitat classification, i.e. differentiating between sites dominated by river red gum versus coolabah, did not significantly influence 2014 assemblage composition, despite previously discussed results revealing seven percent more site fidelity at river red gum sites.

The distance-matrix analyses depart from earlier results in that abundance-based data sets, specifically log-transformed abundance, yielded greater model fit (as measured by the correlation coefficient or adjusted r^2) than presence-absence data, while noting that qualitatively the results are virtually identical. Earlier results had shown that Bray–Curtis similarity values, based on actual densities, were between 7% and 10% smaller than corresponding presence-absence similarities for within- and between-period comparisons, and that tablewide the mean similarity could be as low as 13% for abundance data across all sites. However, provided the same-site similarities are appreciably larger as they were in our study (23–27% higher), then the site self-similarity signal, will be evident using appropriate tests. The results of Maron *et al.* (2005) did not conform strongly to this pattern, with only 4% (abundance) and 8% (presence-absence) greater similarity than the overall between-survey similarity, but nor did three of our habitats, namely black box, grassland and revegetation (7–8% greater similarity, using abundances). We argue that grasslands and successional revegetated sites are special cases where we do not necessarily expect a strong site self-similarity signal. Grasslands have few sedentary passerine species associated with them (Keast 1959; Cody 1985; Kinross 2004), and the structure and composition of grassland plant communities can vary greatly over short time scales due to rainfall, calendar season and grazing (and other disturbance) recent history (McIntyre & Lavorel 1994). These considerations are particularly applicable in the derived (disclimax) or otherwise highly modified grasslands surveyed in this study, and bird species richness was accordingly low (mean = 4.76, se = 0.76) and variable in composition. Bird community composition varies with successional stage in woody vegetation (Ford 1989), and time since establishment was an important predictor of bird assemblage composition in Victorian revegetation (Munro *et al.* 2011), and so it is unsurprising that we observed relatively low site self-similarity in this habitat. There may have been local factors at play in the case of the low site self-similarity observed in black box discussed above.

The role of bird movements

Reviews of the Australian avifauna have noted that the proportion of nomadic species in bird communities increases in drier environments (Keast 1959; Ford 1989), as in any environments with irregular pulses of resource production (Andersson 1980). Like Maron *et al.* (2005) we found that sedentary species showed significantly lower levels of distributional turnover than migrant and nomadic species, and so we anticipate that comparable studies of compositional change in arid regions ought to find lower levels of site self-similarity over time than we have reported. No studies of this type have been documented from arid Australia, but Cody (1994) reported remarkably high levels of compositional predictability in 20, widely dispersed, mulga (*Acacia aneura*) bird communities (located in arid and semi-arid regions of Western Australia, Northern Territory & Queensland), compiled from (single but extended) censuses conducted over a nine-year period. His results would suggest that mulga bird communities will demonstrate high levels of site self-similarity, particularly as most of his ‘core mulga’ species were sedentary passerines (Cody 1994). However, Recher (2018) presented contrasting results from repeat surveys at four mulga sites in Western Australia, with census and foraging studies conducted three years apart, under wet conditions in 1999 followed by dry conditions in 2002; there were considerable changes in assemblage composition and abundance, with fewer species generally and at lower abundance in the latter period, and with nomadic species accounting for most of the changes to community structure.

Although neither Maron *et al.* (2005) nor our study found a significant difference between migrant and nomadic species in the amount of distributional turnover, in both cases there was a trend for nomadic species to exhibit greater turnover than migrants, particularly in ours where the distributional change (D) across all sites was 90% for nomads, significantly higher than that of

migrants (at 76%), compared with 63% for residents. Australian bird movements are varied (Griffioen & Clarke 2002) and imperfectly understood, with some species displaying sedentary, migratory and nomadic behaviour within the one locality/population (*e.g.*, Olsen & Olsen 1987) as well as among locations and populations. We do not claim our classification is perfect, and the one nomadic species, White-browed Woodswallow that displayed significant site fidelity, has often been considered migratory in eastern Australia (Blakers *et al.* 1984; Higgins *et al.* 2006).

