Clumped planting arrangements can improve the ecological function of revegetated eucalypt woodlands

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A thesis submitted in fulfilment of the requirements for the degree of
Doctor of Philosophy

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October 2018

Table of Contents

Published and submitted works included in the thesis

McCallum KP, Lowe AJ, Breed MF and Paton DC (2018) Spatially designed revegetation – why the spatial arrangement of plants should be as important to revegetation as they are to natural systems. *Restoration Ecology* **26**:446-455 https://onlinelibrary.wiley.com/doi/full/10.1111/rec.12690

McCallum KP, Breed MF, Paton DC and Lowe AJ (Accepted October 2018) Clumped planting arrangements improve seed production in a revegetated eucalypt woodland. *Restoration Ecology*

Abstract

Revegetation is a key conservation activity in areas that have been extensively cleared and is undertaken in the hope it will prevent further species losses, mitigate land degradation and return functional ecosystems to degraded areas. Although revegetation has the potential to achieve these outcomes, the field is still relatively young and actively developing in terms of standards and best practice. As a result, the long-term viability, functionality and resilience of many re-planted systems remains uncertain. There have been calls for revegetation to move towards more ecologically informed designs and one way to achieve this is for plantings to mimic the composition and structure of natural vegetation. However, the outcomes of failing to undertake such practice is still poorly understood.

The spatial arrangements of plants are central to natural communities and influence the majority of ecological processes that occur. Consequently, the position of plants within revegetated sites may affect the long-term viability and resilience of these restored systems. Despite this, planting arrangements are rarely considered an important feature of revegetated communities, especially for variables other than overall planting density and this may limit the ecological value of revegetated communities.

The primary aim of this thesis was to examine how planting arrangements influence the ecological processes occurring within revegetated sites, with a focus on reproduction in woodland systems. I first review the available literature and synthesise information from natural ecosystems, plantation communities, and experimental plantings to identify ways plant arrangements may influence the ecological function of revegetated systems and highlight key knowledge gaps. The data chapters of my thesis then evaluate how planting arrangement influences pollination, seed production, plant mating patterns and patterns of gene flow in a revegetated eucalypt woodland in southern Australia. Following this, I document the arrangement of plants within remnant eucalypt woodlands and identify key features that can potentially be incorporated into revegetation design if projects seek to re-create more natural woodland plant arrangements.

I found that plant arrangements have the potential to influence a range of ecological processes, from those at the individual plant level (survival, growth), the population and community level (pollination, seed dispersal) and the ecosystem level more generally (habitat provision, erosion). My experimental results support these expectations and although plant reproduction was highly variable, the spacing between conspecifics and the degree of aggregation influenced seed production and plant mating patterns in the *Eucalyptus* species studied, whereas population abundance had little influence.

Taken together, these findings suggest that woodland revegetation should consider not only the number of each species to be planted, but also the fine-scale arrangement (conspecific spacing, aggregation) of those species, if reproductively productive populations are to be established. One way to achieve this is to re-create more natural plant arrangements, where aggregation is common and large distances between conspecifics are rare. The challenge is now to find ways to effectively incorporate spatially designed revegetation into the planning and planting phases of revegetation and then monitor the outcomes of this approach.

Declaration

I hereby certify that this work contains no material that has been accepted for the award of any other degree or diploma in my name in any university or other tertiary institution. To the best of my knowledge and belief, this work contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint award of this degree. The author acknowledges that copyright of published works contained within this thesis resides with the copyright holder(s) of those works.

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I acknowledge the support I have received for my research through the provision of an Australian Government Research Training Program Scholarship.

Signature: Date: 30-Oct-18

Acknowledgements

Thank you to my primary supervisor David Paton for taking me on as PhD student, for sharing your knowledge of eucalypt woodlands and for introducing me to the Monarto revegetation area. Thanks for all the opportunities and guidance over the past few years and for trusting my ideas and directions.

Thank you to my two co-supervisors Andy Lowe and Martin Breed.
Andy – thank you for agreeing to supervise me again and for pushing me to do more when you knew I could, but for also telling me when to stop when I needed it.

Martin – you were the unlucky one that got most of my first drafts, but I really appreciate your constant enthusiasm, encouragement and advice. Particularly, thank you for all your help with the genetics chapter and tips on data analysis for all the data chapters.

A very big thank you to my sister Chelsea for helping with the field surveys for my natural plant arrangement chapter. These surveys were super tedious, so I understand if you never help me with fieldwork again. But thank you for braving the 40°C days, the rain and the inch ants!

Thanks Aaron for supporting me over the last 3 years and for putting up with me and my very large collection of gumnuts (and for helping me get rid of them all!). Thanks for the days you helped me in the field, for the chats about statistics and for being patient with me, especially over the final weeks.

Over the course of my PhD I have been lucky enough to share an office with Hayley Merigot, Grace Hodder, Hannah Bannister, Casey O’Brien, Victoria McCarron, Tom Hunt, Amanda McLean and Melissa Jensen. I’m glad that I got to share my PhD journey with all of you. Thank you also to all the other members of the Paton and Lowe research groups and the Restoration group, particularly Monique Smith.

Thank you to the following organisations for believing in and supporting my research: BioR, Holsworth Wildlife Research Foundation, Nature Foundation SA, Field Naturalists Society of SA and the Australian Wildlife Society. Without your financial support my research would not have been possible. Thank you also to the Botanic Gardens of South Australia for the Noel and Vivian Lothian Scholarship which helped support me during the first year of my PhD.

Finally, thank you BioR for giving me the opportunity to put into practice what I learnt during my PhD and design the Frahn’s Farm revegetation site for 2017. It was fantastic to try out all the ideas that I developed during my PhD and test how feasible it actually was to manipulate the arrangement of plants during revegetation. It’s great to know that all this work amounts to more than a thesis and it has actually helped to shape a patch of revegetation.

Chapter 1. General introduction

Two-thirds of global terrestrial ecosystems have either been converted for human uses (i.e. agriculture, cities) or are heavily degraded, and as a result biological diversity and land and water health are compromised in many areas around the world (Suding 2011). In light of this, many ambitious targets have been set to restore huge areas of land (Suding 2011; McDonald *et al.* 2016a), such as 150 million hectares of disturbed and degraded land by 2020 under The Bonn Challenge (IUCN 2011).

In many degraded areas large-scale revegetation is required because the protection and restoration of remnant vegetation alone is not enough to prevent further species losses and mitigate land degradation (Vesk *et al*. 2008; Bradshaw 2012; Possingham *et al*. 2015). However, revegetation outcomes can be highly variable and there are concerns that even with best practice revegetation, plantings may fail to reach the desired levels of ecosystem function (Munro *et al.* 2009; Miller *et al.* 2017; Fielder *et al*. 2018).

The spatial arrangement of plants influence the majority of ecological processes that occur within plant communities and their component ecosystems (Dale 1999). The importance of these arrangements has been recognised for decades in natural ecosystems (Watt 1947), but in spite of this knowledge, the spatial positioning of plants for revegetation projects has rarely been considered (Miller *et al.* 2010), and this omission may influence the functional development of revegetated communities.

For example, the spatial arrangement of plant species within revegetated sites may directly affect the attraction, abundance and behaviour of pollinators (Ritchie *et al.* 2017). In animal pollinated species, plants growing at higher densities often receive more pollinator visits because the costs of moving between plants is lower (Kunin 1993, 1997a; McCallum *et al.* 2013). In contrast, dispersed plants may receive fewer pollinator visits, exhibit higher levels of selfing and suffer reduced seed set (de Jong *et al.* 1993; Butcher *et al.* 2005; Llorens *et al.* 2012), and this can limit population persistence (Lamont *et al.* 1993). Planting activities can result in revegetated populations being more widely spaced than natural populations (McCallum *et al*. 2018a), and this is a concern because it may limit the ability of these populations to become self-sustaining.

If pollination and seed production are limited in revegetated populations, population loss, subsequent declines in species diversity and reductions in habitat quality are a risk (Godefroid *et al.* 2011; Schneemann & McElhinny 2012), thereby reducing the ecological value of revegetated sites. Revegetation is a costly and labour-intensive process (Wilson & Lowe 2003; Smith 2008), so it is important that we learn as much as possible from plantings to allow on-going improvement of revegetation practices (McDonald *et al.* 2016b; Broadhurst *et al*. 2017a).

One area that is a priority for revegetation is the temperate eucalypt woodlands of southern Australia (Hobbs 1993; Broadhurst *et al*. 2017b). These woodlands were once widespread, but clearing for agriculture and grazing has resulted in these systems being almost completely lost from the landscape (Yates & Hobbs 1997). Tubestock planting and direct seeding are widely used in this region and it has been estimated that >180,000 ha of revegetation has occurred, with at least 63 million tubestock planted (Broadhurst *et al*. 2017b). *Eucalyptus* species generally dominate these plantings (Dorrough & Moxham 2005; Broadhurst 2013; Prober *et al.* 2016), and they are considered as ‘foundation’ species because they determine the habitat for many other species of plants and animals (Bennett 2016). However, despite the fundamental role that eucalypts play in revegetation across Australia, little is known about the reproductive performance of these populations and if reproductive fitness is influenced by planting arrangement.

Thesis aims and objectives

In this study, I investigate the role of spatial arrangement in woodland revegetation, with the overall research question – “Can manipulating the spatial arrangement of plants improve revegetation outcomes?”

To address this, the following questions are asked.

1. What do we currently know about the role of planting arrangement in revegetated systems and what ecological processes may be affected by planting layout?
2. Does population abundance, conspecific spacing and the degree of aggregation influence seed production, pollination, germination, mating systems and patterns of gene flow in a revegetated eucalypt woodland?

Then, in light of the evidence gathered in response to aims 1 and 2

3. What are the key characteristics of natural plant arrangements and how can they be incorporated into revegetation design?

Thesis structure

Following this general introduction, the main body of this thesis comprises six chapters. Here I briefly summarise each of these chapters and justify the flow of ideas from the review (Chapter 2), through the data chapters (Chapters 3-7), and then finally to the concluding chapter, which synthesises the findings, identifies the limitations of the study, and highlights areas for future research (Chapter 8). The thesis is comprised of a series of papers that have or will be submitted for publication and are written as stand-alone pieces of work. As a result, there is some inevitable overlap in the content of chapters and inconsistencies in style and formatting.

Chapter 2 is a review that has been published in *Restoration Ecology* describing how the spatial arrangement of plants can influence the ecological processes occurring within plant populations and communities, and their component ecosystems more broadly. It identifies current gaps in the knowledge, provides a series of recommendations for how the fine-scale arrangement of plants can be considered during revegetation and introduces the concept of spatially designed revegetation. Research into the role of planting arrangement is still in its infancy, so many gaps exist in the knowledge. However, this review identified a major gap that I tackle in my thesis – what is the influence of planting arrangement on plant reproduction (pollination, seed production and plant mating systems)?

Chapters 3-5 assess how different aspects of planting arrangement can influence plant reproduction in a revegetation context. In Chapter 3 (accepted for publication in *Restoration Ecology*), I evaluate seed production (seeds/fruit) as a function of population abundance (number of conspecifics within a 100 m radius) and nearest neighbour distance for six eucalypt species (*Eucalyptus leucoxylon*, *E. caesia*, *E. incrassata*, *E. platypus*, *E. stoatei* and *E. woodwardii*) in a 40-year-old revegetated woodland. Seed production was highly variable, but despite this variability seed set declined as the distance between conspecifics increased, whereas abundance had little influence on seed production. In light of these results, I focus my subsequent research on the role of plant spacing and conspecific aggregation.

In Chapter 4, I investigate whether reproduction is pollination limited in aggregated and dispersed *E. leucoxylon*. I did this work in the same 40-year-old revegetated woodland as in Chapter 3. On average, seed production was higher in aggregated trees, but both aggregation groups – aggregated and dispersed – showed signs of pollination limitation, with fruit and seed production increasing with addition of outcrossed pollen. However, these differences varied between seasons, most likely because of differences in flowering intensity across years. Germination rates were similar between the two arrangement groups, so it is likely that trees in both arrangements received outcrossed pollen resulting in seed of a similar quality being produced.

In Chapter 5, I further investigate the role of plant spacing and aggregation and look at mating patterns and pollen flow dynamics in the 40-year-old revegetated *E. leucoxylon* population studied in Chapters 3 and 4. Outcrossing rates were consistently higher in aggregated trees (80-100%), whereas outcrossing was more variable with more dispersed arrangements (27-100%). Extensive pollen flow was observed (up to ca 2000 m), and this appears to help overcome spatial isolation, maintaining high levels of outcrossing even in spatially isolated individuals and establishing connectivity between remnant and revegetated *E. leucoxylon* patches. Widespread outcrossing and a lack of spatial genetic structure resulted in the genetic diversity of seed produced being independent of spatial aggregation of the mother tree. Taken together, these results indicate that robust pollination systems have developed in the Monarto Woodlands, even though plantings were undertaken with little consideration of spatial arrangement, but opportunities exist to improve the reproductive performance of revegetated eucalypts by manipulating planting arrangements.

Reproduction in the eucalypts studied was highly variable, but despite this variability, Chapters 3 to 5 highlight that planting arrangements can influence a range of ecological processes, with the spacing between plants a key factor. Consequently, the potential exists to improve the ecological function of revegetated populations. More research is needed to determine optimal planting designs, but as conspecific aggregation is common and large distances between conspecifics are rare in natural communities, using natural vegetation to guide planting layouts has the potential to improve the ecological function of revegetated communities.

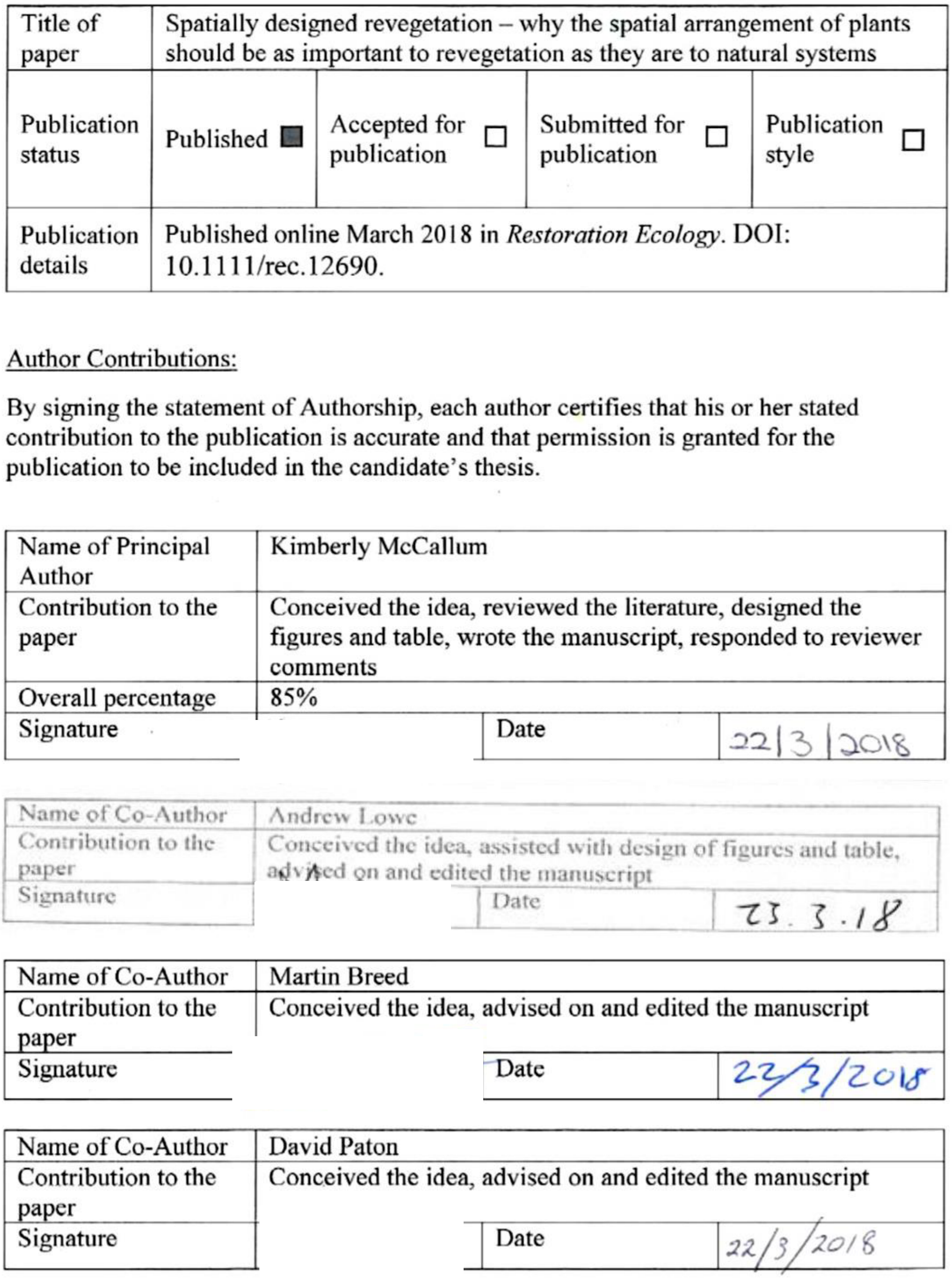
To provide the context for plant spacing and aggregation in natural populations, Chapter 6 describes the natural spatial arrangement of *E. leucoxylon* dominated woodlands in South Australia and identifies key ways that this information can potentially be incorporated into revegetation design.

Finally, Chapter 7 is a short technical paper, which first describes a range of potential options for manipulating the spatial arrangement of plants during revegetation and then documents how one of these options (tubestock planting into individual holes), was trialled during woodland revegetation at Frahn's Farm, South Australia.

In my conclusion, Chapter 8, I reflect on the contribution of Chapters 2 to 7 to understanding the role of planting arrangement to revegetation. I describe the limitations of my study and identify potential areas of future research, which would help to improve our understanding of the role and importance of planting layouts to revegetation.

Chapter 2. Spatially designed revegetation

Statement of authorship



Title of paper	Spatially designed revegetation – why the spatial arrangement of plants should be as important to revegetation as they are to natural systems			
Publication status	Published ■	Accepted for publication ☐	Submitted for publication ☐	Publication style ☐
Publication details	Published online March 2018 in *Restoration Ecology*. DOI: 10.1111/rec.12690.			

Author Contributions:

By signing the statement of Authorship, each author certifies that his or her stated contribution to the publication is accurate and that permission is granted for the publication to be included in the candidate's thesis.

Name of Principal Author	Kimberly McCallum		
Contribution to the paper	Conceived the idea, reviewed the literature, designed the figures and table, wrote the manuscript, responded to reviewer comments		
Overall percentage	85%		
Signature		Date	22/3/2018

Name of Co-Author	Andrew Lowe		
Contribution to the paper	Conceived the idea, assisted with design of figures and table, advised on and edited the manuscript		
Signature		Date	23.3.18

Name of Co-Author	Martin Breed		
Contribution to the paper	Conceived the idea, advised on and edited the manuscript		
Signature		Date	22/3/2018

Name of Co-Author	David Paton		
Contribution to the paper	Conceived the idea, advised on and edited the manuscript		
Signature		Date	22/3/2018

Spatially designed revegetation - why the spatial arrangement of plants should be as important to revegetation as they are to natural systems

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Author contributions: All authors conceived the review; KPM wrote the first draft of the manuscript and all authors contributed to revisions; KPM, AJL designed the table and figures.

Running head: Spatially designed revegetation

Word count: 6831

Manuscript published in Restoration Ecology

https://onlinelibrary.wiley.com/doi/full/10.1111/rec.12690

Abstract

The spatial arrangement of plants, both within and between species, play a key role in natural systems and influence many fundamental ecological processes (e.g. survival, competition, facilitation, pollination and seed dispersal) and ecosystem functions (e.g. habitat value, erosion, water and nutrient capture). Despite this knowledge, fine-scale planting arrangements are rarely considered during restoration plantings, yet manipulation of planting designs have the potential to aid the development of resilient and self-sustaining ecosystems. Here we outline how the spatial arrangement of plants can influence processes at both the vegetation level and more broadly at the ecosystem level. The review is focused on woodland systems, but also draws on key examples from grassland ecosystems. Following this synthesis, we identify research gaps in the revegetation literature that could usefully be addressed to help develop this understudied field of research. Finally, we outline components of population and community level arrangements (e.g. spacing, aggregation, community composition) that can be considered during restoration plantings - spatially designed revegetation - which are likely to lead to improved ecological outcomes of woodland and grassy woodland revegetation.