Complexity in movement behaviours is compounded by the spatial scale over which displacements occur (variable within and between species), while in many loosely migratory species different proportions of a population may move from one year to the next (*e.g.*, Chan 2001). Increasingly, patterns of bird movements are being shown to change systematically in response to both climate change (Chambers *et al.* 2005; Smith & Smith 2012) and the shifting distribution of resources in human dominated landscapes (*e.g.*, migratory frugivores in south-eastern Australian cities). Only long-term studies at multiple locations in contrasting environments will allow movement behaviours to be better classified and understood (Ford 1989), but if we accept that the concept of breeding migrant entails a regular return to the same geographic area in the breeding season (Roshier & Reid 2003), and that for some individuals of some migratory species, at least, a return to the same territory, migratory species ought to show greater site fidelity (lower T) and less distributional turnover (D) than nomadic species. Given many Australian migratory species have been found to return to the same breeding territory, *e.g.*, Rufous Whistler (Bell & Ford 1987; Bridges 1994), Golden Whistler (van Dongan & Yocom 2005) Sacred Kingfisher (Higgins 1999), Rainbow Bee-eater (Boland 2004), Fairy Martin (Magrath 1999) and Noisy Friarbird (Ford 1998) – all recorded in our study (Table S1), and most also occurring in the Wimmera region – future studies of this sort, with a sufficient number of observations (more sites and species), should find statistically significant differences in both D and T for migrants versus nomads. In fact, when we analysed the amount of turnover (T) as a binomial response to the combination of habitat type and movement category in the full data set using generalized linear mixed models (site as random effect, 145 non zero observations), the main effects were highly significant (both $P < 0.0001$), the interaction term was not significant, nomadic species contributed significantly more turnover than migrants ($P < 0.05$), and turnover was significantly less at RRG/Cool sites than the other four habitats.

A lot of the apparent turnover in these studies is caused by non-breeding birds that have been variously described as ‘floaters’, ‘casual’, ‘vagrant’ and ‘transient’ (*e.g.*, Ford 1989) – individuals which are passing through and stopping for a short time only, or which spill over from adjacent habitats (Cody 1994). Where the specific identity of these individuals differs from the sedentary and other breeding species present at sites during bird surveys, they will be a major source of variation in assemblages over time. Cody’s characterisation of species as ‘core’, ‘peripheral’ and ‘casual’ in mulga habitats, a classification gained from long-term study over a broad geographic extent, has potential for making greater sense of studies like ours, and resembles Hanski’s (1982) framework of ‘core’ and ‘satellite’ species (Hanski 1982) which is amenable to testing.

The perceived dynamism in assemblage composition might also be partly or even largely attributed to the stochastic vagaries of census techniques and sampling inefficiencies (including heterogeneous imperfect detectability among species: Boulinier *et al.* 1998; Kellner *et al.* 2018); detection probabilities are frequently below 50%. Contributing to this is the mismatch in spatial scale between census area (1-2 ha in the two studies compared here) and home-range area that can be an order or orders of magnitude greater (Reid 1999). In our study where two short visits (20 minutes each) were paid to each site, usually only one or two days apart, many species with large home ranges were unlikely to be encountered, even if the site were fully included in the home range. An indication of the magnitude of this stochastic variation is given by the Bray–Curtis dissimilarities between each pair of census conducted in 2014 – across all 56 sites the mean dissimilarity, calculated on presence-absence data, between censuses was 55.1% (46.5% at RRG/Cool sites). These values are virtually identical to the corresponding figures for between survey period, same-site dissimilarities (last line of data in Table 7). We conclude that sampling stochasticity probably accounts for much of the

apparent turnover between sites' assemblages over longer periods of time, and that the amount of stochasticity will be inversely related to the comprehensiveness of the census methods.