Key words

Ecosystem function, grassland, planting position, plant spatial pattern, restoration, woodland

Conceptual implications

- Information from natural, plantation and experimental plant communities can be used to guide woodland and grassy woodland revegetation.
- Manipulating planting arrangements has the potential to increase plant survival, maintain species diversity, facilitate pollination, seed dispersal and recruitment, limit weed invasion and erosion, promote water and nutrient capture, and improve habitat value.
- Revegetation may be most effective if planned over a range of scales, from the position of individuals within populations and communities, and the position of communities within the landscape.
- The most effective planting designs will be dependent on the species used, site conditions and restoration goals, and on-going management such as thinning and supplementary planting may be required to achieve the desired planting arrangement.

Introduction

Large scale revegetation is being undertaken in the hope that increasing the cover of native vegetation will help stem land degradation, prevent further biodiversity loss, and return resilient and self-sustaining ecosystems to degraded areas. Although revegetation has the potential to achieve these outcomes, it is often done in an *ad hoc* way and results can be highly variable (Paton & O'Connor 2010; Miller et al. 2017; Gellie et al. 2018).

In particular, ecological principles tend to be overlooked during the planning and planting phases of revegetation, which are likely to hinder the success and functional outcomes of revegetation projects (Bartha et al. 2004; Fazey et al. 2006; Breed et al. 2013). One aspect that is often overlooked is the spatial arrangement of plants. In natural systems, plant arrangements influence a number of community and ecosystem level processes (Watt 1947), so it likely they will play similar a role in revegetated sites.

Revegetation activities such as tubestock planting or direct seeding determine the spatial arrangement of plants (Miller et al. 2010; Stanturf et al. 2014). Tubestock are often planted into individual holes or along ripped rows, which result in haphazard or linear plantings (Munro & Lindenmayer 2011). Similarly, direct seeding is generally done in rows, with the same mix of seeds being applied at a constant rate over an area (Jonson 2010). As a result, revegetated communities often show uniform, linear or random spatial arrangements, rather than the aggregated to random patterns more commonly observed in natural ecosystems (Miller et al. 2010; Paton et al. 2010; Zhao et al. 2015). Revegetation also tends to have different relative abundances of species, fewer understory species, and greater admixture of species than natural systems (Lockhart et al. 2006; Paton et al. 2010; Schneemann & McElhinny 2012; Zhao et al. 2012). These differences in arrangement may influence the ecological processes occurring in revegetated sites (e.g. pollination, competition, herbivory), but the fine-scale position of plants is rarely considered an important characteristic of restored vegetation.

Here we assess how knowledge from natural, plantation and experimentally established plant communities can be applied to the design and management of revegetated systems, specifically in relation to plant spatial arrangement. Our review draws on information from a range of global systems, but is focused on woodland systems and where relevant, also draws on information from grasslands. Due to the broad scope of the paper, we pick out key examples, rather than reviewing all possible studies, to demonstrate how spatial arrangements have the potential to influence revegetation outcomes. We first cover the drivers of spatial arrangements in natural systems, describing their influence at both the vegetation (population and community) and ecosystem levels. We then highlight what is known from revegetated ecosystems, and detail key knowledge and research gaps. Following this, we introduce the concept of spatially designed revegetation and provide practical recommendations for incorporating the fine-scale arrangement of plants into revegetation design.

Drivers of spatial arrangements in natural ecosystems

Plant arrangements are influenced by a number of factors and may result from processes that span many generations (Turnbull et al. 2007). Environment (e.g.

soil type, topography), disturbance (e.g. fire), plant morphology and ecological processes (e.g. seed dispersal, competition, predation, spatial priority effects) all contribute to plant arrangements (Miller et al. 2010; Perry et al. 2013; Young et al. 2017). Climate governs where species survive more generally, while finer-scale environmental components such as soil type and topography determine where individuals establish within this broader area (Woodward 1987). Disturbance can open up new areas for colonization (Gardner & Engelhardt 2008), while plant size and plant-plant interactions (e.g. competition, facilitation) influence spacing between individuals and species co-existence (Perry et al. 2009). These factors can each influence population size (abundance) and spacing (density), community composition and the degree of aggregation and segregation (Fig. 1).

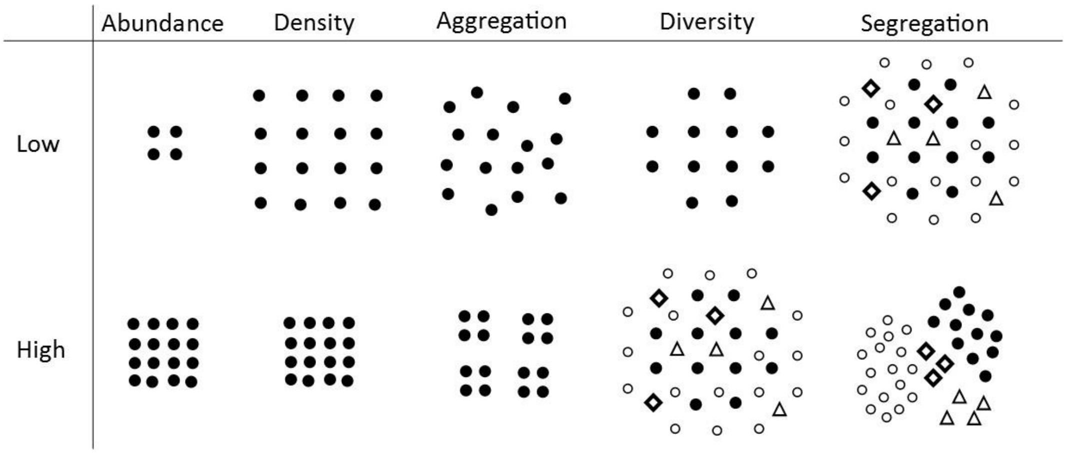


Figure 1. The different components of plant arrangements, including population (abundance, density, aggregation) and community (diversity, segregation) level patterns that can be considered during revegetation. Abundance is the number of individuals planted in an area, density is the spacing between those individuals, aggregation is the degree of clustering of individuals within a population, diversity reflects the number of species in a community and segregation reflects how those different species are spatially arranged (ranging from intermixed to spatially separated conspecific clusters). Adapted from Kunin (1997b).

Effect of spatial arrangements on vegetation

Facilitation and competition

Plants are sessile and interact most strongly with their neighboring plants. Interactions are often localized and grasses tend to interact at the centimeter scale (Yurkonis & McKenna 2014), shrubs at the centimeter to meter scale (Tyler & D'Antonio 1995) and trees at the meter scale (Williams et al. 2006). Interactions can be both positive (facilitation) or negative (competition), with the strength of these interactions tending to decrease as the space between plants increases (Tyler & D'Antonio 1995; Padilla & Pugnaire 2006). Facilitative interactions between plants occur when some individuals change the micro-climate for other individuals by preventing extreme temperature fluctuations, providing shade, buffering wind, improving soil and reducing herbivory (Holmgren et al. 1997; Gómez-Ruiz et al. 2013). However, facilitative interactions may shift to competitive interactions as individuals grow, densities increase or environmental conditions change (Holmgren et al. 1997; Padilla & Pugnaire 2006; Raventós et al. 2010).

Competitive interactions can occur between individuals of the same species or different species and are generally more intense when resources are limited (Holmgren et al. 1997). Competition between individuals may be equal or asymmetric depending on species type, growth form, emergence time and plant size (Freckleton & Watkinson 2001). Competition often increases as plants grow and this can reduce growth rates, limit lateral growth and increase mortality (Phillips & MacMahon 1981; Holmgren et al. 1997). Overtime these competitive interactions can increase plant spacing as weaker individuals are out-competed and this can result in changes from aggregated to random and/or regular patterns as stands age (Phillips & MacMahon 1981). When there are differences in the competitive ability of species, weaker species may be outcompeted, especially at high densities (Stoll & Prati 2001). However, intraspecific aggregation can promote species co-existence in plant communities (Stoll & Prati 2001; Wassmuth et al. 2009), and as a result, species may occur in conspecific clusters, with these clusters spatially separated from other species (Raventós et al. 2010). If interspecific competition occurs over shorter distances than intraspecific competition (heteromyopia), spatial segregation can promote species co-existence (Murrell & Law 2003).

Pollination and seed production

The spatial distribution of individuals within populations and communities can also influence pollination and seed production (Meagher & Vassiliadis 2003). In wind-dispersed species, most successful pollination events occur over short distances, so the proximity of conspecific individuals is a key predictor of reproductive success (Vandepitte et al. 2009). Likewise, in animal-pollinated species, pollinators tend to move more frequently between neighboring plants (Hopper & Moran 1981). Therefore, individuals in areas with higher conspecific densities tend to receive more outcrossed pollen and a greater diversity of pollen, which can increase outcrossing rates, seed set and viability, and offspring fitness (Breed et al. 2012a; Breed et al. 2015a; Lowe et al. 2015). However, strong competition at high densities can cause plants to suppress reproductive output, and in some high density stands, intense intraspecific competition may completely suppress sexual reproduction (Williams et al. 2006; Dwyer et al. 2010). Therefore, trade-offs between pollination and seed production often occur in plant populations (Ghazoul 2005).

Seed dispersal, recruitment and weed invasion

Plant spatial arrangements can influence seed dispersal by affecting frugivore foraging behaviour and wind speeds (Morales & Carlo 2006; Marchetto et al. 2010). Frugivores often remove more fruit as plant population density increases, but this tends to correlate with shorter dispersal distances because animals can forage over smaller areas (Morales & Carlo 2006; Carlo & Morales 2008). Similarly, the spread of seed in wind dispersed plants is often reduced at high densities because dense plant growth reduces wind speeds (Marchetto et al. 2010).

Seeds need to be dispersed into open or low density patches for recruitment to occur, so the size and position of open space is important (Bergelson 1990; Bergelson et al. 1993). Natural regeneration may occur most frequently at intermediate distances from mother plants (i.e. 30 m in *Taxus baccata* (English yew) trees), because competition with the mother is reduced but seed fall remains relatively high (Devaney et al. 2014). In addition, lower density or patchy stands can promote understory species richness and diversity (Chen & Coa 2014). However, lower density or patchy communities can be at greater risk of weed invasion, with greater weed spread through large and closely spaced, open patches (Bergelson et al. 1993; Simmons 2005).

Effect of spatial arrangements on ecosystems

Habitat

Plant communities play a major role in the physical structure of ecosystems and influence the distribution and abundance of animal species (Tews et al. 2004). Heterogeneous systems generally support a greater diversity and abundance of animal species because these systems provide a range of different habitat resources (Tews et al. 2004; Mac Nally 2008; Paton & O'Connor 2010). The spatial arrangement of plants, both within populations and communities, can also influence how animals move around systems, and if preferred plant species are clustered, animals tend to move shorter distances while foraging (Morales & Carlo 2006; Wang et al. 2010). The characteristics of neighboring plants can also influence foraging behaviour and plants may be more likely to be browsed if they occur in patches of vegetation containing high abundances of palatable species and/or low abundances of less-palatable species (Bee et al. 2009).

Abiotic environment

The spatial arrangement and functional diversity of plants can influence the abiotic environment, with effects on water and nutrient capture and release, soil surface temperature and wind speed. Higher densities can reduce erosion, protect against high temperatures and increase water infiltration (Balandier & Dupraz 1999; Yates et al. 2000; Bautista et al. 2007; Loades et al. 2010). High vegetation densities and functional diversities offer more obstructions to the surface flow of water and therefore increased chances for re-infiltration (Bautista et al. 2007; Loades et al. 2010). In addition, fine-scale patchiness (small vegetation patches, short distances between patches and greater connectivity) is more effective in capturing water and sediment flows than coarser arrangements (Bautista et al. 2007). Plant canopies also intercept rain, reduce wind speeds and limit the amount of radiation reaching the soil surface, so higher densities can prevent erosion and decrease evaporative losses (Yates et al. 2000; Breshears et al. 2009; Loades et al. 2010). However, high density stands need to use greater amounts of soil resources (nutrients and water) and this can result in drying of the soil layer and depletion of soil nutrients (Zhu et al. 2003; Chen & Coa 2014).

Spatial arrangement and revegetation

As we have shown, plant arrangements can influence plant fitness and function at both the vegetation (population and community) and ecosystem levels. Consequently, planting arrangements can determine the trajectory of long-term development of restored vegetation (Jonson 2010). In the following section, we detail what is currently known from revegetated systems and highlight gaps in the knowledge. In many cases, the role of spatial arrangement is not the main focus of the research, but studies which detail the effects of overall planting density or that occur in mixed plantings can still contribute to our understanding (Table 1).

Table 1. Theoretical background (from natural, plantation and experimental plant communities), evidence from revegetated sites, and gaps in the knowledge.

	Theoretical background	Revegetation evidence	Research gaps
Vegetation *Facilitation and competition*	• Plants interact with neighboring plants (positive, negative) and the strength of these interactions decrease with increasing distances between plants.	• Spacing can influence survival and growth (Otoda et al. 2013).	• Timing, extent and frequency of thinning to reduce competition and promote growth.
	• Some plants can improve growth in others by buffering against environmental conditions or reducing herbivory.	• Nurse plants can improve survival and growth (Castro et al. 2002) and reduce herbivory (Smit et al. 2006).	• Species that can be used as nurse plants and effectiveness of staggered plantings.
	• When competition occurs between species, weaker species may be outcompeted, but conspecific aggregation and priority effects can prevent competitive exclusion.	• Aggregation (Wassmuth et al. 2009) and priority effects (staggered plantings) can help maintain species diversity (Young et al. 2017).	• Influence of conspecific aggregation and staggered plantings on competition in woodland species and at the field scale.
Pollination and seed production	• Small distances between plants or conspecific aggregation can promote outcrossing, while large distances can result in selfing and reduced seed set.		• Role of intra- and interspecific arrangements (density, aggregation, segregation, relative abundances) on pollen flow and seed production.
	• Random planting arrangements may facilitate pollen transfer between different species (hybridization).		• Pollinator movements and patterns of gene flow in areas that are randomly mixed, compared to those that are aggregated.
	• High densities can suppress reproductive output.		• Trade-offs between pollination and seed production in relation to planting density.

Seed dispersal, recruitment and invasion	• More fruit consumed from higher density populations, but shorter seed dispersal.		• Manipulating plant density and areas of open space to promote seed dispersal.
	• Gaps and low density areas can promote recruitment and natural regeneration.	• Planting density and recruitment (Vesk et al. 2008).	• Position of gaps that promote regeneration of natives, but minimize weed invasion.
	• Large and closely spaced gaps can promote weed invasion, but high densities of natives or small, conspecific patches can suppress weeds.	• Weed invasion and patchiness (Bergelson et al. 1993), and conspecific patches and resistance to invasion in grasses (Yurkonis and McKenna 2014).	• Manipulating the size and arrangement of conspecific patches in woodland revegetation to minimize weed encroachment.
Ecosystem *Habitat*	• Heterogeneous sites that are structurally diverse and have a range of densities can enhance habitat value.	• Planting density and habitat development (Vesk et al. 2008).	• Thinning to improve habitat value, and the frequency and extent of thinning required. • Supplementary planting to increase structural diversity of revegetated sites.
	• The spatial arrangement of plants can influence how animals use and move around plant communities, and aggregation of plant species can concentrate resources and reduce foraging distances.	• Micro-habitat features for woodland birds (Allan 2016).	• Habitat value and foraging behaviour in sites with conspecific aggregation vs. mixed plantings.
Abiotic environment	• High densities and/or functional diversities can promote water and nutrient capture and limit run-off, but drying of the soil layer and depletion of nutrients may occur in high density stands. • Low density stands can suffer from wind and water erosion.	• Soil stability, water infiltration and nutrient cycling in woodlot and ecological plantings (Munro et al. 2012). • Soil stability as a function of plant cover (Herrick et al. 2006).	• Planting densities, patch arrangement and combinations of species that optimize water and nutrient cycling and limit erosion. • Position and arrangement of high density patches to increase water infiltration and minimize erosion.

Vegetation level

Facilitation and competition

If restoration attempts are unsuccessful because of harsh environmental conditions or strong herbivory, nurse plants can be used to improve fitness of target species (reviewed by Padilla & Pugnaire 2006). Although not widely used, there have been some successful attempts, with success often occurring when nurse plants, such as early successional shrubs, are already established (Castro et al. 2002; Gómez-Aparicio et al. 2004). For example, re-forestation in the Sierra Nevada area of Spain found that the survival and growth of seedlings planted under shrubs was higher than those planted in the open and this was stronger in dry years compared to wetter years (Gómez-Aparicio et al. 2004). While, growing next to unpalatable plants significantly increased sapling survival of spruce (*Picea abies*) in pasture ecosystems in Switzerland, by reducing grazing (Smit et al. 2006).

Similarly, in single cohort plantings, individuals can buffer each other from harsh abiotic conditions. For example, survival rates of two tree species (*Pinus sylvestris*, *Populus alba*), in afforestation plots in the Ordos Desert, northern China, decreased as the spacing between individuals increased (3, 5 and 7 m). At larger spacings, wind blew away more sand, exposing the roots of widely spaced individuals, reducing their survival (Otoda et al. 2013). In contrast, three succulent species were found to compete with each other when planted in multi-species clumps during mine site restoration in South Africa. In this desert environment, facilitation was predicted to outweigh competition, with clumped individuals expected to perform better than those planted alone. However, survival was higher for single plants because the three succulent species had similar resource requirements and thus competed with each other (Blignaut & Milton 2005).

Competition may be a problem in revegetated sites, particularly for sites that are direct seeded as seed mixtures often contain species with different competitive abilities and succession strategies. If some species germinate and establish before other species, these species can gain a competitive advantage (spatial priority effects) and this will influence community assembly and diversity (Young et al. 2001; Porensky et al. 2012). As a result, the abundances of species in direct seeded sites often differs from what would be expected based on the proportions of seed used (Pyke & Archer 1991; Schneemann & McElhinny 2012). For example, in a direct seeded woodland in south-eastern Australia, species richness was found to decline with time from sowing. This decline was attributed to a small number of dominant overstorey species (*Acacia* and *Eucalyptus*) monopolizing site resources, leading to the progressive loss of many less dominant mid- and understory species (Schneemann & McElhinny 2012).

The chance of species co-existing in revegetated sites can be improved by determining species competitive relationships, using a diversity of species and growth forms and/or manipulating spatial arrangements or planting times (Pyke & Archer 1991; Stoll & Prati 2001; Porensky et al. 2012). At this stage, little has been done to determine combinations of species that can successfully co-exist, particularly in woodland systems. However, there are some examples from grassland ecosystems (e.g. Pyke & Archer 1991; Francis & Pyke 1997). Similarly, the role of spatial arrangement, specifically conspecific aggregation, in maintaining species diversity has received more research attention in grassland communities than woodlands (Stoll & Prati 2001; Porensky et al. 2012; Yurkonis & McKenna 2014). Field margins sown with native species (annual grasses and forbs) in Germany showed that conspecific aggregation prevented the loss of weaker species and resulted in higher numbers of individuals of all species, compared to randomly mixed areas (Wassmuth et al. 2009). Planting in conspecific clusters can increase the time taken for weak and competitive species to interact, allowing weaker species to become established (Porensky et al. 2012). Alternatively, weaker species can be planted before more competitive species to prevent competitive exclusion (Young et al. 2017). However, much of this research has been conducted in small experimental grass plots and generally over short periods (<3 years), so these designs need to be extended to field-scale projects (Yurkonis & McKenna 2014) and longer lived perennial species.

Pollination and seed production

Pollination is a critical ecosystem service but is rarely investigated as a measure of revegetation success (Ritchie & Krauss 2012), with research into how restoration methods alter pollination still in its infancy (Menz et al. 2011; Ritchie et al. 2017). A few studies have started to investigate patterns of pollen flow in revegetated systems, but these studies are generally focused on gene flow and connectivity between remnant and revegetated populations, with the majority undertaken in Australian woodland systems (Ritchie & Krauss 2012; Broadhurst 2013; Frick et al. 2014). Although these studies can provide some insight into patterns of pollen flow within revegetated patches, the role of fine-scale planting arrangements have not been explicitly considered. Therefore, further research into pollen flow, fruit and seed production and plant mating systems are required in relation to within patch planting arrangements, such as planting density, conspecific spacing and the degree of aggregation and segregation.

Seed dispersal, regeneration and invasion

The role of seed dispersal in revegetation has received some attention but is often focused on dispersal into degraded areas (passive revegetation), rather than dispersal within revegetated systems. Despite this lack of research, planting clusters of trees and shrubs in open areas has been promoted as a way to facilitate seed dispersal and promote natural regeneration in these sites (Robinson & Handel 1993). Existing vegetation often benefits the establishment of new individuals (Felinks & Weigand 2008), so a combination of planting and spontaneous succession can be used to restore areas (Prach & Hobbs 2008). This option is generally cheaper, results in structurally diverse vegetation and allows more natural aggregated arrangements to develop (Felinks & Weigand 2008).

More is known about how planting arrangements influence regeneration and recruitment in revegetated sites, but overall planting density has been the focus, rather than finer-scale measures, such as patch size and position. For example, modelling by Vesk et al. (2008) found that recruitment was nearly three times more likely for trees and five times more likely for shrubs in low density (<250 stems/ha) compared to higher density sites (>250 stems/ha) in revegetated woodlands in south-eastern Australia (Vesk et al. 2008). However, there is a trade-off between maximizing regeneration and growth of understory species and managing weeds (Jones et al. 2015).

Weeds are often a problem for revegetated sites, because disturbance during preparation and planting can favor weed growth (Reid et al. 2009). Furthermore, large and continuous spaces, which often occur in direct seeded sites, can facilitate the spread of invasives (Yurkonis et al. 2010). A number of studies have researched weed invasion and spatial arrangements in experimental grass and forb communities (Bergelson et al. 1993; Liao et al. 2014; Seahra et al. 2016), and although these are often done with a restoration focus, there is a need to extend these designs to field-scale projects and other vegetation types. In grassland systems, it has been shown that the spatial distribution of bare ground can influence invasion rate, with faster spread through large and closely spaced gaps (Bergelson et al. 1993). In addition, larger conspecific patches of native grasses are invaded more often than small patches, particularly in the period following seeding (Seahra et al. 2016), while planting in small conspecific clusters can reduce weed invasion (Yurkonis et al. 2012).

Ecosystem

Habitat

Many studies have assessed habitat quality of revegetated sites at the stand scale, with structurally diverse vegetation generally supporting a greater diversity of fauna (e.g. Fletcher Jr & Koford 2002; Watts & Gibbs 2002; Munro et al. 2007). Using a range of planting densities can increase habitat value, but the development of key habitat features such as large boughs, tree hollows and fallen timber may be delayed in high density stands (Vesk et al. 2008; Munro & Lindenmayer 2011). However, finer scale, within stand variables, such as the distribution of open space, degree of aggregation and the spatial arrangement of individuals within populations and communities are rarely considered. Nonetheless, research into the microhabitat features of a revegetated woodland in southern Australia, found that spatial variation (clustering) of trees and shrub species, as well as areas of open space, have the potential to improve habitat value (Allan 2016).

Abiotic environment

Practical methods for determining ecosystem function in revegetated sites are rare (Munro et al. 2012), and to the best of our knowledge, never applied to the spatial arrangement of plants within populations and communities. However, ecosystem function has been assessed in woodlot (only overstorey) and ecological plantings (trees and shrubs) in regards to soil stability, water infiltration and nutrient cycling, with no differences found between the two planting types (Munro et al. 2012). In addition, soil stability has been assessed as a function of plant cover (shrubs, perennial grasses, annuals) in restored mine sites in Wyoming, USA. Soil stability was reduced in sites with lower perennial plant cover and as a result these sites were more susceptible to soil erosion (Herrick et al. 2006). There has also been some consideration during on-ground works, with higher density tree plantings used along contour lines to improve the capture and infiltration of rainfall (Jonson 2010), but the outcomes of this are yet to be documented.

Practical recommendations

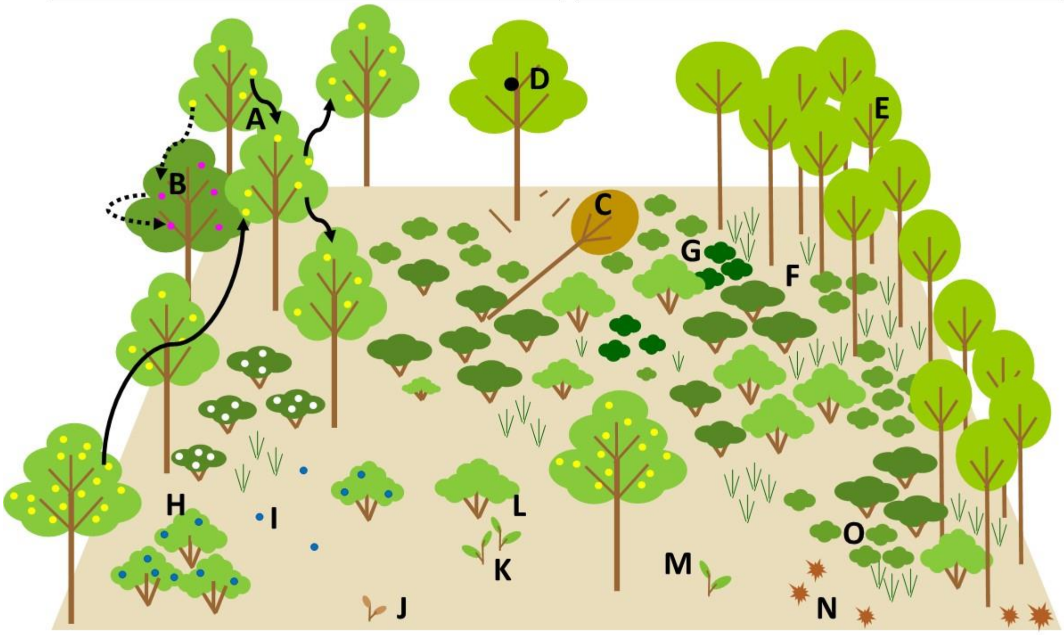
Revegetation designs may be most effective if planned over a range of scales, from the position of individual plants within a population, the position and distribution of populations within communities and the position of communities in the landscape (Jonson 2010). There have been calls for more detailed consideration of spatial arrangements during revegetation, particularly for grasslands, but achieving this at the field-scale presents challenges (Yurkonis & McKenna 2014). In addition, recommendations for considering spatial arrangement during woodland revegetation are often simple and may underestimate the complexity of natural ecosystems (Bartha et al. 2004). For example, Munro & Lindenmayer (2011) recommended that plantings should replicate the variability in density seen in natural systems, by having dense and sparse patches as well as small clearings. In the following section, we build on these recommendations and detail how the fine-scale arrangements of plants can be more thoroughly considered during revegetation (Fig. 2). The most effective planting arrangements will be dependent on the species used, sites conditions and restoration goals, and may require on-going management such as supplementary planting and/or thinning (Fig. 2).

Planting species in clusters can promote cross-pollination (A). In contrast, mixed plantings can result in selfing and/or interspecific pollen transfer (B).

High density plantings can buffer against weed invasion, abiotic conditions (i.e. wind) and prevent erosion, but can deplete soil water and nutrients, affect plant growth form and limit understory diversity (E).

Thinning can help relieve strong competition, improve structural diversity, and if thinned timber is left on the ground can provide habitat for animals (C).
Hollow formation may occur more readily in thinned or widely spaced stands (D).

Fine-scale patchiness using a mix of plant types can increase water and nutrient capture and decrease run-off (F).
Intraspecific aggregation can maintain species diversity by preventing competitive exclusion of weaker species (G).



Aggregating flowering, fruiting or palatable species can reduce foraging costs for animals (H), but larger spacing can increase seed dispersal distances (I).

Patchy or low density areas can promote regeneration and patches may be most effective at intermediate distances from mother plants (M).

Large distances between plants can result in abiotic stress and reduced survival (J), while growing near other seedlings (K) or established plants can buffer against these conditions (L).

Gaps and open space can promote weed invasion, particularly large and closely spaced gaps (N).
Small conspecific patches of natives can reduce weed invasion (O).

Figure 2. Spatially designed revegetation - key recommendations for incorporating the fine-scale arrangements of plants into woodland and grassy woodland revegetation design.

Planting individuals closer together can increase survival, particularly in seedlings and small species and this may be most beneficial in areas exposed to harsh abiotic conditions, such as strong winds or frost (Balandier & Dupraz 1999; Bhattacharjee et al. 2010; Otoda et al. 2013). Similarly, planting palatable species next to or under unpalatable species (e.g. spiny) can increase survival if herbivory is a problem (Smit et al. 2006), and this may be most effective if staggered plantings are used. However, planting individuals close together can result in strong competition and reduced growth as stands develop, particularly when environmental conditions or herbivory do not limit performance (Padilla & Pugnaire 2006). Therefore, thinning of revegetated sites may be an important management options as stands age and may be most effective if multiple thinning events are used as the vegetation matures (Stanturf et al. 2014). Thinning can also provide additional benefits if thinned timber is left on the ground, because it increases the structural diversity of sites and provides additional habitat features (Vesk & Mac Nally 2006; Dwyer et al. 2010; Horner et al. 2010; Stanturf et al. 2014).

Areas at risk of erosion (wind or water) and weed invasion can benefit from higher density plantings (Simmons 2005; Bautista et al. 2007; Breshears et al. 2009). The best way to achieve this may be through buffers of dense vegetation along patch boundaries (Dwyer et al. 2010), or in areas at risk of erosion, such as along slopes (Jonson 2010), because high density plantings can deplete soil water and nutrients (Chen & Coa 2014), reduce habitat value and delay hollow formation (Vesk et al. 2008), and limit recruitment and understory species richness (Chen & Coa 2014). Consequently, high density plantings may be most suitable where erosion or weed invasion is a problem but may be detrimental if entire sites are planted at high densities.

Aggregated plantings (intraspecific clustering, interspecific segregation) have the potential to maintain community level diversity, facilitate pollination, limit weed invasion and improve habitat value. Aggregated arrangements can be achieved by planting seedlings in intraspecific clusters or sowing seed in conspecific patches (Jonson 2010). Supplementary plantings can be used to maintain or manipulate the degree of aggregation as stands age, while targeted thinning can also be used to achieve aggregated arrangements (Stanturf et al. 2014). Creating aggregated arrangements of species can reduce competitive exclusion if species with different competitive abilities are planted together (Wassmuth et al. 2009; Porensky et al. 2012). Planting in small, conspecific clusters can also reduce the risk of weed invasion because it increases the fine-scale heterogeneity of vegetation patches (Bergelson et al. 1993).

Aggregated arrangements can also influence plant-animal interactions. Clustered plantings can facilitate pollination and may have additional benefits if seedlings from a range of mother plants are used, because this has the potential to increase the diversity of pollen received and reduce mating between related individuals (Ritchie & Krauss 2012). Aggregated arrangements can also reduce foraging costs (Morales & Carlo 2006; Wang et al. 2010), so clustering individuals of the plant species used by animals has the potential to increase habitat value for target species.

Patchy systems or larger distances between plants can promote seed dispersal and recruitment (Carlo & Morales 2008; Vesk et al. 2008) and this can result in the development of more structurally diverse, self-sustaining vegetation. Therefore, incorporating areas of open space into revegetation design may be equally as important as manipulating the spatial arrangement of plant populations and communities. Alternatively, thinning of established stands can promote natural regeneration and allow mid- and understory layers to develop (Dwyer et al. 2010; Jones et al. 2015), while supplementary plantings can be used to increase structural diversity and aid the establishment of mid- and understory vegetation (Stanturf et al. 2014).

Fine-scale vegetation patchiness (small patches, short distances between patches) can be beneficial in areas prone to water run-off and erosion (e.g. slopes), because it promotes the capture and infiltration of water (Herrick et al. 2006; Bautista et al. 2007). In addition, planting a diversity of plant forms (i.e. trees, shrubs, grasses) can aid water capture, minimize erosion and lower soil surface temperature because they offer more obstructions to the flow of water, reinforce the soil, intercept rain and provide greater soil surface cover (Bautista et al. 2007; Loades et al. 2010). Therefore, the species used, and their spatial arrangement can be tailored to site conditions and structurally diverse plantings, including fine-scale patchiness, may be beneficial in areas at risk of erosion or evaporative losses (Fig. 2).

Conclusions

The spatial arrangement of plants within populations and communities, influence many processes including growth, facilitation, competition, pollination and recruitment, habitat value, and water and nutrient cycles. Thus, the position of individual plants and their position relative to others has the potential to influence the functional outcomes of revegetation in terms of self-sustainability, biodiversity value and resilience. As such, there are opportunities to improve woodland and grassy woodland revegetation through greater consideration of the fine-scale arrangement of plants during the planning, planting and maintenance phases of revegetation. The most effective planting designs will be dependent on the species used, sites conditions and restoration goals, and achieving these designs may require on-going management, including thinning and supplementary planting. Although research into planting arrangements and revegetation is still relatively new, we identify key ways that the spatial arrangement of plants can be incorporated into revegetation design and introduce the concept of spatially designed revegetation. The challenge now exists to find effective ways to incorporate these principles into on-ground works. More research into the role of planting arrangements within revegetated sites is required, but we hope this review provides a strong basis for further research into this understudied field.

Acknowledgements

This work was supported by the Noel and Vivian Lothian Scholarship awarded to KPM and Australian Research Council funding to AJL and MFB (DE150100542; DP150103414). KPM was supported by an Australian Postgraduate Award during this study. We thank three anonymous reviewers and the editors for comments that greatly improved this manuscript.

Chapter 3. Seed production

Statement of authorship

Title of paper	Clumped planting arrangements improve seed production in a revegetated eucalypt woodland			
Publication status	Published ☐	Accepted for publication ☐	Submitted for publication ■	Publication style ☐
Publication details	Submitted to *Restoration Ecology* February 2018, currently under review			

Author Contributions:

By signing the statement of Authorship, each author certifies that his or her stated contribution to the publication is accurate and that permission is granted for the publication to be included in the candidate's thesis.

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Percentage (%)	90%		
Signature		Date	22/3/2018

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Contribution to the paper	Assisted with study design and data analysis. Advised on and edited the manuscript		
Signature		Date	22/3/2018

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Contribution to the paper	Assisted with study design and species selection. Advised on and edited the manuscript		
Signature		Date	22/3/2018

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Contribution to the paper	Assisted with study design. Advised on and edited the manuscript		
Signature		Date	23.3.18

Clumped planting arrangements improve seed production in a revegetated eucalypt woodland

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Running head: Planting arrangement and seed production

Author contributions: All authors conceived the ideas and developed the experimental design; KPM carried out field work and data collection; KPM competed data analysis, with assistance from MFB; KPM wrote the first draft of the manuscript and all authors contributed to revisions.

Word count: 6006

Manuscript accepted for publication in Restoration Ecology

Abstract

The arrangement of plants within revegetated sites is rarely considered an important characteristic of these communities. However, in natural systems, plant spatial arrangements can influence a range of ecological processes, including pollination and seed set. Pollinators tend to preferentially visit larger and/or more closely spaced populations, with plants in these populations generally receiving more outcrossed pollen, resulting in increased seed set and better quality seed. Similar trends may occur in revegetated populations, but little is known about the influence of planting arrangement on seed production in restored systems. Here we quantified the effect of plant abundance (number of conspecifics within 100 m) and distance to nearest reproductive conspecific on the level of seed set for six eucalypt species (n = 422 trees in total) in one year and for one of these species (*Eucalyptus leucoxylon*), across three additional years. Seed number per fruit was highly variable both between individuals and within individuals across years. Despite this variability, there was a consistent trend of higher seed production (seed number per fruit) when another reproductive conspecific was within 20 meters. In contrast, plant abundance had little influence on seed production. Further investigation of nearest neighbor arrangements found the distance to either the first, second, third or fourth reproductive neighbors were the key predictors of seed production. Therefore, revegetation designs that consider plant spacing and aggregation, rather than only planting to overall density criteria (i.e. trees/ha), at least for the eucalypts studied here, has the potential to improve seed production in revegetated populations.

Key words

Ecosystem function, nearest neighbor distance, plant spatial pattern, pollination, reproduction, restoration planting

Implications for practice

- Using a standard number of plants per hectare to guide revegetation, without consideration of the spatial arrangement of those plants, may limit the reproductive output of restored populations.
- Planting designs that consider the spacing between conspecifics (avoiding large distances between plants) and conspecific aggregation, have the potential to increase seed production and seed quality in revegetated populations.
- Aggregated arrangements are common in natural systems, so planting designs that mimic natural patterns of conspecific spacing and aggregation may improve seed production and the self-sustaining nature of revegetated populations.

Introduction

Revegetation is the most common method used to restore degraded land, especially where there has been extensive clearing (Wortley et al. 2013). Increasing the cover of native vegetation has the potential to mitigate land degradation and reverse biodiversity loss (Munro et al. 2009). However, revegetation goals are often poorly defined, with success generally measured by the number of plants established or the total area planted, rather than ecological outcomes (Corr 2003; Wortley et al. 2013; Thomas et al. 2014). As a result, revegetation may create simplified plant communities (Bartha et al. 2004), and concerns have been raised about the ability of these replanted systems to become self-sustaining and resilient to environmental change (Ruiz-Jaén & Aide 2005a; McCallum et al. 2018b).

Restoring ecosystem functions (e.g. pollination, seed dispersal, nutrient cycling), is vital to create self-sustaining revegetated populations, and studies have shown that the spatial arrangement of plants may influence these functions (Ruiz-Jaén & Aide 2005b; Miller et al. 2010; Menz et al. 2011; Munro et al. 2012; McCallum et al. 2018a). In natural systems, plants often display aggregated spatial patterns (Condit et al. 2000; Perry et al. 2008) due to environmental (e.g. climate, soil type, topography) and ecological factors (e.g. dispersal, facilitation, recruitment) (Bartha et al. 2004; Alados et al. 2009; Gaston & Garcia-Vinas 2013). However, revegetation designs rarely consider the fine-scale spatial layout of plants, which is a concern since the position of individual plants in revegetated systems are largely determined by on-ground implementation methods (SERI 2004; Miller et al. 2010). Plantings are often done in a haphazard or linear way (Jonson 2010;

Munro & Lindenmayer 2011), and this can cause revegetated populations to be more regularly spaced or dispersed than natural populations (Zhao et al. 2015; McCallum et al. 2018a). Consequently, the ecological function of revegetated populations may be compromised under such current practices.

In natural systems, individuals in higher density or aggregated populations generally receive a greater diversity of pollen because pollinator movements between plants are more common (Kunin 1993; Yates et al. 2007; González-Varo et al. 2009a; Breed et al. 2012a). Similarly, plants with more mobile pollinators, such as birds, may receive a higher diversity of pollen, because these pollinators can forage over greater distances (Ottewell et al. 2009; Breed et al. 2015a; Krauss et al. 2017). A greater diversity of pollen and higher levels of outcrossing tend to correlate with higher seed production, seed quality and offspring fitness (Burrows 2000; González-Varo et al. 2009b; Breed et al. 2012a, 2014). In contrast, plants in small or low density populations often suffer from elevated inbreeding, which can lower fruit set and seed production due to inbreeding depression (Wilcock & Neiland 2002; González-Varo et al. 2009b). In some small or dispersed populations, regeneration may be limited by insufficient seed production, putting populations at risk of extinction (Lamont et al. 1993). Species loss and subsequent declines in species diversity are risks if seed production and seed quality are limited in revegetated populations, but at this stage, the reproductive output of revegetated populations has rarely been assessed (McCallum et al. 2018b).

Here, we examine the role of fine-scale planting arrangements on seed production in a revegetated eucalypt woodland. We expect that individuals growing in areas with a higher number of conspecifics and/or closer neighbors will produce more seeds per fruit, while seed production will be limited in individuals with fewer conspecifics nearby. In addition, we predict that these responses will vary between species with potentially different pollen vectors and between flowering seasons, if floral production varies from year to year.

Our specific research questions are: (1) Does plant abundance and the distance to the nearest reproductive neighbors influence seed production (seeds per fruit) in eucalypts? (2) Are these trends consistent across species with different floral morphologies and therefore potentially different pollinators? (3) Are these trends consistent across years, where it is common for flowering intensity to vary from season to season?

Methods

Study system

We studied the influence of fine-scale spatial arrangements on seed production of six eucalypt species planted at the Monarto Woodlands, approximately 70 km south-east of Adelaide, South Australia (139.1°E, 35.1°S). This area was cleared and used for agriculture before being revegetated in the mid to late 1970s by the South Australian Government. Approximately 1850 ha were revegetated with a mix of 250 species (largely eucalypts), making it the most extensive revegetation project in South Australia. The area was revegetated with a mix of local endemics, Australian natives planted outside their natural range, and exotic species (Paton et al. 2004a, 2010), but little information is available regarding seed sourcing for the project.

Figure 1. The revegetated Monarto Woodlands during the study, showing clear signs of the linear planting arrangements despite being planted in the 1970s (>40 years ago). [photo credit: Kimberly McCallum, 2016]

Planting of tubestock was undertaken at 4 - 6 m spacing along lines spaced 4 - 6 m apart, resulting in a density of approximately 400 plants/ha at establishment. Although there has been recruitment and mortality in the system (current density ca 200 plants/ha), these linear plantings were still evident at the time of sampling (Fig. 1). Recruitment of *Eucalyptus* sp. has been rare in the Monarto revegetation and mortality of planted individuals is generally much greater than establishment of new individuals (Paton unpub. data).

Study Species

Eucalyptus species were chosen as the focus of our research because these long-lived trees are widely used for revegetation across southern Australia (Broadhurst 2013). Eucalypts have a high reproductive capacity and individual trees often produce large numbers of flowers (Ottewell et al. 2009), but flowering intensity is known to vary from year to year (Paton 2008). Eucalypt flowers are relatively unspecialized and although some species are predominantly bird or insect pollinated, it is common for eucalypts to be pollinated by a range of generalist bird and insect pollinators (Hopper & Moran 1981; Ottewell et al. 2009).