Random redistribution of most species vs high levels of site fidelity

In both studies, for most species recorded in both survey periods, the null hypothesis that a species was randomly distributed among sites in period 2 compared with period 1 could not be rejected. Of 54 species recorded in both periods, Maron *et al.* (2005) found only five that were significantly likely to be found at the same sites in the second survey. Two of these species, Brown Treecreeper and Yellow Thornbill, were also significantly site faithful in our study, and a third, Variegated Fairy-wren, was at the margin of significance ($P < 0.1$), while the others were either not recorded at all or too infrequently (Spiny-cheeked Honeyeater). We found many species, albeit a minority, that were significantly site faithful, *e.g.*, 25 of 56 species that were detected at two or more sites in both surveys. However, this strict frequentist approach belies the real situation. There were 37 species in our study with sufficient site records in both periods to enable us to examine their distribution in the 1000 Monte Carlo randomisations of expected number of matching sites in the two 50 percentiles of the null distribution. For only one species, Cockatiel, did the actual number of matching sites fall in the lower 50 percentile. That is, 36 of 37 species fell in the upper 50 percentile of the null distribution, of which 24 species lay in the upper 10 percentile. We conclude there was a strong tendency for most frequently recorded species to exhibit site fidelity. Infrequently detected species, dispersing birds, 'floaters', 'transients' and truly nomadic species, which do redistribute themselves across landscapes, independently of season, and according to the current distribution of food, mainly, and other resources (both within and outside of the study region), account for most of the noise in our data.

What should be the general expectation from repeat surveys?

The lack of significant differences in bird abundance and species richness between the two survey periods in our study was likely a consequence of the similar antecedent weather conditions, particularly rainfall, in the 1–2 years prior to our two surveys. This was fortuitous, and should not be a general expectation of Australian communities. Bird abundances at both species and community levels can vary markedly in response to fluctuating rainfall and the ensuing pulsed nature of resource flows, and the mass movement and irruptions of nomadic species where and when conditions permit are defining characteristics of the Australian avifauna (*e.g.* Keast 1959; Recher *et al.* 1983, 1985; McGoldrick & Mac Nally 1998; Burbidge & Fuller 2007; Smith 2015). Even in studies with large changes in abundance as reported by Maron *et al.* (2005) we expect the site self-similarity signal to be evident and highly significant with the application of appropriate (*e.g.* Mantel) tests. We argue that a high degree of site tenacity should be the norm for landbirds (particularly passerines) in most Australian habitats characterised by long-lived woody vegetation (see Introduction). We therefore believe the results of Maron *et al.* (2005) are anomalous in this respect, and that it would be timely to repeat the surveys at their sites.

We propose two explanations for the findings of Maron *et al.* (2005). First, it is possible that in highly fragmented landscapes, such as the buloke system in western Victoria, high rates of patch extinction in periods of severe drought (Reid 1999; Mac Nally *et al.* 2009; Watson 2011) could lead to marked changes in occupancy patterns, such that if recolonization events in the ensuing ameliorated conditions were at least partly random as is assumed by metapopulation theory (Hanski 1982), then this could constitute a reset of the patch-occupancy distribution which might then be relatively stable until another broad-scale major perturbation. Second, and linked to the first through island biogeography theory, given the extreme relictual nature of their study landscape – most of their sites were located in small (mostly <20 ha in size) buloke remnants, which were 'very isolated from other areas of native vegetation by cropping land' (Maron *et al.* 2005) – it is possible the remnants are comparable to oceanic islands and subject to high rates of both local extinction and immigration (MacArthur & Wilson 1967), explaining the essentially random re-assortment of bird assemblages across sites over 7 years. The second explanation differs from the first in predicting that patch composition will be found to be in a continual state of flux, and so the second theory could be readily testable with a renewed and extended round of surveys.

Recommendations for future survey design (timing, frequency, etc.) and analysis/interpretation of results

The two studies compared here (Maron *et al.* 2005 and our study) present contrasting hypotheses about the site-fidelity and stability of Australian landbird assemblages in the medium term (7 years). If the hypothesis of Maron *et al.* (2005) about the random re-assortment of site-based landbird assemblages generally holds over periods of several years, then many of the conclusions of studies of Australian landbird communities based on snapshot surveys are suspect. There are doubtless many datasets in existence that can be used to evaluate the competing hypotheses addressed in this paper, and we would encourage Australian ornithologists and ecologists to use our approaches based on matrix regression, Mantel tests and the hypergeometric distribution to interrogate their data to see which hypothesis is generally applicable. Given the popularity of bird watching and the quasi-professional approach of many amateur ornithologists, citizen science could be a useful means of exploring this question further. However, rigorous survey methods are required to ensure the integrity of the data collected.

Lippia paper

This study investigated the influence of moisture availability and increasing density of a widespread invasive weed species on germination and seedling growth of a keystone tree species found in riparian zones across much of inland eastern Australia. In this study, moisture availability and weed cover impacted river red gum germination and growth in somewhat predictable ways. Knowledge of the competitive interactions between invasive and native species in different scenarios can inform ecosystem management and restoration actions that improve ecosystem condition. In this instance, management of lippia may increase river red gum regeneration rates, particularly at those locations where large old remnant trees are senescing or succumbing to dieback and are currently not being replaced due to low recruitment rates. However, given lippia is an extremely successful weed, a high degree of continued human intervention is likely to be required to see significant improvements in natural recruitment rates.

Impact of moisture availability and weed cover on tree seed germination and growth

The germination data revealed a competition effect due to high lippia cover in the wet treatment, resulting in low river red gum germination and early survival. This was surprising and in disagreement with the suggestion of Richardson (1994) as the soil moisture data was not affected by increasing lippia cover in either the wet or dry treatments. Competition for light was likely as opposed to water, as suggested by Earle (2003), as the soil was saturated in the wet treatment and therefore not a limiting factor. The lippia plants in the wet treatment differed in their morphology forming a low, prostrate ground cover in the dry treatment, while in the wet treatment, a more erect form shaded the river red gum germinants and reduced light availability. Soil fertility is high in the riparian vertosol soils used for this experiment, so competition for nutrients (e.g. nitrogen) is unlikely (Smith *et al.* 2015). Competition also impacted the growth of seedlings once established in both the wet and dry treatments.

In contrast, the presence of intermediate levels of lippia cover appeared to facilitate river red gum germination in the dry treatments, with the highest germination rates recorded in the 25%, 50% and 75% lippia cover treatments. There may have been differences in temperatures and humidity with intermediate lippia cover that the river red gum seeds found conducive to germination and early survival. Once lippia cover progressed to 90% in the dry treatment, competition again reduced river red gum germination or early survival. Competition between plant roots for space may have reduced tree seed germination or early survival in the high lippia cover in both moisture treatments. Contrasting roles of competition and facilitation on germination and early survival have been documented previously and appear to be dependent on seasonal conditions and resource abundance. For example, when investigating competitive interactions between grasses and tree seedlings, Good *et al.* (2014) reported that tree seedling growth (but not survival) was impacted by competition when water was not limiting in a glasshouse trial, but survival and growth of tree seedlings was facilitated by grasses during winter on a grey vertosol floodplain. In other words,

when conditions were favourable, competition was the dominant process dictating plant survival and growth, but when conditions were stressful, facilitation processes became important.

Grime's C-S-R model states that there is a positive correlation between competition intensity and resource abundance, productivity and neighbour biomass (Grime 1988). This certainly rang true in the case of the germination of river red gum seed with increasing density of lippia in the wet treatment in this study. However, our seedling growth data indicated that competition was higher in the dry treatment once seedlings were established, with significantly lower mean height of seedlings in the dry treatments compared to the wet treatments. Davis et al. (1998) proposed that gross resource supply or neighbour biomass are not the important factors, but net water supply (i.e. gross water supply minus demand by herbaceous vegetation) is what dictates competition intensity. However, given there was no difference in soil moisture with increasing lippia cover, the current study does not support this theory. Neither Grime (1988) or Davis et al. (1998) fully explain the results found in this study. However, other authors (Tilman 1988; Grubb 1985) suggest that competition intensity may be high in stressful environments, as was found in our seedling growth data. All three points of view are required to explain the results recorded in this study, indicating that competitive interactions between invasive and native species are complicated. The notion that lippia may be allelopathic (Daley et al. 2005) has not been ruled out in this study. However, the fact that there was a facilitation effect with increasing lippia cover in the dry treatments that the impact of allelopathic chemicals (terpenes) produced by lippia is having no impact on river red gum germination.