Our study was largely focused on *Eucalyptus leucoxylon* (South Australian blue gum or yellow gum). *Eucalyptus leucoxylon* grows as a small, multi-stemmed mallee tree in more arid areas (such as Monarto) and to a large single-stemmed tree up to 30 m in height in more mesic areas (Nicolle 1997). Individual trees often produce heavy flower crops, with medium sized flowers, which range in color from cream through to red (Ottewell et al. 2009). The species flowers predominantly in winter and spring but has been recorded to flower year-round in the Adelaide region (Paton et al. 2004b; Merigot & Paton 2018).
Eucalyptus leucoxylon is primarily bird pollinated (e.g. honeyeaters and lorikeets) but is also visited by a range of insects, particularly the introduced honeybee, *Apis mellifera* (Paton & Ford 1977; Paton 2008; Ottewell et al. 2009).

In addition to *E. leucoxylon*, we also characterized seed production of five other eucalypts – *E. caesia*, *E. incrassata, E. platypus* (subsp. *platypus* and subsp. *congregata*), *E. stoatei* and *E. woodwardii*. These six study species were chosen to represent a range of flower sizes and colors (Fig. 2). All species are from the subgenus *Symphyomyrtus*, with *E. leucoxylon* in Section *Adnataria*, *E. incrassata*, *E. stoatei* and *E. woodwardii* in section *Dumaria* and *E. caesia* and *E. platypus* in section *Bisectaria*.

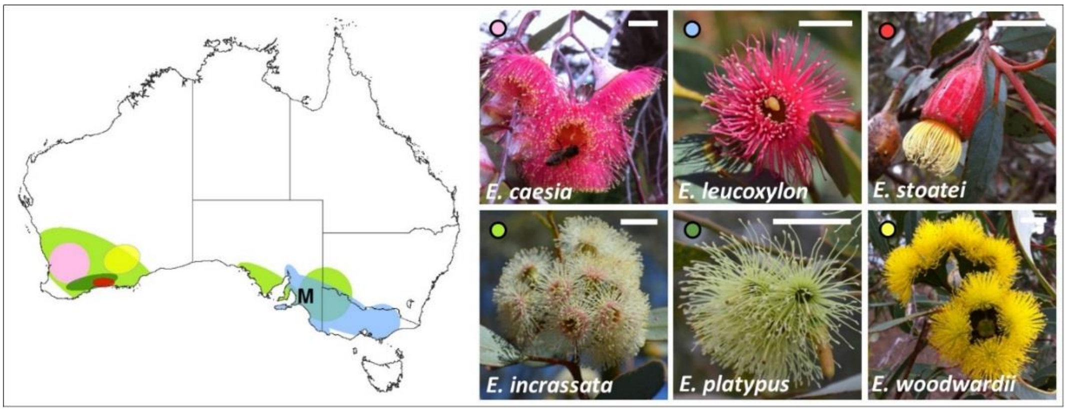


Figure 2. Natural distribution and flowers of the six *Eucalyptus* study species. Distributions are approximate and based on occurrence records from the Atlas of Living Australia (www.ala.org.au; accessed 22 August 2017). Distribution data were used to show the natural distribution relative to the revegetation site. The position of the revegetated Monarto Woodlands, South Australia, is shown as 'M'. The white bars on top right of the photos represent a 10 mm scale bar [photo credits: Kimberly McCallum]

Eucalyptus incrassata is native to the Monarto region and occurs across the arid southern parts of Australia. *Eucalyptus incrassata* grows as a multi-stemmed mallee up to 8 m in height, and has small cream and pink flowers, which occur in clusters of seven. It is primarily pollinated by honeyeaters and to a lesser degree insects (Bond & Brown 1979; Breed et al. 2015b). The four other species – *E. caesia*, *E. platypus* (subsp. *platypus* and subsp. *congregata*), *E. stoatei* and *E. woodwardii* – are endemic to Western Australia and were planted outside their natural range (Fig. 2). *Eucalyptus caesia* often has a weeping form and grows to 6 m in height, with a relatively open canopy. It flowers during winter and produces large, pink flowers, which are predominantly pollinated by birds (Hopper 1981; Bezemer et al. 2016). *Eucalyptus platypus* generally grows as a mallee and can reach 10 m in height. It produces clustered, white to cream-yellow-green flowers and is visited by insects and birds (Paton 2008; DPAW 2017). *Eucalyptus stoatei* is a slender tree, growing to 8 m in height and its flowers are predominantly bird pollinated. It flowers intermittently throughout the year, with peak flowering from spring to autumn. *Eucalyptus stoatei* produces single yellow flowers, but the bright red hypanthium is the main attractant (Hopper & Moran 1981). *Eucalyptus woodwardii* is a slender tree, reaching heights of 6 to 15 m. It flowers during winter and spring and has large, bright yellow, clustered flowers which are visited by birds and insects (DPAW 2017).

Sample collection

We recorded the spatial position of individuals of the six target species in ca 300 ha of Monarto Woodlands with a hand-held Garmin GPSmap 62, recording the position of ca 2500 trees and their reproductive status. Trees were considered as reproductive if they held fruits from the previous flowering season. The target species occurred within a matrix of other species (predominantly eucalypts), but as we only assess population level arrangements here, the position of all other species within the survey area were not recorded as part of this study. The density (reproductive trees/ha) of the target species varied across the survey area, with *E. leucoxylon* ranging from ca 0-35 trees/ha, *E. caesia* from 0-10 trees/ha, *E. incrassata* from 0-13 trees/ha, *E. platypus* from 0-10 trees/ha, *E. stoatei* from 0-12 trees/ha and *E. woodwardii* from 0-8 trees/ha.

Previous research has shown that eucalypt pollen is often dispersed within 100 m (Potts et al. 2003), so abundance was estimated as the number of reproductive conspecifics within a 100 m radius. Study trees were selected across the range of abundance values (range = 0 to 125 trees within 100 m) and nearest neighbor (NN1) distances (range = 4 to 120 m to nearest reproductive conspecific) available. Surveys were completed within a 100 m radius of each study tree or until the nearest five reproductive neighbors (NN1-NN5) were recorded. Isolated trees (nearest neighbor >50 m) were uncommon across all six species, so only fruits from one to thirteen isolated individuals could be collected per species.

Fruits were collected from across the canopy of each study tree with extendable loppers (up to 6 m), with only mature fruits from the previous flowering season collected (refer to Table 1 for sample sizes). Diameter at breast height (DBH), % canopy cover (percent of branches with foliage - as an indicator of tree health), and fruit crop (number of fruit from the last flowering season) were recorded for every tree sampled. Fruit crop varied widely between individuals within species (>10 to 10,000s), so crop size was estimated by counting the number of fruits on an average branch and multiplying this by the number of branches on the tree that held fruits. Fruits were collected over four years for *E. leucoxylon* (2014-2017), and one year (2016) for *E. caesia*, *E. incrassata, E. platypus*, *E. stoatei*, and *E. woodwardii* (Table 1). Fruits collected from each tree were stored in individually labelled paper bags and left to dry at room temperature until open. Fruits were placed in a plastic container and shaken vigorously to expel contents, and each fruit was then examined to ensure all contents had been released. Seeds were separated from chaff and capsule material by hand, with seeds distinguishable from chaff based on shape, size and color. Seeds were counted and mean seed number per fruit determined for each study tree.

Table 1. Study species information, showing the number of study trees sampled per year, the mean number and range of fruit collected and the mean number and range of seeds per fruit.

Species	Year collected	Number of trees sampled	Mean number of fruits collected per tree (range)	Mean number of seeds per fruit (range)
Eucalyptus caesia	2016	50	15 (1-44)	29.8 (0.8-83.5)
Eucalyptus incrassata	2016	54	40 (3-151)	1.9 (0.1-7.9)
Eucalyptus leucoxylon	2014	75	40 (2-139)	7.4 (0-33)
Eucalyptus leucoxylon	2015	48	25 (1-70)	7.2 (0.5-29)
Eucalyptus leucoxylon	2016	148	30 (1-140)	9.0 (0-49)
Eucalyptus leucoxylon	2017	112	20 (1-75)	8.4 (0-40)
Eucalyptus platypus	2016	84	75 (6-256)	3.9 (0-11.6)
Eucalyptus stoatei	2016	71	10 (1-24)	8.7 (0.5-40.1)
Eucalyptus woodwardii	2016	60	25 (1-106)	5.2 (0.3-20.6)

Data analysis

We used generalized linear models (GLMs) to estimate the effect of spatial arrangements and other plant characteristics on seed production (variables described below). We used a negative binominal link function in R v. 3.4.4 (R-Core Team 2018) for the seed number per fruit data because this count data was over-dispersed (i.e. variance exceeded the mean) (Ver Hoef & Boveng 2007). Seed number per fruit was the response variable in all models, and nearest neighbor distance (distance to the nearest reproductive conspecific, NN1), abundance (number of reproductive conspecifics within 100 m), DBH (diameter breast height), health (percent of branches with foliage) and fruit crop (number of fruit from the last flowering season) were the predictor variables. Nearest neighbor distance (NN1) and abundance were somewhat correlated ($r^2 = 0.4$) but since we were interested in testing their relative importance, we included both predictors in our model set. All trees were planted at about the same time, but since tree size, health and reproductive output varied, these variables were also included in our model set.

A multivariate model with all predictor variables was run for all species and for each year of the *E. leucoxylon* data because the number and spatial arrangement of reproductive trees varied across seasons. Following this, a backwards elimination procedure was used to obtain the final models based on the step-wise elimination of terms that were not significant at $P = 0.05$ (Brys et al. 2008; Dalgaard 2008). The models were re-run (removing one non-significant term at a time) until all non-significant terms were removed. ANOVAs were run on each model using the

χ^2 argument to justify model reductions and to rank the significant values based on the variance explained (Dalgaard 2008).

Following this, general linear models with a maximum likelihood, multi-model inference framework (Burnham & Anderson 2002) were run in the base statistics package in R v. 3.4.4 (R-Core Team 2018) to further investigate nearest neighbor arrangements. This method was chosen because it is able to handle correlated predictor variables and rank their relative importance. A separate model was run for each species and for each year of the *E. leucoxylon* data. The response variable was seed number per fruit and the predictor variables were the distance to the nearest neighbor (NN1) and the distance to the second (NN2), third (NN3), fourth (NN4) and fifth (NN5) nearest neighbors (m). We estimated Akaike's Information Criterion corrected for small sample sizes (AIC*c*) and Akaike weights (*w*AIC) for each model (Burnham & Anderson 2002). To assess relative importance of each of the nearest neighbor predictor variable, we derived the index of the relative importance of predictor variable *i* (AIC*i*), which is the sum of Akaike weights for all models that included parameter *i* (Burnham & Anderson 2002; Giam & Olden 2016). Greater AIC*i* (where AIC*i* varies 0-1) implies parameter *i* has greater importance in predicting variation in response variable *j* (seeds/fruit) than parameters with smaller AIC*i*. The data sets were square-root transformed to meet assumptions of normality of model residuals.

Results

Inter-species trends for 2016

Seed production, as measured by seed number per fruit was highly variable, but the highest values were recorded when there was another reproductive conspecific within 20 m for all six species (Fig. 3). Nearest neighbor distance was a better predictor of seed production than abundance in all species and was the strongest predictor of seed production in all six species studied (Fig. 3; Appendix 1). There was a negative relationship between seed count and increasing nearest neighbor distance. However, seed production was more variable at shorter distances (ca 0-50 seeds/fruit) and became less variable with increasing neighbor distances (ca 0-10 seeds/fruit).

In addition to these spatial variables, DBH was a significant negative predictor of seed production in *E. leucoxylon* and *E. platypus*, health was a significant positive predictor for *E. caesia* and *E. platypus* and fruit crop was a significant positive predictor in *E. incrassata*, *E. platypus* and *E. stoatei* (Appendix 1).

Further analysis of nearest neighbor arrangements found that the distance to the first nearest neighbor was the strongest predictor of seed production for *E. incrasssata*, *E. platypus* and *E. stoatei*, the distance to the second nearest neighbor was the key predictor for *E. caesia* and the distance to the fourth neighbor was the strongest predictor for *E. leucoxylon* and *E. woodwardia* (Appendix 1).

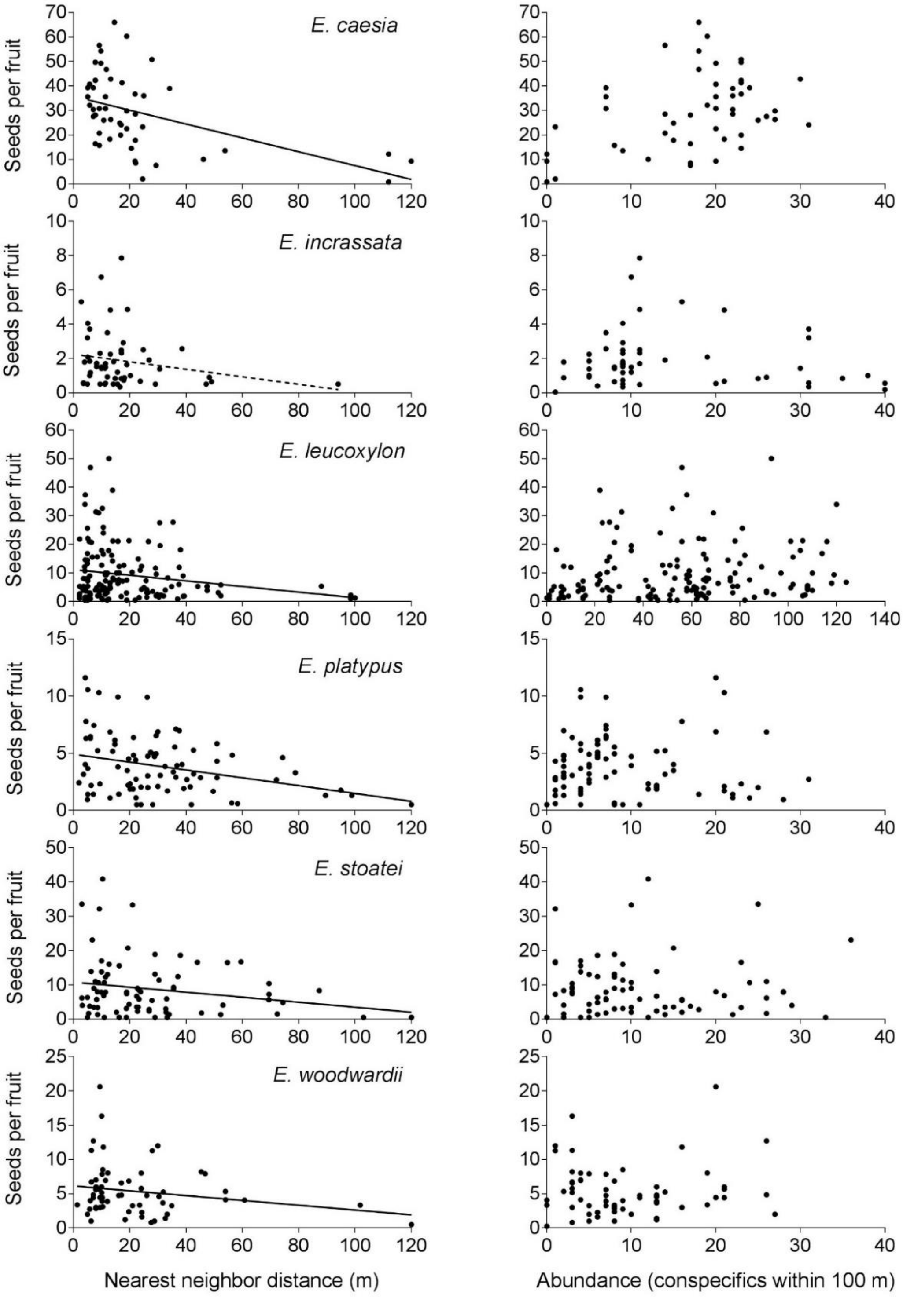


Figure 3. Seed production (seed number per fruit) against nearest neighbor distance (m) and abundance (conspecifics within 100 m) for all six of the *Eucalyptus* species sampled in 2016. Fitted lines show significant linear correlations between seed number and arrangement (solid line: P <0.05; dashed line: P = 0.05-0.1; S1). Note *E. leucoxylon* occurs over a greater abundance range (ca 0-125), than the other five species (ca 0-40).

Inter-year trends in Eucalyptus leucoxylon

Seed production was highly variable for *E. leucoxylon* individuals across years, with average seed number per fruit varying by up to 30 seeds per fruit on an individual tree basis. Despite the highly variable nature of seed production, similar overall trends with planting arrangement were observed in *E. leucoxylon* across the four years (Fig. 4). Nearest neighbor distance was the strongest predictor of seed production in each year, whereas abundance was not a significant predictor in any of the years (Appendix 1). However, seed production was consistently lower for *E. leucoxylon* when there were fewer than 20 conspecifics within 100 m (Fig. 4).

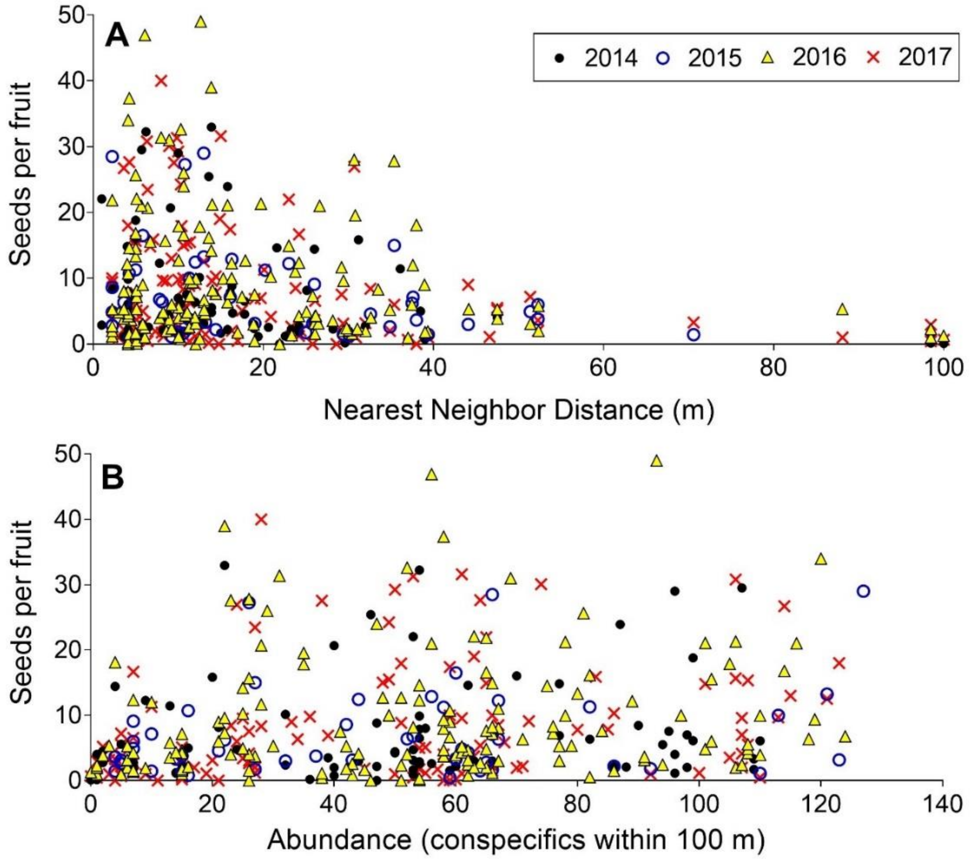


Figure 4. Seed production for *Eucalyptus leucoxylon* individuals across the four years sampled (2014-2017). Seed production against nearest neighbor distance – the distance from the study tree to the closest reproductive *E. leucoxylon* (A); and abundance – the number of reproductive *E. leucoxylon* within a 100 m radius (B).

Analysis of the five reproductive nearest neighbor distances showed that the relative importance of these arrangements varied between the years. In 2014 the distance to the nearest neighbor (NN1) was the strongest predictor of seed number, compared to the distance to the third nearest neighbor (NN3) for 2015, the fourth neighbor (NN4) for 2016 and the second neighbor (NN2) for 2017 (Appendix 1).