Recommendations for management and restoration

Price et al. (2011a) indicated that restoring historic flood regimes could favour native species over the exotic lippia. As opposed to the current study, their research was conducted in a wetland situation where the ground may be inundated with water levels maintained above the soil surface for long periods of time. In the current study, both the native tree species and lippia responded similarly to high soil moisture conditions (not inundation). Regular thinning of lippia was required, particularly in the wet treatment, to maintain cover at the required treatment densities. As such, one might conclude that increasing soil moisture may favour increased lippia cover in the absence of other, more competitive native ground cover species resulting in no benefit to river red gum recruitment. On the other hand, if lippia can be eradicated from a site using long-duration floods (Price et al. 2011a), increased moisture availability will improve river red gum growth rates, as indicated in the current study with significantly taller tree seedlings recorded in the wet treatment. Strategic grazing management has been suggested as an alternative method to control or manipulate herbaceous species composition and reduce the dominance of exotic species (Davies et al. 2009; Fensham et al. 1999; Firn et al. 2010). However, initial investigations into the utilisation of grazing as a method to control lippia indicate that the response of lippia to rest from grazing pressure is complex, and certain grazing regimes may increase lippia dominance (Price et al. 2011c). As such, further research is required to determine whether grazing management can be used in lippia management. In the meantime, landholders continue to use herbicides and cultivation to manage lippia (Crawford 2008). While this is an effective method to reduce lippia dominance, these methods are harmful to other flora and fauna existing at the site and are expensive and labour intensive methods of control. A combination of herbicide control, direct seeding or tubestock tree planting and irrigation of seedlings to facilitate early establishment may be the most effective option to increase tree cover until alternative methods of lippia management, such as biological control or an effective grazing management regime can be found.

Study limitations

This study set out to investigate the competitive interactions between lippia and river red gums in the context of sites where the weed is already established and thriving, to explain low tree seedling recruitment rates. As a secondary aim, we wished to inform the level of effort required to improve germination and recruitment rates to ensure persistence of river red gums in these landscapes into the future. Lippia has already invaded large areas of riparian lands in the MDB, with low river red gum recruitment rates also observed in many of these sites. We did not examine lippia invasion at

sites already supporting established river red gum seedlings, and we did not investigate competitive interactions when both species germinate simultaneously, as may occur following prolonged flooding when ground cover is drowned out and the site may have little live herbaceous ground cover remaining. Future research may investigate these situations.

While we appreciate that undertaking this study in a glasshouse setting has limitations, we perceived there to be too many confounding factors to conduct this study in the field, at least initially. The biggest criticism of this study is likely to be that we have created an artificial environment with constrained soil space, and therefore we have forced these two species to interact and compete. As such, our results may be considered to be context-specific.

The results of this study show that lippia, where present, is likely to be contributing to low river red gum recruitment rates and is therefore not just an innocent by-stander. However, the impact of river regulation and reduced flood frequency is also a significant contributor, facilitating lippia dominance and resulting in low soil moisture status. Natural recruitment of river red gums is unlikely except in large or repeated flood events. Therefore significant input in the form of lippia control, tree planting and irrigation is likely to be the most appropriate approach to increasing tree cover in riparian zones in most parts of the MDB at this point in time.

Seed germination paper

Jensen et al. (2008) showed that semi-arid floodplain eucalypts (*E. camaldulensis* and *E. largiflorens*) produced less seed when impacted by dieback caused by moisture stress, with orders of magnitude difference in the number of seeds produced by parents from three different canopy density classes. The current study indicates that in addition to the impact of dieback on seed production already reported, the quality of the seed produced may also be compromised by dieback and moisture stress, with differences in seed germination rates and time to germination, as well as seedling vigour of offspring compromised as a result of inferior seed produced by dieback-affected trees. The implications of this study are significant for seedling recruitment and canopy recovery at sites impacted by dieback as a result of prolonged drought or rapid climate change, river regulation or floodplain development. Not only is the extant generation of trees impacted by dieback, but future generations may also be compromised, as well as restoration or plantation projects using seed sourced from moisture-stressed or dieback-affected parent trees. Data collected by Grose and Zimmer (1958) found no difference in germination responses of seedlots of river red gums collected from riparian vs floodplain populations and no influence of east-west climatic gradient on germination response, suggesting that the impacts of moisture stress reported in the current study are as a result of recent changes to resource availability or abiotic conditions as opposed to long term conditions under which the tree established.

Factors influencing seed size, germinability and seedling vigour

River red gum canopy cover naturally varies according to weather conditions, and leaf shedding is a common feature associated with short-term drought while shedding of large branches occurs during severe or prolonged water stress. This adaptation allows the trees to deal with variable soil moisture availability in heavy clay soils in the semi-arid regions they occupy (Roberts and Marston 2000). Canopy cover is restored through epicormic growth following cessation of drought conditions, but this process can be taxing following successive periods of stress and recovery as the tree depletes its carbohydrate reserves (Sala et al. 2012; Waring 1987). This process of stress and recovery may result in reduced seed production and smaller seed sizes as parent trees prioritise resources for functions relating to survival and restoring tree canopy, rather than reproduction (Landsberg and Cork 1997). This may explain the interesting result of decreased seed size and seedling leaf area as a result of increasing parent canopy density, as many of the canopies were composed of epicormics growth of various ages, but this was not recorded or analysed in this study.

Many of the trees included in this study had also been impacted by a recent widespread outbreak of psyllids in the region and were experiencing moisture stress at the time of seed collection, with a rainfall deficit recorded in the region during the period corresponding to seed set. As such, the trees

were struggling to restore their canopies following insect damage and may have been using carbohydrate stores to survive. The significant decrease in seed size as a result of increasing leaf damage and decreasing water availability also confirms that trees were allocating resources to canopy restoration rather than reproduction.

Fragmentation or isolation of populations brought about by tree dieback may also result in reduced quantity and quality of seed, increased inbreeding, and shifts in vegetation composition and structure (Broadhurst et al. 2017; Aguilar et al 2016). Given dieback and moisture stress results in reduced flowering and therefore pollen production, plant–pollinator interactions may be disrupted in a similar manner in dieback-affected stands to that following ecosystem fragmentation, leading to decreased pollination, increased inbreeding and therefore declines in seed production and seedling fitness (Aguilar et al. 2006; Broadhurst et al. 2017). Eucalypts are self-compatible, with a mixed mating system showing preferential outcrossing (House 1997). However, studies have shown that self-pollination results in production of fewer viable seeds and reduced seedling vigour (Burrows 2000). It is possible that tree dieback at the wider site or landscape scale is impacting pollinators, thereby resulting in a higher proportion of self-pollination, with subsequent impacts on seed germination and growth rates. Further investigation into outcrossing rates in dieback versus healthy stands of trees is required to improve our understanding of the impact of dieback pollination, offspring genetics and resilience.

Implications for management and restoration

The impacts of tree dieback and forest loss on both humans and natural systems are many and varied. Forests play a significant role in regulating Earth’s climate through their ability to sequester and store large amounts of carbon (Goodale et al. 2002; Pan et al. 2011), and exert a strong regulating force on local and global hydrological cycles (Bond et al. 2008; Myers 1997). Carbon emissions to the atmosphere as a result of large-scale forest loss are already and will continue to be an important driver of global land cover and climate change (DeFries et al. 2002). Further biodiversity loss and declines in the provision of a range of forest ecosystem services can be expected as forest dieback continues (Breshears et al. 2011; Trumbore et al. 2015). Simultaneously, loss of biodiversity, ecosystem functions and services such as natural pest control, pollination, seed dispersal and altered soil nutrient balances provide a feedback to accelerate tree dieback and reduce regeneration.

River red gums are an important ecosystem engineer, with the riparian ecosystems it dominates forming structurally complex, productive and biodiverse woodlands and forests in otherwise simplified landscapes and providing a number of important ecosystem services (Colloff 2014). River red gums are also an economically important species grown in forestry situations in a variety of climates around the world and favoured for their fast growth rates and hardwood timber qualities (Boland et al. 2006; Doran and Wongkaew 2008; Smith et al. 2017). Dieback will impact forestry and revegetation projects by downgrading seed quality and therefore seedling establishment and growth rates. Provenance considerations feature prominently in seed collection strategies for revegetation, particularly in regards to fragmentation and genetic implications (Byrne et al. 2011, Breed et al. 2012; Broadhurst et al. 2017). However, few studies have investigated the importance of broad parent tree health or stress parameters on revegetation outcomes.