Discussion

Plant-pollinator interactions are sensitive to both the number and spatial arrangement of plants within populations, and although these measures are often correlated, they can influence pollination differently (Kunin 1997a; Mustajarvi et al. 2001). In some populations, the number of individuals may have little influence on pollination, with spacing between individuals a more important variable (Kunin 1997a). Our findings support this observation and highlight that similar trends are evident in the revegetated Monarto woodlands, across species and years. To the best of our knowledge, this is the first work to document this trend in a revegetated system. We found that nearest neighbor distance was a better predictor of seed number per fruit than overall plant abundance (number of conspecifics within 100 m). We observed a consistent trend for seed production to be highest when there was another reproductive conspecific within 20 m and then decrease with increasing nearest neighbor distance, despite differences in floral morphology, natural distribution (species native to South Australia or Western Australia) and overall abundance of the six eucalypt species studied. Consequently, in order to promote pollination and seed production in revegetated stands, we recommend that revegetation designs consider conspecific spacing (avoiding large distances between conspecifics), rather than simply aiming for an overall number of individuals within an area to promote seed production and potentially improve the long-term sustainability of revegetated populations.

Reductions in seed set with increasing nearest neighbor distances have also been recorded in natural *Eucalyptus* populations. For example, in *Eucalyptus melliodora*, trees separated by at least 50 m produced half the number of seeds per fruit of closely spaced woodland trees (Burrows 2000). Similar trends are also evident in a range of other species from around the world, with examples of wind and animal pollinated species, across plant types (i.e. herbs, shrubs, trees), and from multiple biomes (e.g. Ghazoul et al. 1998; Tomimatsu & Ohara 2002; Severns 2003; Burgos et al. 2008). Accordingly, planting arrangement has the potential to influence reproduction not only in eucalypts but also a range of other species used for revegetation around the world. Furthermore, research has shown that reproduction in eucalypts can be more resistant to the impacts of fragmentation than other species because of strong outcrossing and regular long-distance pollen flow (Byrne et al. 2008; Breed et al. 2015b). Therefore the influence of dispersed planting arrangements may be more pronounced in other species, particularly those with less mobile pollinators. On-going research is now required to examine whether the patterns observed here hold true for other species and other systems.

In natural communities, some plant species appear more resistant to the effects of dispersed or fragmented arrangements (Krauss et al. 2007; Ottewell et al. 2009; Vesk et al. 2010; Breed et al. 2015a), and one potential reason for this is pollinator mobility, with different responses appearing in bird and insect pollinated species (Breed et al. 2015a, b; Krauss et al. 2017). More mobile bird pollinators can forage over greater areas and cover larger distances between plants, allowing similar levels of seed production to be maintained in more dispersed populations (Breed et al. 2015a; Lowe et al. 2015; Krauss et al. 2017). Floral syndromes, such as flower color, shape and size have evolved to attract specific groups of pollinators (Baker et al. 1998; McCallum et al. 2013). However, despite differences in floral morphology, we detected similar trends in seed production with spatial arrangement across all six species (flowers ranged from small, white and clustered (presumably insect pollinated) to large, single and pink (presumably bird pollinated)). We found that the presumably insect pollinated species appeared to show the same pattern as those pollinated by insects and birds and those species considered predominantly bird-pollinated. As such, our results suggest that the six eucalypt species included here were probably visited by a similar suite of generalist pollinators, with pollinator movements that seem to be most common between plants separated by < 20 m.

The number of flowers produced by eucalypts often vary from year to year on an individual tree and population basis (Paton 2008), and this may influence pollinator foraging behavior because pollinators often forage more widely when less flowers are available (Carthew 1994). We found nearest neighbor distance to be a stronger predictor of seed production than abundance across all four years of *E. leucoxylon* data. However, the relative importance of the distance to the first to fifth nearest neighbors differed across the four seasons. This suggests that pollinator foraging behavior varied across the years in response to resource availability, at a fine scale, but despite this variation, the distance between conspecifics remained a better predictor of seed production than abundance.

In order for revegetated sites to become self-sustaining, it is not only pollination and seed set that need to occur, but also regeneration (Godefroid *et al.* 2011). In the Monarto Woodlands, eucalypts have been flowering and setting fruit for at least 20 years, but recruitment is rare (Paton *et al*. unpubl. data), and a lack of recruitment has also been observed in other revegetated systems (Schneemann & McElhinny 2012; Neldner & Ngugi 2017). While manipulating planting arrangements can have positive benefits for reproductive output, it will only be worthwhile if recruitment failure in these systems is also addressed. Therefore, to aid in the creation of self-sustaining systems, on-going research into both reproductive output and the recruitment dynamics of revegetated populations is required.

Implications for revegetation

Here we show that, at least for our study species, using a standard number of plants per hectare, without consideration of their spatial arrangement, to guide revegetation may fail to produce reproductively healthy, and therefore self-sustaining populations. We show that seed production in our six eucalypt species is improved by planting conspecifics close together. However, this recommendation needs to be tempered by the effect of very close plantings (<4 m), which can affect tree structure (Alcorn et al. 2007) and reduce reproductive output due to strong competition (Williams et al. 2006; Paton 2008). The genetic quality of seed also needs to be considered and planting in pairs or planting conspecific individuals very close together (i.e. canopies touching) may result in seed crops being dominated by one pollen donor (Krauss 2000), reducing seed quality and offspring fitness (Breed et al. 2012a, 2014).

In natural eucalypt woodlands it is common for individuals to have a neighbor of the same species and many conspecifics nearby (McCallum et al. 2018a). Spatial aggregation is also commonly observed in a range of other ecosystems around the world (e.g. Condit et al. 2000; Davis et al. 2005; Miller et al. 2010). Therefore, planting designs that mimic natural patterns of conspecific spacing and aggregation have the potential to facilitate pollinator movements between conspecifics increasing both the quantity and quality of seed produced. Although on-going research is required, aggregated arrangements may also help maintain community level diversity, limit weed invasion and improve habitat value in restored systems (McCallum et al. 2018b).

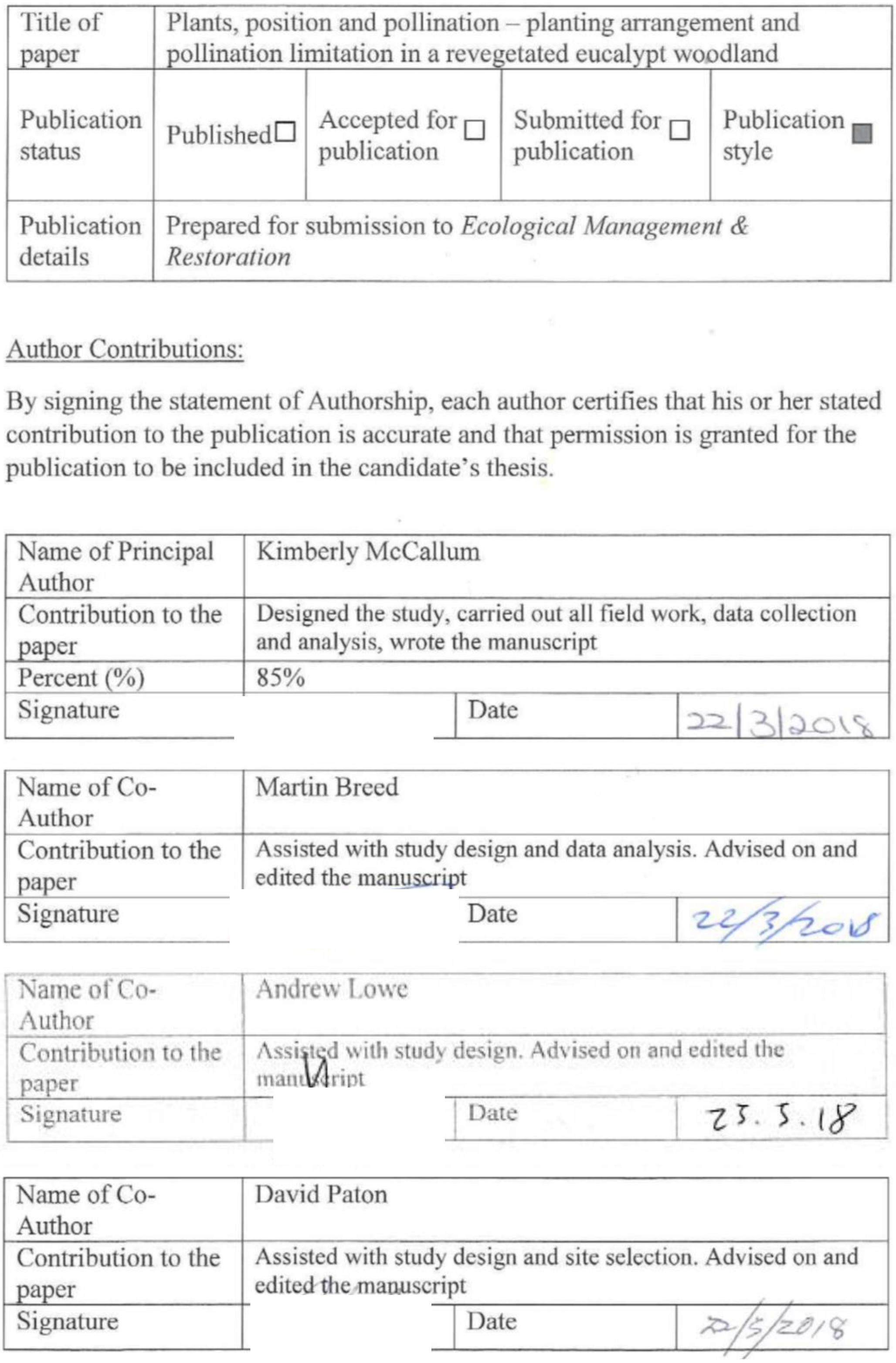
Aggregated arrangements will be easier to achieve with tubestock planting, as the position of every plant can be controlled (Jonson 2010). However, tubestock plantings may not be a viable option for some large-scale revegetation projects (Corr 2003; Munro & Lindenmayer 2011), and in these cases, a combination of direct seeding and tubestock planting may be most cost-effective (Jonson 2010). Direct seeding of common species may be adequate because individuals are more likely to have conspecifics nearby, simply because they are more abundant (although thinning may be required if high density stands establish). On the other hand, tubestock planting may be the best option for rare species, because large distances between conspecifics can be avoided.

Acknowledgements

This research was supported by funding from the Australian Wildlife Society, BioR, Field Naturalists Society of South Australia, Holsworth Wildlife Research Endowment and the Nature Foundation of South Australia awarded to KPM, and Australian Research Council funding to AJL and MFB (DE150100542; DP150103414). KPM was supported by an Australian Postgraduate Award and the Noel and Vivian Lothian Scholarship. We thank Aaron Miatke for field and statistical support, Rachel Ladd and Hayley Merigot for providing access to vegetation survey data and Geoff Brooks for providing access to vegetation with the Monarto Zoological Park. We also thank two anonymous reviewers whose comments greatly improved the manuscript.

Chapter 4. Pollination

Statement of authorship



Title of paper	Plants, position and pollination – planting arrangement and pollination limitation in a revegetated eucalypt woodland			
Publication status	Published ☐	Accepted for publication ☐	Submitted for publication ☐	Publication style ☒
Publication details	Prepared for submission to *Ecological Management & Restoration*			

Author Contributions:

By signing the statement of Authorship, each author certifies that his or her stated contribution to the publication is accurate and that permission is granted for the publication to be included in the candidate's thesis.

Name of Principal Author	Kimberly McCallum		
Contribution to the paper	Designed the study, carried out all field work, data collection and analysis, wrote the manuscript		
Percent (%)	85%		
Signature		Date	22/3/2018

Name of Co-Author	Martin Breed		
Contribution to the paper	Assisted with study design and data analysis. Advised on and edited the manuscript		
Signature		Date	22/3/2018

Name of Co-Author	Andrew Lowe		
Contribution to the paper	Assisted with study design. Advised on and edited the manuscript		
Signature		Date	23.5.18

Name of Co-Author	David Paton		
Contribution to the paper	Assisted with study design and site selection. Advised on and edited the manuscript		
Signature		Date	22/5/2018

Plants, position and pollination – planting arrangement and pollination limitation in a revegetated eucalypt woodland

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Running head: Plants, position and pollination

Author contributions: All authors conceived the ideas and developed the experimental design. KPM carried out field work and data collection. KPM competed data analysis, with assistance from MFB. KPM wrote the first draft of the manuscript and all authors contributed to revisions.

Abstract

The spatial arrangement of plants is a key determinant of pollination services in natural ecosystems. Despite this, plant arrangements are rarely considered an important characteristic of revegetated communities and this may be limiting successful pollination dynamics in these systems. We assessed if aggregated planting arrangements improved pollination success by measuring fruit set, seed production and germination in a revegetated eucalypt woodland. We show that on average, aggregated *Eucalyptus leucoxylon* produced more seeds per fruit than dispersed individuals across the three seasons, but this difference was most pronounced in years of greater flowering intensity. Germination rates were similar for seeds from aggregated and dispersed trees, which suggests outcrossed pollen was received by trees in both arrangements. However, pollination limitation was evident, with the addition of outcrossed pollen increasing fruit and seed set in both aggregated and dispersed trees. Consequently, we propose that revegetation plantings that establish conspecific clusters can improve seed production in revegetated woodlands, but this will only be effective if there are enough pollinators in the system and those pollinators deliver an adequate quantity and quality of pollen.

Key words

Aggregation, germination, plant spatial pattern, restoration planting, seed production, revegetation design, seed production

Introduction

Plant reproduction is often linked to the interaction between plant spatial arrangement and pollinator behaviour (Meagher & Vassiliadis 2003; García-Meneses & Ramsay 2012). In animal pollinated species, pollinator movements determine patterns of pollen flow (Jones 1997; Wilcock & Neiland 2002), and plants growing at higher densities often receive more pollinator visits than widely spaced individuals because the cost of moving between plants is lower (Kunin 1993, 1997a; McCallum *et al*. 2013). Consequently, closely spaced individuals tend to receive higher quantities of outcrossed pollen and a greater diversity of pollen than more dispersed individuals, increasing reproductive fitness (Butcher *et al*. 2005; Breed *et al.* 2014).

In low density populations, plants often receive fewer pollinator visits due to the increased cost of moving between individuals (Kunin 1997a; Field *et al.* 2005; Ottewell *et al.* 2009). As a result, dispersed or isolated plants may exhibit higher levels of selfing (de Jong *et al.* 1993; Butcher *et al*. 2005; Breed *et al*. 2012b), and this can cause plant reproduction (fruit and seed set, seed viability) and offspring fitness to decline (Cunningham 2000; Vesk *et al*. 2010; Breed *et al.* 2012a). These declines can put plant populations at risk of extinction if they do not set adequate seed to replace themselves (Lamont et al. 1993; Wilcock & Neiland 2002).

Human activities can result in changes to the spatial arrangement of plant populations and this may influence pollination by directly changing pollinator and plant abundance, or by changing pollinator behaviour (Lamont *et al.* 1993; Cunningham 2000; Ghazoul & Shaanker 2004). Fragmented systems often experience such effects, with clearing generally resulting in smaller and more dispersed populations, and this can negatively impact plant reproduction (Lamont et al. 1993; Aguilar *et al.* 2006; Breed *et al.* 2015a). It can be hypothesised that similar trends will occur in revegetated communities if planting activities generate populations that are smaller and/or more dispersed than those that occur naturally. There is some evidence to support this hypothesis with the distance between plants a predictor of seed production in revegetated woodlands (McCallum *et al.* accepted). However, on-going research is needed to better understand how planting arrangements influence pollination services and seed quality in revegetated systems.

Here we assessed fruit set, seed production, germination and pollination limitation in revegetated *Eucalyptus leucoxylon* individuals as a function of spatial aggregation. The specific research questions were: (1) Do aggregated *E. leucoxylon* set more fruit and more seeds per fruit, and produce more viable seed than dispersed individuals? (2) Is reproductive output constrained by pollination limitation, and is this limitation greater in dispersed trees? (3) Do these trends vary between flowering seasons as flowering intensity often varies from year to year in long-lived *Eucalyptus* species?

Methods

Study system

We targeted the revegetated Monarto Woodlands for our study, approximately 70 km south-east of Adelaide, South Australia (Fig. 1). The Monarto Woodlands were revegetated in the 1970s by the Government of South Australia in an effort to reduce dust and erosion. A mix of 250 tree and large shrub species were used, including local endemics, Australian natives planted outside their natural range, and exotic species. Approximately 1,850 ha were revegetated with tubestock planted 4 - 6 m apart in rows spaced 4 - 6 m apart (Paton *et al.* 2004a, Paton *et al.* 2010), resulting in a density of ca 400 plants/ha at establishment. The revegetation is dominated by eucalypt species (ca 60-70% of plants; Fig. 2), and the density of these individual species can vary widely across the revegetation area.

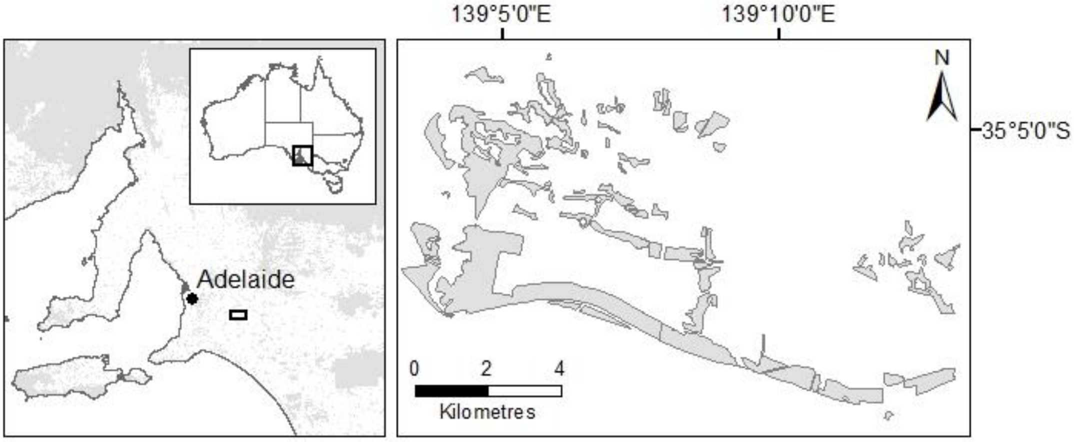


Figure 1. The location of the revegetated Monarto Woodlands, South Australia, showing the location and extent of remnant vegetation (left) and the size and distribution of the revegetation areas (right). Data source: Department of Environment, Water and Natural Resources; Regional City of Murray Bridge.

Figure 2. Planting arrangement at the Monarto Woodlands showing the linear planting arrays (4 - 6 m spacing with 4 - 6 m spaced contour rows), which are still evident ca 40 years after planting (A). The woodlands are eucalypt dominated and conspecifics generally occur within a matrix of other species (B). [Photo credits – Kimberly McCallum 2016].

Study species

Eucalyptus leucoxylon is widely used in revegetation across southern Australia and is relatively abundant in the Monarto Woodlands (ca 1-35 trees/ha). The species has been recorded flowering year round in the Adelaide region, but flowering is generally most abundant during winter and spring (Nicolle 2013; Merigot & Paton 2018). Flowers range in colour from cream to red and are visited by a range of birds, primarily honeyeaters and lorikeets, and small insects, including bees, flies and wasps (Paton & Ford 1977; Ottewell *et al*. 2009). However, the species is thought to be predominantly bird pollinated (Ottewell *et al*. 2009; Zilko *et al.* 2017). *Eucalyptus leucoxylon* is a preferential outcrosser that is also capable of selfing. Flowers are bisexual and male reproductive organs mature before the female organs, but flowers develop sequentially, so geitogamous self-pollination can occur (Ottewell *et al.* 2009). In *Eucalyptus* sp. self-pollination can lower seed set and seed quality (Burrows 2000; Butcher *et al*. 2005).

Field survey

Field work was completed between March 2015 and May 2017. Targeted surveys were undertaken to record the position and reproductive status (presence of fruit, buds, flowers) of *E. leucoxylon* individuals with a hand-held GPS (Garmin GPSmap 62) across approximately 100 ha, leading to 1100 mapped *E. leucoxylon* individuals. Of these individuals, 74 were selected as study trees, based on presence of mature fruit from the previous flowering season (identified by colour and surface texture), number of buds, spatial arrangement and canopy height (canopy height (from ground) range 0-3 m, tree height range 2-10 m). Study trees were either aggregated, where 5 reproductive *E. leucoxylon* were within 30 m (n = 51), or dispersed, with no reproductive *E. leucoxylon* within 30 m (n = 23). Trees were re-surveyed each year to assess reproductive status. Aggregated trees were more common in the woodlands and, as a result, more aggregated than dispersed trees were used in the study. Not all study trees were sampled in every season because the spatial arrangement of reproductive trees varied across seasons.

Natural fruit production, seed set and germination

To determine how spatial aggregation influenced natural seed production of open-pollinated *E. leucoxylon*, we collected fruits from the 2014, 2015 and 2016 flowering seasons, with fruits collected in the following year (i.e. fruits from the 2014 flowering season collected in 2015). *Eucalyptus leucoxylon* holds its fruits for more than one year, so fruits from the most recent season were identified by colour and texture (greener and less woody). Approximately 25 (± 2 SEM) mature fruits were collected from each study tree per year, when available. Fruits were collected from across the canopy and at a variety of heights (up to 6 m with ladder and extendable loppers).

Fruits from each tree were stored in an individually labelled paper bag and left to dry at room temperature (approximately 25°C) until capsules opened. Fruits were placed in a plastic container and shaken vigorously to release contents, before seed was separated from chaff and capsule material. Seeds were distinguishable from chaff based on size (seeds were larger), shape (seeds were rounder) and colour (seeds were darker). Seeds were counted and the mean number of seeds per fruit determined for each tree.

The percent conversion of buds to fruits was estimated for the 2015 and 2016 flowering seasons to assess fruit set. On average, three branches (range = 1-7) on each study tree were marked with flagging tape. Branches were tagged before the start of the flowering season, the number of buds present on each branch counted and any fruits from previous flowering seasons removed. Approximately 200 buds (range = 60-675) per tree were assessed. After flowering finished, fruits were left to develop (ca 6 months), before the number of fruits was recorded.

Seed from a subset of the study trees from the 2015 (17 aggregated, 15 dispersed) and 2016 (19 aggregated, 15 dispersed) flowering seasons were selected for germination trials. Fifty seeds per tree were germinated in petri dishes in glasshouse conditions (when <50 seeds were available, all seeds were germinated). Seeds were placed between two sheets of moist filter paper on a bed of vermiculite. Germination experiments were undertaken in autumn of 2017 in a temperature controlled glasshouse (ca 25°C). The number of seeds germinated was recorded daily over a 14 day period, and seeds were removed from the petri dishes as they germinated (cracked seed coats, emerging cotyledons). The petri dishes were randomly shuffled every one to two days to minimise glasshouse location effects.

Pollination manipulations

A subset of the study trees from the 2015 and 2016 flowering seasons were selected for pollination manipulations. In addition to the open-pollinated branches (described above), three branches were treated with outcrossed pollen (ca 150 buds per tree) and two branches were bagged (with fine voile material) to exclude pollinators (ca 120 buds per tree). In 2015, eight aggregated and six dispersed trees were used, and in 2016, 21 aggregated and 16 dispersed trees were used (Table 1). Fewer study trees were sampled in 2015 because fewer trees flowered, and those that did flower produced fewer flowers.

Table 1. Number of *Eucalyptus leucoxylon* study trees used to track the open-pollinated conversion rates of buds to fruit for the 2015 and 2016 flowering season in each arrangement class (Open) and for the pollination experiments (Experimental). The plants used for the pollination experiments were a subset of the open-pollinated plants.

	Aggregated		Dispersed	
Year	Open	Experimental	Open	Experimental
2015	25	8	19	6
2016	44	21	19	16

Hand-pollination was undertaken in August and September in 2015 and from May to October in 2016. Hand-pollination was undertaken on 11 days in 2015 and branches were treated ca four times. In 2016, hand-pollination was undertaken on 20 days and branches were treated ca five times. The branches used for hand-pollination were left open, so experimental cross-pollination was additional to any pollination occurring naturally. Donor pollen was sourced from revegetated *E. leucoxylon* individuals, with trees treated with pollen from at least three different donors. The stamens of donor flowers were rubbed in a circular motion over the style of the experimental flowers. Once treated, flowers were marked with a small dot of paint (on the peduncle) to allow identification of fruits resulting from hand-pollination.

Fruits were left to develop for ca 6 months before they were counted and collected. The conversion rate of buds to fruits and the number of seeds per fruit were determined for both treatments (bagged, hand-pollinated). Seeds were germinated from 8 aggregated (4 bagged, 8 pollinated) and 6 dispersed trees (3 bagged, 6 pollinated), from 2015 and 10 aggregated (7 bagged, 10 pollinated) and 9 dispersed trees (6 bagged, 9 pollinated) from 2016. Not all trees produced fruits when branches were bagged, so fewer bagged treatments were used in the germination experiments.

Data analysis

Two-way ANOVAs were used to compare open pollinated fruit set, seed number per fruit and germination, with arrangement and year as the fixed factors. Reproductive response data were square-root transformed before analysis to normalise the distribution of residuals. Tukey multiple comparison tests were run when the ANOVA was significant at P=0.05.

Linear mixed effect models were used to compare reproductive responses between arrangement classes and pollination treatments, with individual tree as a random factor to account for the non-independence of the three treatments performed on the same individual. Separate models were run for 2015 and 2016 data. We used nlme (Pinheiro et al. 2017) to run the mixed effect models and lsmeans (Lenth 2016) to run multiple comparison tests on significant factors using least square means. Data analysis was undertaken in R v 3.3.2 (R-Core Team 2016).

Plants can re-allocate resources among flowers, therefore, increased fruit and seed production due to hand pollination may be confounded with plant responses to additional pollen (Ashman et al. 2004). We checked for the possibility of resources being redirected away from untreated flowers by comparing the outputs (fruit and seed set) from flowers left open to pollinators on trees that did and did not have flowers hand-pollinated and bagged using unpaired T-tests. Data were square-root transformed before analysis to normalise the distribution of the residuals.

Results

Natural fruit production, seed set and germination

In 2015, trees set ca 490 buds (± 92 SEM) buds, which was significantly fewer than the 980 buds (± 180 SEM) set during 2016 (Table 2, $P<0.05$), but bud production was similar between the arrangement classes within years (2015 dispersed 570 (± 197 SEM), 2015 aggregated 450 (± 70 SEM), 2016 dispersed 1030 (± 231 SEM), 2016 aggregated 1025 (± 227 SEM)). In 2015, 13.5% (± 1.6 SEM) of tagged buds developed into fruits, which was significantly higher than the 6.9% (± 1.1 SEM) that developed into fruits in 2016 (Table 2, $P<0.001$). There were no significant differences in fruit set between the two arrangements within years ($P=0.9$, Table 2, Fig. 3A).

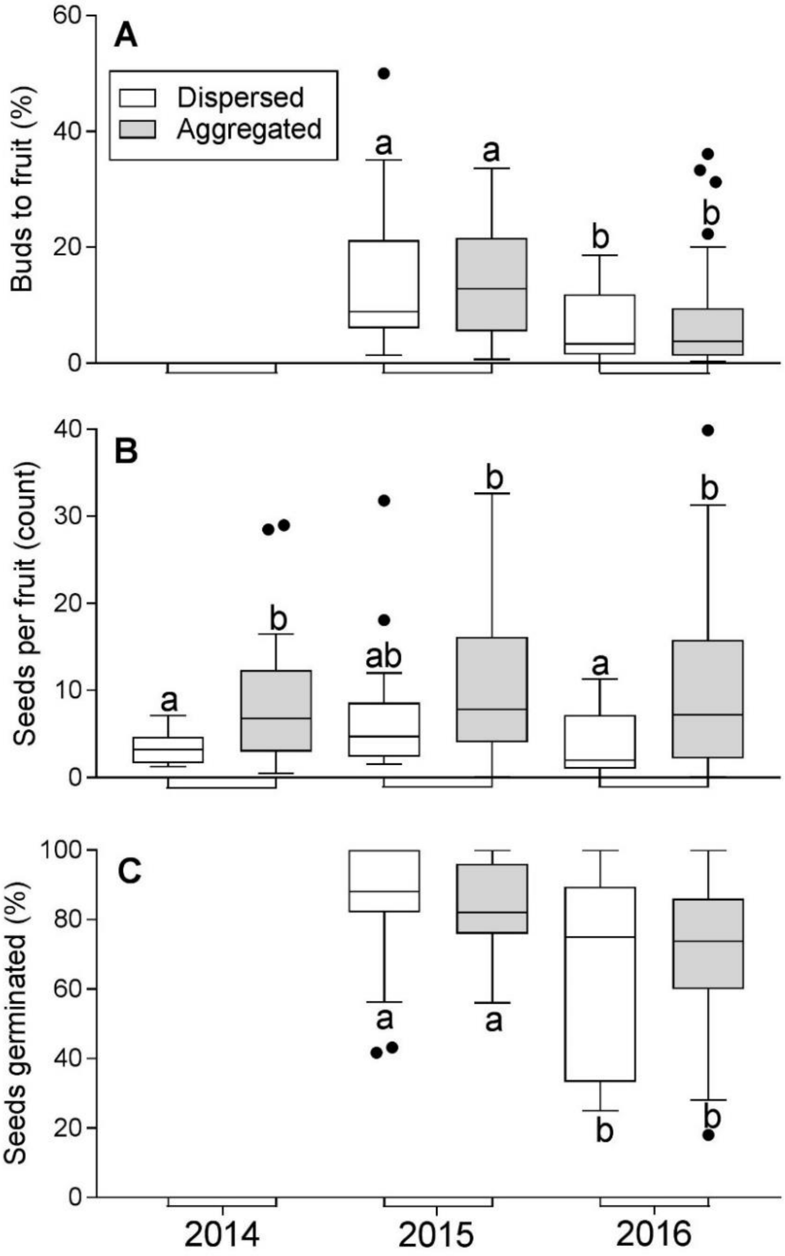


Figure 3. Percent conversion of buds to fruits (A), seed number per fruit (B) and percent of seeds germinated after 14 days (C) for open pollinated aggregated and dispersed *Eucalyptus leucoxylon* in the 2014 to 2016 flowering seasons. Sample sizes (number of trees), 2014 (dispersed n = 14, aggregated n = 25), 2015 (dispersed n = 19; aggregated n = 25) and 2016 (dispersed n = 19, aggregated n = 44). Tukey box and whisker plots, with line at the median, box from 25th to 75th percentiles, minimum to maximum shown by whiskers and outliers shown by dots. Significant differences at P=0.05 represented by letters.

Table 2. 2-way ANOVA of natural bud and fruit production, seed set and germination. Bud number – total number of buds per tree, conversion buds to fruit - % buds that developed into fruit, seeds per fruit – mean number of seeds per fruit and germination rate - % of seeds germinated after 14 days. Arrangement*year represents the interaction between the two variables. Bold denotes significant differences at P=0.05.

Source of variation	df	SS	*F*	*P*
Bud number				
Arrangement	1	23.7	0.13	0.72
Year	1	1996.0	10.97	**<0.05**
Arrangement*year	1	6.5	0.035	0.85
Error	103	18729.7		
Conversion buds to fruit				
Arrangement	1	0.006	0.003	0.96
Year	1	36.33	17.59	**<0.0001**
Arrangement*year	1	0.052	0.025	0.87
Error	103	212.71		
Seeds per fruit				
Arrangement	1	31.29	18.58	**<0.0001**
Year	2	3.04	1.81	0.167
Arrangement*year	2	1.88	1.12	0.329
Error	166	1.69		
Germination rate				
Arrangement	1	0.392	0.19	0.67
Year	1	19.08	9.18	**<0.01**
Arrangement*year	1	0.214	0.10	0.75
Error	62	128.82		

Aggregated trees produced more seeds per fruit than dispersed trees across all three seasons (Table 2, Fig. 3B). This difference was significant in 2014 and 2016 (2014 dispersed 3.4 (± 0.4 SEM), aggregated 8.5 (± 1.7 SEM), Tukey P<0.05; 2016 dispersed 3.3 (± 0.8 SEM), aggregated 10.4 (± 1.5 SEM), Tukey P<0.01). In 2015, seed number per fruit was similar in aggregated and dispersed trees (dispersed trees 6.8 (± 1.6 SEM), aggregated 10.8 (± 1.5 SEM), Tukey P=0.5). The percent of seeds that germinated in 2015 (84% ± 3 SEM) was significantly higher than 2016 (67% ± 4 SEM) but was similar between the arrangements within years (Table 2; Fig. 3C).

Pollination experiments

In 2015, the percent of buds converted into fruits was similar between dispersed and aggregated trees, but differed between the pollination treatments (Table 3, Fig. 4). Less than 1% of buds on bagged branches developed into fruit (0.6% dispersed trees, 0.9% aggregated trees), 10.5% (± 1.8 SEM) of open buds developed into fruit (8.2% dispersed trees, 12.4% aggregated trees), and 22% (± 3.7 SEM) of hand-pollinated buds developed into fruit (16.3% dispersed, 26.3% aggregated). There was a significant difference in conversion of buds to fruits between all pollination treatments (Least squares multiple comparisons, Bag-Open $P<0.001$, Bag-Pollinated $P<0.001$, Open-Pollinated $P<0.01$; Fig. 4A).

The same trend was observed in 2016, with the percent of buds to fruits similar between the arrangements but differing between the pollination treatments (Table 3, Fig. 4). Bagged branches set 3.8% (± 1.4 SEM) of fruit (4.2% dispersed, 3.6% aggregated), open branches set 6.7% (± 1.4 SEM) of fruit (6.3% dispersed, 7.1% aggregated) and hand-pollinated branches set 17.3% (± 2.2 SEM) of fruit (22.8% dispersed, 13% aggregated). There was a significant difference between all treatments (Least squares multiple comparisons, Bag-Open $P<0.01$, Bag-Pollinated $P<0.001$, Open-Pollinated $P<0.001$; Fig. 4B).

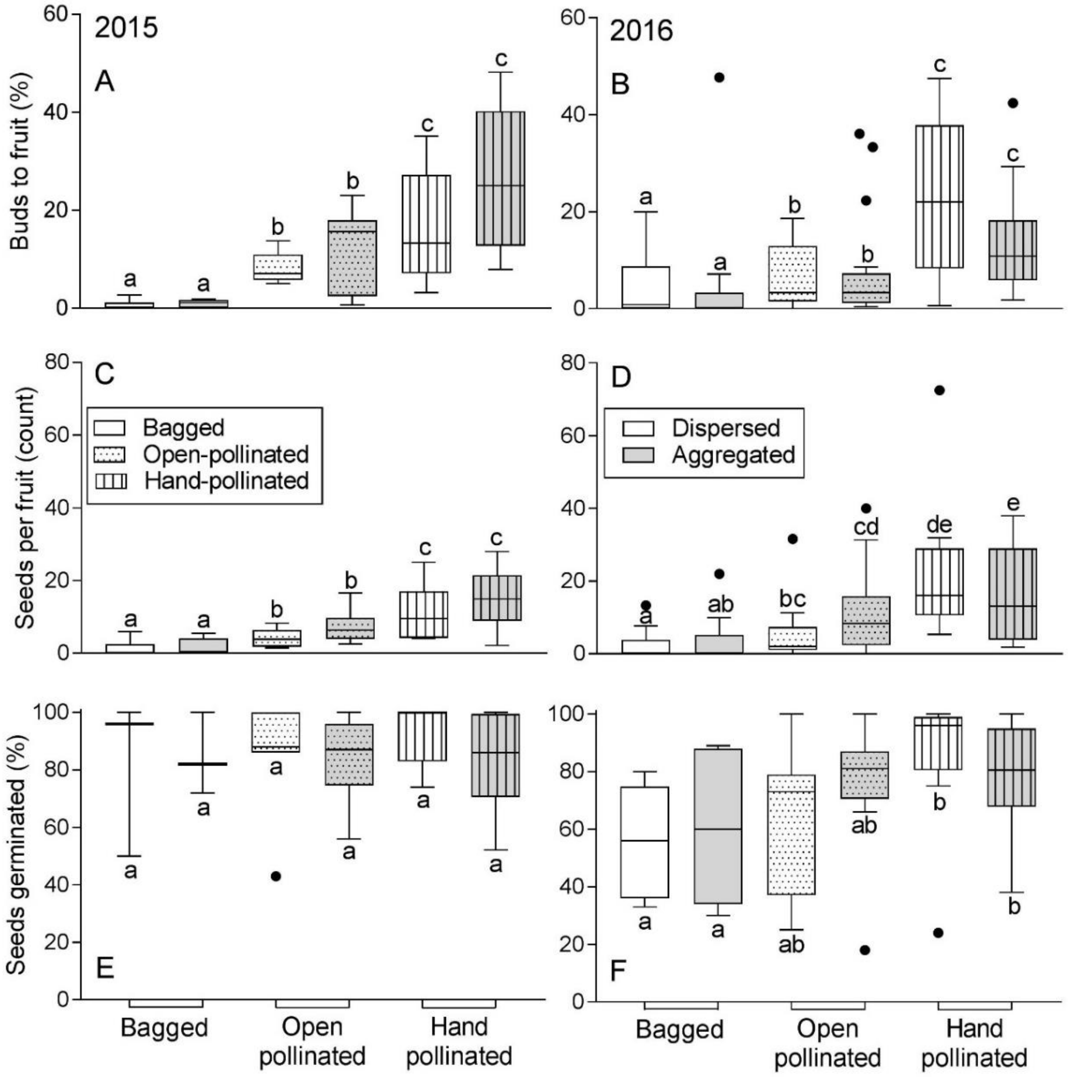


Figure 4. Conversion rate of buds to fruits, seed number per fruit and percent of seeds germinated at day 14 for aggregated and dispersed trees across the three pollination treatments (bagged, open-pollinated, hand-pollinated) and two flowering seasons (2015, 2016), for *Eucalyptus leucoxylon*. Tukey box and whisker plots, with line at the median, box from 25th to 75th percentiles, minimum to maximum shown by whiskers and outliers shown by dots. Shading distinguishes the different arrangements - dispersed (white) and aggregated (grey). Patterns show the different pollination treatments, with bagged (no pattern), open (dots) and pollinated (vertical lines). Significant differences at P=0.05 represented by different letters.

In 2015, seed number per fruit was significantly different between the pollination treatments but was similar between the two arrangements (Table 3, Fig. 4). Bagged branches set 1.4 (± 0.6 SEM) seeds/fruit, open branches set 6.0 (± 1.1 SEM) seeds/fruit and hand-pollinated branches set 13.4 (± 2.2 SEM) seeds/fruit.

There were significant differences between the three treatments (Least squares multiple comparisons, Bag-Open P<0.001, Bag-Pollinated P<0.001, Open-Pollinated P=0.01; Fig. 4C).

Table 3. Generalized linear mixed model for pollination treatments in the two study years. Arrangement*treatment refers to the interaction between the two. Significant differences at P=0.05 are shown in bold.

Source of variation	2015				2016			
	df	SS	*F*	*P*	df	SS	*F*	*P*
Conversion buds to fruit								
Arrangement	1	1.4	1.5	0.25	1	2.9	1.7	0.20
Treatment	2	109.4	60.1	**<0.0001**	2	68.7	40.3	**<0.0001**
Arrangement* treatment	2	1.5	0.8	0.44	2	2.8	1.6	0.21
Seeds per fruit								
Arrangement	1	2.3	2.3	0.16	1	2.8	2.0	0.18
Treatment	2	53.0	26.3	**<0.0001**	2	169.9	59.9	**<0.0001**
Arrangement* treatment	2	0.3	0.2	0.86	2	14.4	5.1	**<0.01**
Germination rate								
Arrangement	1	1.2	0.3	0.57	1	0.4	0.2	0.67
Treatment	2	1.7	0.2	0.79	2	16.0	4.3	**<0.05**
Arrangement* treatment	2	1.4	0.2	0.83	2	3.8	1.0	0.37

In 2016, there was a significant difference between the treatments but not between the arrangement classes (Table 3). Bagged branches set 2.7 (± 0.8 SEM) seeds/fruit, open branches set 8.2 (± 1.6 SEM) seeds/fruit and hand-pollinated branches set 18.5 (± 2.3 SEM) seeds/fruit. There was a significant difference between all three treatments (Least squares multiple comparisons, Bag-Open P<0.001, Bag-Pollinated P<0.001, Open-Pollinated P<0.05). In 2016, there was also a significant interaction between treatment and arrangement (Table 3). The open pollinated dispersed treatment was not significantly different from one of the bagged treatments and the open pollinated aggregated treatment was not significantly different from one of the hand-pollinated treatments (Fig. 4).

The percent of seeds germinated was similar across arrangements and treatments for 2015 with ca 85% (± 2.7 SEM) of seeds germinating after 14 days (Table 3, Fig. 4). In 2016, the percent of seeds germinated was significantly higher for the hand-pollinated treatment (82% ± 5 SEM) compared to the bagged treatment (58% ± 6 SEM), but neither differed from the open pollinated treatment (69% ± 6 SEM; Fig. 4F).

Resource re-allocation

There was no significant difference between flowers left open to pollinators on trees that did and did not have flowers hand-pollinated and bagged, and so no evidence of resource re-allocation (Table 4).

Table 4. Unpaired t-test outputs to check for resources being redirected away from untreated flowers by comparing outputs (% buds to fruit, seeds/fruit) from flowers left open to pollinators on trees that did (experimental) and did not (non-experimental) have flowers hand-pollinated and bagged. SEM in parentheses.