Dieback compromises the ability of trees to regenerate naturally. Not only is there less seed produced, but the quality of the seed that is produced is compromised. In situations where dieback is impacting extant trees, human intervention may be required to restore tree cover using external seed sources. Effort should be made to restore natural disturbance regimes to support natural regeneration processes. However, in many cases this may be unrealistic, for example, restoring historic flood regimes will not be possible in many instances as competition for water resources between environmental and economic uses intensifies.

Dieback impacts both extant trees and future generations of trees by compromising both the quantity and quality of seed available for regeneration. This has serious consequences for global

forest cover into the future, with predictions of further climate change and therefore water stress and insect herbivory accelerating dieback processes. The impacts of forest loss on humans and other biodiversity through concurrent changes in ecosystem service provision make tree dieback a significant global issue worthy of further research and immediate action to address contributing factors. The feedback of further climate change and hydrological disruption as a result of forest loss should raise considerable concern amongst world leaders and policy makers.

5. Please describe any:-

- a) technical advances achieved (eg commercially significant developments, patents applied for or granted licenses, etc.);**
- b) other information developed from research (eg discoveries in methodology, equipment design, etc.); and**
- c) required changes to the Intellectual Property register.**

Updates to the IP register will be made as papers are published and made available to CRDC.

Conclusion

6. Provide an assessment of the likely impact of the results and conclusions of the research project for the cotton industry. What are the take home messages?

Tree dieback is a natural process, and highlight complex, with multiple interacting factors implicated, and contributing factors likely to be context-specific. Human management, including regulation of river systems, drawdown of groundwater aquifers, use of broad spectrum insecticide chemicals and introduction of competitive exotic species has exacerbated the frequency, extent and severity of dieback events, stressing trees further than they are evolved to cope with in already variable environments. Changing climatic conditions are also likely to intensify tree dieback processes, including increasing moisture stress and insect herbivory. Riparian ecosystems hold a variety of environmental, social and economic values. Unfortunately there is no silver bullet answer to immediately improve tree health and management is likely to be context-specific. However, a variety of management options are available to growers that wish to improve tree health, as outlined in the tree dieback identification and management guide available through Cottoninfo. Commitment to improving tree health on cotton farms for a variety of outcomes, including biodiversity conservation and ecosystem service provision, demonstrates the industry's dedication to sustainability and environmental stewardship.

Extension Opportunities

7. Detail a plan for the activities or other steps that may be taken:

- (a) to further develop or to exploit the project technology.**
- (b) for the future presentation and dissemination of the project outcomes.**
- (c) for future research.**

The extension opportunities already afforded this project have been many and varied. Presentations have been made at three conferences (2016 Regional Universities Network [RUN] conference in Rockhampton, Restore, Regenerate, Revegetate in Armidale in 2017 and the Australian cotton conference 2018), at two kayaking field days on the Murrumbidgee (2017), at the 2018 Royal Easter Show as part of the Farm of the Future exhibit, and on various occasions at the UNE SMART Farm to travelling dignitaries, including the NSW Governor David Hurley and Mrs Hurley (<http://au.ictinternational.com/governor-of-nsw-applauds-une-smart-farm/>) Prime Minister of Australia (Hon. Malcolm Turnbull - <http://www.armidaleexpress.com.au/story/4358133/business-innovates-on-global-stage/>), Deputy Prime Minister (Hon. Barnaby Joyce), Agriculture Minister (Hon David Littleproud), Rabobank meeting, Parliamentary Inquiry into Agricultural Innovation and to the then Agriculture Minister's Parliamentary Advisory Committee. Students undertaking the Cotton and the Environment unit as part of the industry's Cotton Production Course delivered at UNE have seen data collected as part of this project in lectures, as have students in various other units at UNE, include Ecosystem Rehabilitation and Fundamentals of Ecology. Media releases went out following the meeting with the Prime Minister and publication of a paper on carbon sequestration by river red

gums (from a previous project). Both media releases were picked up by various media organisations, including ABC Radio and various local and regional newspapers. Regular social media coverage (Twitter and Facebook) has documented project outcomes through various UNE (Cotton Hub, UNE Environment, UNE Agriculture and The University of New England) and industry (Cotton Rivercare) pages. Contributions have also been made to Spotlight magazine throughout the project and recently (2018) to the Australian Cottongrower magazine. A comprehensive dieback identification and management guide has been produced through Cottoninfo and NWLLS (see below).

**9. A. List the publications arising from the research project and/or a publication plan.
(NB: Where possible, please provide a copy of any publication/s)**

Four papers produced during this project are currently at advanced draft stage, with one paper (the first bird survey paper) currently undergoing a second round of reviews with *Wildlife Research*. The second bird paper will be submitted to CRDC for comment before the end of 2018, while the lippia paper and seed germination paper will be submitted to CRDC for comment in January 2019. A fifth paper on the causes of river red gum dieback will be submitted for publication before June 2019, with an early draft of this paper currently being finalised. The sixth paper reporting the outcomes of the restoration trial at Wallah will be attempted once data is available for that site. Copies of these publications will be provided to CRDC once finalised.

A seventh paper detailing environmental management at 'Kilmarnock' and the ecosystem service and economic benefits flowing from works undertaken by the Watson family has been submitted to CRDC and is also available from the Ecological Management and Restoration website:

<https://onlinelibrary.wiley.com/doi/10.1111/emr.12331>

B. Have you developed any online resources and what is the website address?

1. A tree dieback identification and management guide has been produced for CottonInfo and is available at: www.cottoninfo.com.au/publications/tree-dieback-guide

2. A recording of the presentation given at the 2017 Restore, Regenerate and Revegetate conference, along with presentations by various other cotton industry researchers (including Nancy Schellhorn, Cate Paul, Vesna Gagic, Sam Capon, Peta Zivic, Andrew Watson, Stacey Vogel, Heidi Kolkert and Karrar Al-Hajiya) is available at: www.une.edu.au/about-une/faculty-of-science-agriculture-business-and-law/school-of-environmental-and-rural-science/ers-news-and-events/restore-regenerate-revegetate-conference-2017

Part 4 – Final Report Executive Summary

Provide a one-page summary of your research that is not commercial in confidence, and that can be published on the internet. Explain the main outcomes of the research and provide contact details for more information. It is important that the Executive Summary highlights concisely the key outputs from the project and, when they are adopted, what this will mean to the cotton industry.

This project investigated a range of factors leading to dieback and low recruitment rates of river red gums in cotton-growing regions of the northern Murray-Darling Basin. Our research echoes the sentiments of previous research, confirming that multiple complex interacting factors are driving tree dieback and recruitment processes in these landscapes and many factors are context-dependent. Tree dieback is a natural process, however, human management, including regulation of river systems, drawdown of groundwater aquifers, use of broad spectrum insecticide chemicals and introduction of competitive exotic species has exacerbated the frequency, extent and severity of dieback events, stressing trees further than they are evolved to cope with in already variable environments. Changing climatic conditions are also likely to intensify tree dieback and recruitment processes, including increasing moisture stress and insect herbivory.

A dieback identification and management guide has been produced and is available through the Cottoninfo website (www.cottoninfo.com.au/publications/tree-dieback-guide). Growers are urged to

seek out that document for more information on how dieback can be addressed in specific situations. A range of scientific publications will be available in coming months and will be made available through CRDC.

Riparian ecosystems hold a variety of environmental, social and economic values. Unfortunately there is no silver bullet answer to immediately improve tree health and management is likely to be context-specific. However, a variety of management options are available to growers that wish to improve tree health, as outlined in the tree dieback identification and management guide available through Cottoninfo. Commitment to improving tree health on cotton farms for a variety of outcomes, including biodiversity conservation and ecosystem service provision, demonstrates the industry's dedication to sustainability and environmental stewardship.