	Experimental (± SEM)	Non-experimental (± SEM)	*t*	*df*	P
2015					
% buds to fruit	10.6 (± 1.8)	14.8 (± 2.3)	1.11	46	0.3
Seeds/fruit	6.3 (± 1.1)	8.3 (± 1.6)	0.63	43	0.5
2016					
% buds to fruit	6.4% (± 1.1)	9.1% (± 2.9)	0.91	57	0.4
Seeds/fruit	8.2 (± 1.2)	7.4 (± 1.6)	0.33	57	0.7

Discussion

In natural plant communities, individuals in closely spaced or aggregated populations often receive a greater quantity and diversity of pollen because pollinator movements between plants are more common (Kunin 1993; Yates *et al.* 2007; Breed *et al.* 2012a). Accordingly, aggregated plants often show higher levels of cross-pollination and increased reproductive output (González-Varo *et al.* 2009a); but despite these general trends, it is also common for reproductive output to vary widely between plants, and within and between flowering seasons due to fluctuations in floral resources (Burd 1994; Carthew 1994; Burrows 2000).

Here we show that these pollination trends are also evident in the revegetated Monarto Woodlands. Fruit set was comparable between the two arrangement classes, but aggregated *E. leucoxylon* produced more seeds per fruit than dispersed trees across all three years of investigation. Seed production was highly variable, but despite this variation, aggregated trees produced significantly more seeds per fruit than dispersed trees in the two years of higher flower production (2014, 2016). In 2015, when fewer trees flowered and those that did flower produced fewer flowers, seed production was similar in aggregated and dispersed trees. Pollen is often dispersed more widely during times of low flower production (Carthew 1994), so it is likely that pollinators foraged over larger areas in 2015, allowing dispersed trees to produce a similar number of seeds per fruit to aggregated trees.

Self-pollination in eucalypts can result in a reduction in both the number of seeds produced and the viability of those seeds (Burrows 2000; Butcher *et al.* 2005). Although it was common for dispersed trees to produce fewer seeds per fruit than aggregated trees, there was no difference in the percent of those seeds that germinated. It is therefore likely that trees in both arrangements received outcrossed pollen, and this is supported by the pollination experiments as open-pollinated branches set more fruit and more seeds per fruit than bagged branches. In addition, there may also be efficient selection against self-pollination such that only relatively outcrossed seeds mature (Krauss *et al.* 2007), resulting in seed of a similar quality being produced by trees in both arrangements.

Eucalyptus leucoxylon is predominantly pollinated by honeyeaters and lorikeets (Ottewell *et al.* 2009; Zilko *et al.* 2017), and surveys in the Monarto Woodlands have recorded a range of these species, with densities of birds comparable to those using nearby remnant vegetation (Paton *et al*. 2004a). These birds are capable of moving distances of 10-15 km in relatively short periods (Paton *et al*. 2004a; Willoughby 2005), so movements between trees separated by 4-88 m is likely (nearest neighbour distance range of study trees), facilitating outcrossing even in dispersed trees.

However, field surveys in the Monarto region have shown that eucalypt floral resources often exceed the numbers of birds available to exploit these resources (Paton 2008), and this may contribute to the pollination limitations observed. In addition, the mixed nature of plantings at Monarto may result in pollinators moving between individuals of different flowering species, facilitating inter-species pollen flow and reducing the quality of pollen received (Paton & Ford 1977; Kunin & Iwasha 1996). Consequently, opportunities exist to reduce pollination limitation of plant reproduction in revegetated systems through greater consideration of the needs and foraging behaviour of pollinators.

Implications for Revegetation

Almost 90% of the 400,000 flowering plant species worldwide rely on animals for pollination (Krauss *et al*. 2017), so opportunities exist to improve the reproductive performance of revegetated populations by planting in conspecific clusters. For *E. leucoxylon*, planting at least 5 conspecifics within 30 m can improve reproductive performance. However, aggregated arrangements will only be effective if revegetated systems attract and support an adequate number and diversity of pollinators.

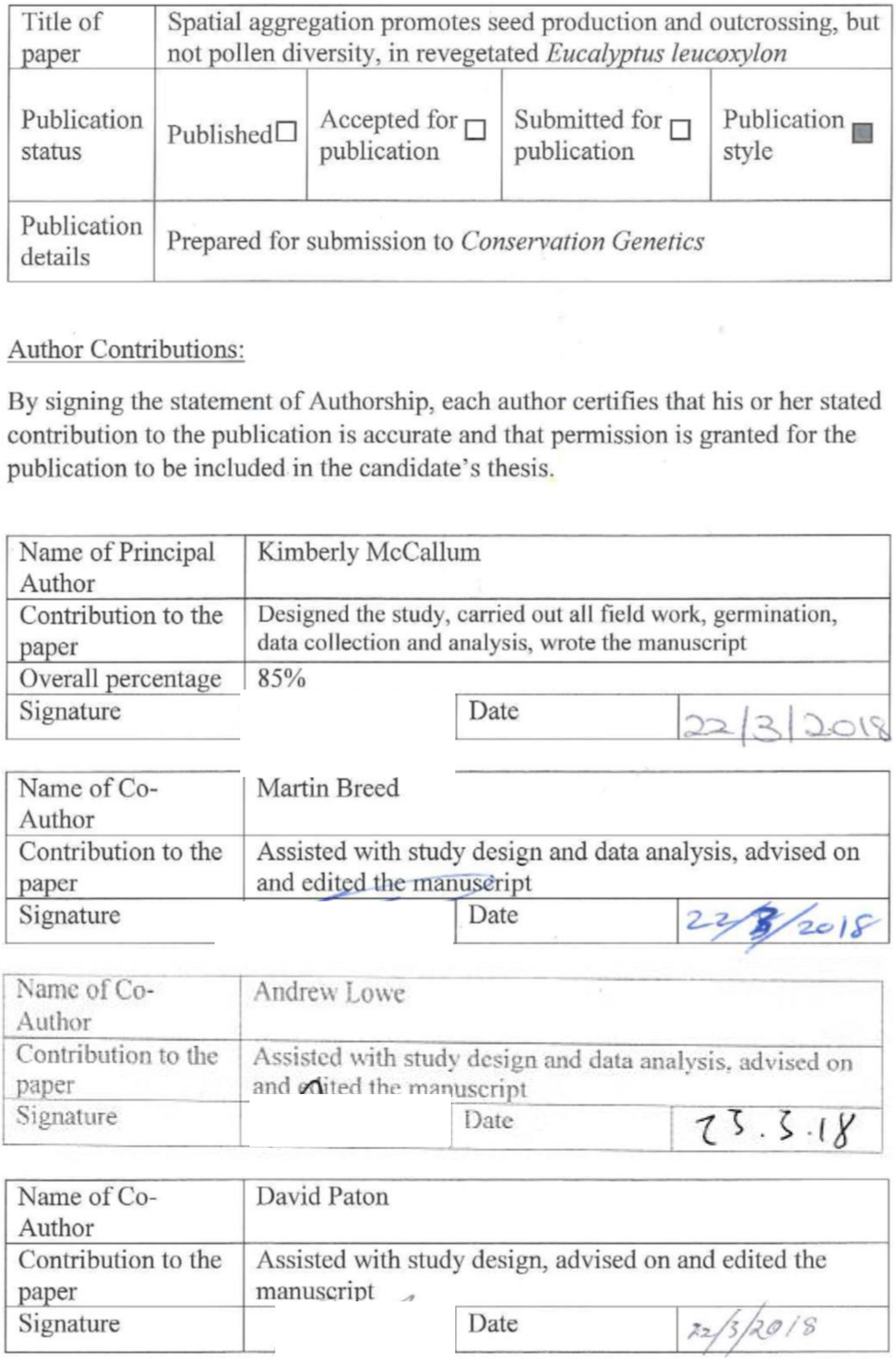
Structurally diverse communities that include a variety of plants, including species that provide large quantities of nectar and flower for a long time can help to attract and sustain pollinators (Menz *et al.* 2011; Munro *et al.* 2011; Cusser & Goodell 2013). The creation of biodiverse systems is now widely promoted for revegetation (McDonald *et al.* 2016a), and it is common for a wide variety of species to be planted (Jonson 2010; Haby & Klein 2012). Diverse plantings may result in conspecifics occurring within a matrix of other species, potentially facilitating inter-species pollen flow. Consequently, reinstating diverse systems with conspecific clusters of plants may help restore pollination services in revegetated communities (McCallum *et al.* 2018b), and will be beneficial for plants and their animal pollinators.

Acknowledgements

This research was supported by funding from the Australian Wildlife Society, BioR, Field Naturalists Society of South Australia, Holsworth Wildlife Research Endowment and the Nature Foundation of South Australia awarded to KPM, and Australian Research Council funding to AJL and MFB (DE150100542; DP150103414). KPM was supported by an Australian Government Research Training Program Scholarship and the Noel and Vivian Lothian Scholarship, provided through the University of Adelaide. We thank Geoff Brooks for allowing access to vegetation with the Monarto Zoological Park.

Chapter 5. Genetics

Statement of authorship



Title of paper	Spatial aggregation promotes seed production and outcrossing, but not pollen diversity, in revegetated *Eucalyptus leucoxylon*			
Publication status	Published ☐	Accepted for publication ☐	Submitted for publication ☐	Publication style ☒
Publication details	Prepared for submission to *Conservation Genetics*			

Author Contributions:

By signing the statement of Authorship, each author certifies that his or her stated contribution to the publication is accurate and that permission is granted for the publication to be included in the candidate's thesis.

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Overall percentage	85%		
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Contribution to the paper	Assisted with study design and data analysis, advised on and edited the manuscript		
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Contribution to the paper	Assisted with study design, advised on and edited the manuscript		
Signature		Date	22/3/2018

Spatial aggregation promotes seed production and outcrossing, but not pollen diversity, in revegetated *Eucalyptus leucoxylon*

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Running head: planting arrangement and pollen flow

Author contributions: All authors conceived the ideas and developed the experimental design. KPM carried out field and glasshouse work and data collection. KPM completed statistical analysis with assistance from MFB. KPM wrote the first draft of the manuscript and all authors contributed to revisions.

Abstract

The arrangement of plants within revegetated communities are rarely considered an important characteristic of these systems, but it is likely that these arrangements may have fitness impacts via shifts in gene flow and mating system dynamics. Here we assessed mating patterns, pollen flow, seed production and germination as a function of spatial aggregation in a revegetated *Eucalyptus leucoxylon* population (n = 26 maternal families). Seed production, germination and outcrossing were positively associated with greater spatial aggregation. However, extensive pollen flow (up to ca 2000 m) was observed across the population and this appears to overcome important aspects of dispersed arrangements in this revegetated system, facilitating outcrossing even in spatially isolated individuals, and allowing the genetic diversity of seed produced to be independent of spatial aggregation. These results indicate that robust pollination systems have been established in this revegetated woodland, even though plantings were undertaken with little consideration of spatial arrangement. However, opportunities exist to improve the reproductive performance of *E. leucoxylon* by planting in conspecific clusters.

Key words

Paternity analysis, plant mating patterns, planting arrangement, pollen flow, pollination services, restoration, revegetation

Introduction

Revegetation is widely used to restore degraded land, especially in areas that have been extensively cleared, and as a result tubestock planting and direct seeding are key conservation activities globally (Wortley *et al.* 2013; Broadhurst *et al.* 2017b). Revegetating land with tubestock planting or direct seeding largely determines the spatial arrangement of species within revegetated communities, and both approaches can result in linear, random or dispersed arrangements of species (Miller *et al.* 2010; Zhao *et al.* 2017; McCallum *et al.* 2018a). These arrangements may influence the ecological processes (e.g. survival, pollination, seed dispersal) occurring within revegetated systems (McCallum *et al.* 2018b), but the importance of planting arrangements are poorly understood, especially in regard to pollination and plant mating systems.

The spatial arrangement of plants plays a central role in pollination, with most successful pollination events occurring relatively close to the parent tree (Lowe *et al.* 2003; Meagher & Vassiliadis 2003; Sinclair & Hobbs 2009). As a result, aggregated plants, or those in areas with a higher conspecific density, tend to receive a more diverse suite of pollen, exhibit higher levels of outcrossing (Breed *et al.* 2014; Lowe *et al.* 2015), and show increased offspring fitness (Breed *et al.* 2012a).

In contrast, dispersed or fragmented populations often suffer from increased selfing and a limited diversity of pollen donors, which leads to reduced reproductive output and can limit recruitment into these populations (Ellstrand 1992; Lamont *et al.* 1993; Aguilar *et al.* 2006). This is a concern because revegetation activities can result in dispersed arrangements of species (McCallum *et al.* 2018a). However, pollination is rarely investigated as a measure of revegetation success (Dixon 2009; Ritchie & Krauss 2012), and the role of planting arrangement on plant mating patterns has never been addressed.

Tools are available to assess patterns of pollen flow and mating patterns (Mijangos *et al.* 2015), but these are often underutilized in revegetation contexts (Dixon 2009; Ritchie & Krauss 2012). The most direct approach for examining pollen flow is through paternity analysis (Ellstrand 1992; Smouse & Sork 2004). Paternity analyses can be used to determine important mating pattern statistics, such as the number of males contributing pollen to a female, selfing rate and pollen dispersal distances (Smouse & Sork 2004), and these are important measures because they influence the quantity and diversity of seed produced.

Here, we examine the role of fine-scale planting arrangements on seed production, germination, mating patterns and patterns of pollen flow in a revegetated *Eucalyptus leucoxylon* population. We specifically asked: (1) Does planting arrangement influence seed production, germination and plant mating patterns in this revegetated population? (2) Are seed crops dominated by neighbouring trees? (3) Does the spatial arrangement of these plants influence pollen flow distances?

Methods

Study species

Eucalyptus leucoxylon was chosen as the study species because it is heavily relied on for revegetation across south-eastern Australia (Bonney 2003). The species is visited by birds (primarily honeyeaters and lorikeets), and insects (bees, flies and wasps), but it is considered to be predominantly bird pollinated (Paton & Ford 1977; Ottewell *et al.* 2009; Zilko *et al.* 2017). *Eucalyptus leucoxylon* flowers are bisexual (male reproductive organs develop before female organs) and individual flowers on the same tree develop at different times, allowing geitonogamous selfing to occur (Ottewell *et al.* 2009; Zilko *et al.* 2017). Previous research into the mating system of fragmented *E. leucoxylon* found mixed mating to strong outcrossing (t_m = 28% to 100%) (Ottewell *et al.* 2009).

Study system

We targeted the revegetated Monarto Woodlands for our study, approximately 70 km south-east of Adelaide, South Australia (Fig. 1). Revegetation was undertaken in this region by the South Australian Government during the 1970s, with plantings carried out on ca 1,850 ha of land. A large diversity of tree and large shrub species were used (250 sp.), which included a mix of local endemics, Australian natives (planted outside their natural range), and exotic species (Paton *et al.* 2004a; Paton *et al.* 2010). Little is known about seed sources. Revegetation occurred in several patches, often adjacent to remnant vegetation (Fig. 1). Planting was done in rows spaced 4 - 6 m apart, with tubestock planted at 4 - 6 m intervals (Paton *et al.* 2010). Although relative species abundances vary between patches, planting methods were consistent across all patches.

Figure 1. Location of the Monarto Woodlands, South Australia (A), showing the position and distribution of the revegetated and remnant vegetation (B). A portion of the sampling area, with the position of the mother trees (seed and leaf collected), potential father trees (leaf collected) and the adjacent unsampled *Eucalyptus leucoxylon* (C). Data sources: Department of Environment, Water and Natural Resources; Regional City of Murray Bridge.

Sample collection

This research follows on from that undertaken in Chapter 3 and uses a subset of the *E. leucoxylon* individuals sampled for fruit in 2016 from the 2015 flowering season (ca 220 ha of Monarto Woodlands surveyed, recording the position of 1100 *E. leucoxylon* trees, fruit collected from 148 trees). Approximately 30 (± 1 SEM, range 10-90) mature fruit were collected from each tree, with fruit collected from a number of branches around the canopy and at a range of heights. Fruit were left to dry at room temperature until contents were released (seed and chaff) and seeds were then separated from chaff and counted.

ArcMAP (ArcGIS, ESRI) and SpPack (Perry 2004) were used to determine the distance to, and the bounding area of, the five nearest neighbours for the 148 trees originally sampled. The distance to the nearest neighbour (NND) and the area of the five nearest neighbours (NN5A) were used to split the study trees into three aggregation classes (Appendix 2).

Of the 148 adult *E. leucoxylon* sampled for Chapter 3, 26 of these were selected for further study (i.e. paternity and cohort analysis) (Appendix 2). Trees were selected across the range of nearest neighbour distances (4-100 m), aggregation patterns (aggregated, intermediate, dispersed) and seed production (0.1-50 seeds/fruit) available. Tree selection was further refined by location, such that trees from the same aggregation class were not all collected from the same vegetation patch (i.e. clustering all aggregated trees together was avoided).

Due to the high numbers of *E. leucoxylon* planted, not all potential pollen donors could be sampled. Therefore, leaf material was collected from the 26 mother trees specified in Appendix 2 (Fig. S1), and at least the five closest reproductive *E. leucoxylon* neighbours (Fig. 1). Leaf material was collected from 125 adult trees in total, including 10 remnant *E. leucoxylon* (i.e. the remnant cohort). Leaves were dried in the field using silica gel. In some areas, overlap in the five nearest potential fathers occurred between mother trees, with mother trees also acting as potential pollen donors (Fig. 1).

A sample of 50 seeds per tree was selected for germination (when <50 seeds were available, all seeds were germinated). Germination was undertaken in autumn 2017 in a temperature controlled glasshouse (T ~ 25^{o}C). Petri dishes were kept moist but not wet, and the number of germinated seeds (cracked seed coats, emerging cotyledons) were recorded daily over a 14 day period. Petri dishes were randomly shuffled every one to two days to minimise glasshouse location effects. Once germinated, seedlings were transferred to seedling trays and grown under glasshouse conditions for three months. Leaves from fifteen seedlings per mother tree were sampled for genotyping (when <15 seedlings were available because of limited seed, poor germination, or low seedling survival, all available individuals were sampled). Final numbers of seedlings sampled across the 26 families ranged from 8 to 15 (mean 13.6; Table 1).

Microsatellite genotyping

Genomic DNA was extracted from 30 mg of adult leaf tissue and 100 mg of seedling leaf tissue, with DNA extracted from 125 adult and 354 seedling samples. Leaf tissue was extracted using the Machery-Nagel Nucleospin Plant II Kit at the Australian Genome Research Facility (AGRF, Adelaide, Australia).

In total, twelve microsatellite markers were selected. Seven markers (EL01, El07, El13, El14, El16, El18, El28) were selected from the set Ottewell *et al.* (2005), developed for *E. leucoxylon*, and five markers (EMBRA1990, EMBRA1928, EMBRA1382, EMBRA914, EMBRA1924) were selected from the set of EST-derived loci by Faria *et al*. (2010). A single 7 µL multiplex PCR was run at AGRF for each sample and contained 2 µL template DNA (ca 10 ng/µL), 0.6 µl 10x Immolase Buffer (Bioline), 0.24 µL 50mM MgCl2 (Bioline), 0.03 µL Immolase DNA Polymerase (Bioline), 0.12 µL dNTP Mix 10mM (Bioline), 0.3 µL primer mix at 10mM concentration and 3.71 µL water.

PCR conditions were as follows: initial denaturation at 95°C for 7 min, then cycling at 94°C for 60 s, annealing at 55°C for 60 s, and extension at 72°C for 60 s for a total of 35 cycles. All PCRs were run in Veriti 96-well thermocyclers (Life Technologies Australia Pty Ltd., Victoria, Australia). Eight replicates were included per plate and, across the six plates, twelve negative controls and five positive controls were used. Fragments were separated on an AB3730 genetic analyser with a 48 capillary, 50 cm array (Applied Biosystems, Foster City, CA, USA) and LIZ500 size standard was added to the samples before running.

Alleles were sized using the Microsatellite Plug-in for Geneious v11.0.2 (Biomatters Ltd). Two loci (El01, El16) were discarded because of poor amplification or inconsistent peaks. Peaks were automatically binned and double-checked manually, with manual checks also used to ensure that all seedlings had at least one maternal allele at each locus. The adult cohort was used to check for genotyping errors due to allelic dropout and null alleles using MICRO-CHECKER v 2.2.3 (Van Oosterhout *et al.* 2004). Linkage disequilibrium and deviations from Hardy-Weinberg's equilibrium were assessed in GENEPOP (http://genepop.curtin.edu.au), using sequential Bonferroni correction for multiple testing where appropriate. None of the remaining ten loci showed significant allelic dropout, null alleles, linkage disequilibrium or significant excesses of observed heterozygotes. There was evidence of heterozygote deficits in five of the loci, but these deficits were not significant across the three spatial arrangement classes and remnant cohort. All samples were successfully amplified across at least six loci, with the majority of samples amplifying for all 10 loci (421 out of 479).

Genetic diversity

To assess genetic diversity in the adult and offspring cohorts from the three aggregation classes, we estimated the *Na* (number of alleles), *Ne* (effective number of alleles), H_E (expected heterozygosity), H_O (observed heterozygosity) and *F* (fixation index) in GENALEX v 6.502 (Peakall & Smouse 2006; Peakall & Smouse 2012). Allelic richness (AR) was estimated in HP-RARE to rarefy the mean number of alleles per locus to account for variation in sample sizes (Kalinowski 2005). All of the above measures were also estimated for the remnant cohort. Principal coordinate analysis (PCoA) was undertaken in GENALEX using the genetic distance between individuals to assess genetic clustering of individuals within and between arrangement classes and the remnant cohort. Spatial genetic structure (SGS) within the revegetated population was estimated by spatial autocorrelation analysis in GENALEX, using distance classes of 4 m with 50 classes in total.

Mating system analysis

We estimated mating system parameters for the three aggregation classes as well as on a family-by-family basis. We estimated the multilocus outcrossing rate (t_m), single-locus outcrossing rate (t_s), biparental inbreeding (t_m-t_s), and the multilocus correlated paternity (r_p) using MLTR (Ritland 2002). Multilocus outcrossing rate is an estimate of the proportion of outcrossed progeny, including mating between relatives and unrelated individuals. Measures of single-locus outcrossing rate (t_s) only include mating between unrelated individuals. The difference between the two estimates (t_m-t_s) provides a measure of biparental inbreeding (Ritland 2002). Correlated paternity (r_p) is a measure of the proportion of pairs of outcrossed siblings that are full siblings, as opposed to half siblings. The effective number of pollen donors was estimated as $1/r_p$ (Ritland 2002). Families were bootstrapped 1000 times to calculate the variance estimates for each parameter for the three aggregation classes. For family level analysis, individuals within families were bootstrapped 1000 times.

Paternity assignment

Paternity of seedlings was assigned to potential pollen donors using the maximum likelihood approach in CERVUS v3.0.7 (Marshall *et al.* 1998; Kalinowski *et al.* 2007). Logarithm (base 10) of odds (LOD) scores were calculated for all potential pollen donors, as every reproductive individual in the population was a potential pollen donor. Confidence in assignments was measured as the difference (Δ) in LOD scores between the candidate sire with the highest score and the candidate sire with the second highest score. Strict confidence levels were set at 95% and relaxed confidence levels at 80%. Data simulations were run with 10,000 cycles, assuming 1% of loci were mistyped and 15% of potential fathers were sampled (125 of ca 800 potential fathers sampled). These direct paternity assignments were used to derive a second estimate of outcrossing rate for all maternal trees. When the pollen donor of an offspring was assigned to the maternal genotype with >80% confidence level, it was assumed that the offspring derived from self-fertilization. Paternity assignment was used to determine patterns of pollen flow with distances determined in ArcMap using the measure tool, as well as an estimate of the number of fathers contributing to the seed crop.

Data analysis

One-way ANOVAs were used to test for significant differences in genetic diversity and reproductive output between the three aggregation classes. Where significant, Tukey’s multiple comparison tests were used. The seed production data needed to be transformed (natural log) before analysis to normalise the distribution of the residuals. No transformations were required for the germination data.

General linear models with a maximum likelihood, multi-model inference framework (Burnham & Anderson 2002) were run in the base statistics package in R v. 3.3.2 (R-Core Team 2016). Two sets of models were run to assess reproductive fitness and then plant mating patterns. The response variables for the first model set were seeds per fruit and germination rate, and predictor variables were nearest neighbour distance (NND), bounding area to the 5 nearest neighbours (NN5A), outcrossing rate (t_m), bi-parental inbreeding (t_m-t_s) and correlated paternity (r_p). The response variables for the second set of models were the family-level mating pattern parameters (t_m, t_m-t_s and r_p), and predictor variables were NND and NN5A. We estimated Akaike's Information Criterion corrected for small sample sizes (AICc) and Akaike weights (wAIC) for each model (Burnham & Anderson 2002). To assess relative importance of the predictor variables to each response variable, we derived the index of the relative importance of predictor variable i (AICi), which is the sum of Akaike weights for all models that included parameter i (Burnham & Anderson 2002; Giam & Olden 2016). Greater AICi (where AICi varies 0-1) implies parameter i has greater importance in predicting variation in response variable j than parameters with smaller AICi. The following transformations were used to meet assumptions of normality of model residuals: seeds per fruit (natural log), germination rate (no transformation), t_m (squared), t_m-t_s (no transformation) and r_p (natural log). Shapiro-Wilks tests were used to confirm that the residuals were normally distributed using the base statistics package in R.

Results

Genetic diversity

Genetic diversity was similar between the three aggregation groups for both the adult and offspring cohorts, and there were no differences in genetic diversity between generations or between remnant and revegetated cohorts (ANOVA; F= 0.3-1.3, DF=478, P=0.11-0.95; Table 1).

Table 1. Genetic diversity and fixation index of *Eucalyptus leucoxylon* across different spatial arrangements and generations, and in the remnant cohort (SEM in parentheses).

	Spatial arrangement			
	Dispersed	Intermediate	Aggregated	Remnant
Adults				
n	24	27	64	10
Na	8.3 (1.1)	7.7 (1.0)	8.7 (1.3)	7.3 (0.8)
Ne	4.6 (0.7)	4.2 (0.5)	4.6 (0.7)	4.8 (0.3)
AR	4.7 (0.4)	4.5 (0.4)	4.3 (0.3)	5.2 (0.5)
H_E	0.75 (0.03)	0.68 (0.04)	0.72 (0.03)	0.74 (0.04)
H_O	0.67 (0.03)	0.75 (0.07)	0.67 (0.03)	0.75 (0.06)
F	0.04 (0.05)	0.06 (0.04)	0.04 (0.05)	-0.002 (0.04)
Seedlings				
n	122	78	154	
Na	10.9 (1.6)	8.8 (1.1)	9.8 (1.5)	
Ne	4.6 (0.7)	5.0 (0.5)	4.0 (0.6)	
AR	4.6 (0.4)	4.3 (0.3)	4.2 (0.3)	
H_E	0.75 (0.03)	0.71 (0.04)	0.71 (0.03)	
H_O	0.66 (0.03)	0.67 (0.03)	0.70 (0.03)	
F	0.07 (0.04)	0.12 (0.03)	0.002 (0.02)	

n = number of samples, Na = number of alleles, Ne = effective number of alleles, AR = allelic richness, H_E = expected heterozygosity, H_O = observed heterozygosity, F = fixation index (where F = 0 indicates the genotype frequencies are in Hardy-Weinberg Equilibrium).

Using the genetic distance between individuals, there was no clear genetic structuring of adult trees from the three revegetated arrangement classes or of the remnant cohort (Fig. 2). There was no evidence of significant spatial genetic structure in the revegetated population (Appendix 2).

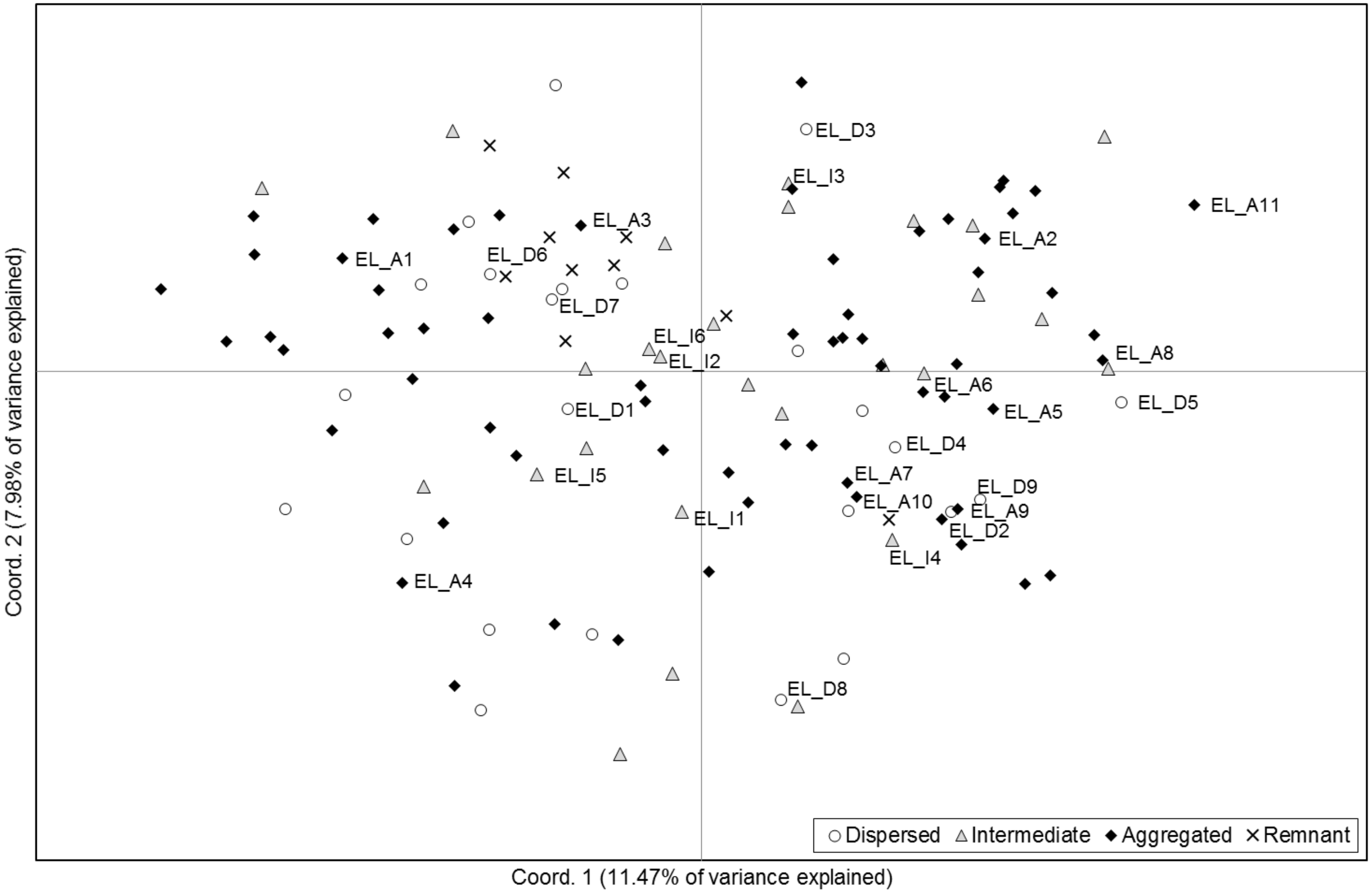


Figure 2. Principal coordinate analysis (PCoA) based on the genetic distance of all adult trees sampled (n=125), for the three revegetated arrangements and the remnant cohort. The 26 mother trees are labelled (refer to Table 2 below for family level information).

Seed production and germination

Seed production (seeds per fruit) was highly variable, with mean seed number per fruit ranging from 0.9 to 35.5 seeds (Table 2). Seed production was greater in aggregated trees (19.8 ± 1.0 SEM seeds/fruit), followed by intermediate trees with 6.4 (± 0.7 SEM seeds/fruit) and dispersed trees with 3.2 (± 0.2 SEM seeds/fruit) (ANOVA, F=13.3, DF= 23, P<0.0001; Tukey's multiple comparisons - Dispersed vs. Intermediate: P=0.7; Dispersed v Aggregated: P< 0.001; Intermediate vs. Aggregated: P<0.01; Table 3). The percent of seeds germinated after 14 days was similar between the three arrangement classes (Aggregated = 80.6% (± 1.3 SEM); Intermediate = 75.1% (± 3.1 SEM); Dispersed = 67.9% (± 2.3 SEM; ANOVA, F=1.3, DF= 23, P=0.3; Table 3).

Mating system

Based on the MLTR estimates, outcrossing rate was higher in aggregated (t_m = 0.96) than intermediate (t_m = 0.89) and dispersed trees (t_m = 0.84), but this difference was not significant (ANOVA, F=1.3, DF=23, P=0.3). Bi-parental inbreeding (t_m-t_s) was highest in dispersed trees (t_m-t_s = 0.148), followed by intermediate (t_m-t_s = 0.106) and aggregated trees (t_m-t_s = 0.091), but not significantly (ANOVA, F=0.4, DF=23, P=0.7). Correlated paternity was lower in intermediate trees (r_p = 0.190), than aggregated (r_p = 0.248) and dispersed trees (r_p = 0.279), but this difference was not significant (ANOVA, F=0.7, DF=23, P=0.6; Table 3).

Based on CERVUS estimates, the outcrossing rate was 97% for the aggregated trees (range 80-100%), 84% for the intermediate trees (range 38-100%), and 79% for the dispersed trees (range 27-100%), but there was no significant difference between categories (ANOVA, F=2.6, DF=23, P=0.1; Tables 2, 3). The estimated number of pollen donors contributing pollen to each mother was similar between the arrangements with 8.2 (range 3-12) for aggregated trees, 8.7 (range 4-14) for intermediate trees and 8.1 for dispersed trees (range 3-13; ANOVA, F=0.09, DF=23, P=0.9).

MLTR and CERVUS outcrossing rates were strongly correlated (Table 2; R^2 = 0.76). The estimated number of sires contributing pollen to each mother was more variable between the two analyses, but still correlated (Linear regression, R^2 = 0.37).

Table 2. Family level mating patterns and gene flow estimates for the 26 mother trees, arranged from least to most aggregated down the table. Class: D - dispersed, I – intermediate and A – aggregated. NND: nearest neighbour distance (m), seeds per fruit: mean number of seeds per fruit, % G: percent of seeds germinated after 14 days. MLTR estimates: t_m: proportion outcrossed, t_m-t_s: biparental inbreeding, r_p: correlated paternity and 1/r_p: number of sires. CERVUS estimates: Offspring assigned: number of seedlings directly assigned (number resulting from self-pollination), Outcrossing rate: proportion of seedlings that were outcrossed, # sires: number of different father trees contributing pollen and Median pollen flow distance: median distance between mother and father tree (range), in metres. No values are provided when pollen flow distances could not be calculated, because no fathers were assigned, or all assigned seedlings resulted from selfing. MLTR estimates based on 1000 bootstraps, with individuals resampled within families.

						MLTR Estimates				CERVUS Estimates			
Family	Class	NND	n	Seeds per fruit	% G	t_m	t_m-t_s	r_p	1/ r_p	Offspring assigned (selfs)	Outcrossing rate	# sires	Median pollen flow distance (m) (range)
EL_D1	D	100	12	1.27	68	1.00	0.32	0.18	6	6 (2)	0.83	8	135 (135-163)
EL_D2	D	98	15	2.28	26	0.56	0.06	0.15	7	8 (8)	0.47	8	
EL_D3	D	52	10	5.79	56	1.00	0.26	0.36	3	8 (0)	1.00	6	240 (68-1655)
EL_D4	D	52	15	2.00	69	0.93	0.32	0.46	2	7 (3)	0.80	11	911 (188-1513)
EL_D5	D	51	15	3.09	96	0.47	0.31	0.70	1	11 (11)	0.27	3	
EL_D6	D	48	10	3.85	62	0.91	0.02	0.21	5	0 (0)	1.00	7	
EL_D7	D	48	15	5.28	94	0.75	0.15	0.25	4	7 (2)	0.87	11	299 (48-479)
EL_D8	D	32	15	1.97	66	1.00	0.35	0.40	3	11 (1)	0.93	6	32 (32)
EL_D9	D	26	15	3.18	74	1.00	0.29	0.10	10	3 (1)	0.93	13	246 (218-274)
EL_I1	I	37	8	0.94	72	0.78	0.33	0.40	2	5 (5)	0.38	4	
EL_I2	I	33	15	4.02	78	0.94	0.06	0.11	9	4 (1)	0.93	13	50 (33-114)
EL_I3	I	29	15	9.60	80	0.93	0.13	0.23	4	4 (1)	0.93	10	29 (29)
EL_I4	I	26	15	7.32	98	1.00	0.17	0.12	9	4 (0)	1.00	12	270 (37-1900)
EL_I5	I	24	15	4.26	80	0.75	0.04	0.42	2	13 (3)	0.80	4	24 (24-248)

EL_I6	I	24	10	12.35	42	0.91	0.15	0.99	1	3 (1)	0.90	9	80 (24-135)
EL_A1	A	14	15	7.61	80	0.87	0.44	0.48	2	9 (3)	0.80	8	155 (38-166)
EL_A2	A	14	11	21.21	72	1.00	0.54	0.14	7	1 (0)	1.00	8	103 (103)
EL_A3	A	10	15	5.57	66	0.88	0.00	0.05	15	8 (2)	0.87	10	39 (14-258)
EL_A4	A	10	15	17.88	68	0.94	0.01	0.29	3	8 (0)	1.00	5	14 (10-55)
EL_A5	A	8	15	31.38	64	1.00	0.13	0.46	2	7 (0)	1.00	8	14 (14-1750)
EL_A6	A	4	15	35.54	96	0.94	0.22	0.38	3	6 (0)	1.00	7	1682 (17-1753)
EL_A7	A	4	14	26.00	64	1.00	0.22	0.37	3	3 (0)	1.00	10	13 (11-13)
EL_A8	A	4	12	34.00	90	1.00	0.37	0.46	2	6 (0)	1.00	3	12 (4-232)
EL_A9	A	4	15	9.95	94	1.00	0.18	0.07	14	8 (0)	1.00	12	53 (13-561)
EL_A10	A	4	15	8.03	98	1.00	0.25	0.12	9	6 (0)	1.00	11	179 (12-1798)
EL_A11	A	4	12	20.70	94	1.00	0.29	0.14	7	2 (0)	1.00	8	37 (24-49)

Table 3. Mating patterns for the revegetated *Eucalyptus leucoxylon* population at Monarto Woodlands, South Australia, across three spatial arrangement classes. Standard error in parentheses. Superscript letters show significant differences at P=0.05. Where no letters are shown, there are no significant differences between the classes.

Class	Seeds per fruit $^+$	% Germinated	MLTR estimates				CERVUS estimates		
			t_m	t_m-t_s	r_p	1/ r_p	n	Proportion outcrossed	Number of sires
Dispersed	3.2 (0.5)^a	67.9 (6.9)	0.835 (0.026)	0.148 (0.026)	0.279 (0.018)	3.6	56	0.79 (0.08)	8.1 (1.0)
Intermediate	6.4 (1.9)^a	75.1 (7.5)	0.889 (0.031)	0.106 (0.029)	0.190 (0.023)	5.3	29	0.84 (0.07)	8.7 (1.6)
Aggregated	19.8 (3.3)^b	80.6 (4.3)	0.962 (0.032)	0.091 (0.010)	0.248 (0.016)	4.0	55	0.97 (0.01)	8.2 (0.8)

Dispersed (n mother = 9, n seedlings = 122), intermediate (n mother = 6, n offspring = 78), aggregated (n mother = 11, n offspring = 154).

% Germinated, percent of seeds germinated after 14 days; t_m, multilocus outcrossing rate; t_m-t_s, biparental inbreeding estimate; r_p, correlated paternity; 1/r_p, estimated number of pollen donors; n, number of pollen donors assigned.

MLTR variance estimates were calculated from 1000 bootstraps, with resampling at the family level.

$^+$ Seeds per fruit data based on a subset of samples (26) from the originally collected 148 (results from the entire data set dispersed = 3.4 (0.48)^a, intermediate = 8.1 (1.29)^{ab}, aggregated=10.6 (1.05)^b).

Predicting reproductive output and mating patterns

Distance to the nearest neighbour (NND) was the strongest predictor of seed production and germination rate, where it showed a negative association in both cases (Tables 4, 5). The two spatial predictors (NND and NN5A) were the best predictors of seed production. Whereas, for germination rate, distance to the nearest neighbour (NND), correlated paternity (r_p) and bi-parental inbreeding (t_m-t_s) were the top predictors (Table 4). Seed production was negatively correlated with NND and NN5A. Germination rate was negatively correlated with NND and t_m- t_s and positively correlated with r_p. NND explained approximately 53% of the variance for seed production and approximately 22% for germination rate (Tables 4, 5).

Table 4. Reproductive output patterns investigated with general linear models. Seed: seed number per fruit, G: percent of seeds germinated after 14 days, NND: nearest neighbour distance (m), NN5A: bounding area of the five nearest neighbours (ha), t_m: outcrossing rate, t_m- t_s: bi-parental inbreeding and r_p: correlated paternity. % DE: percent deviance explained by the model; ΔAIC_c, indicator of difference between model Akaike's Information Criterion corrected for small samples sizes (AIC_c) and the minimum AIC_c in the model set; *w*AIC, weight that show the relative likelihood of model *j*; *k*, the number of parameters; only models with a $\Delta AIC_c < 4$ are shown. Refer to Appendix 2 for full table.

Model	% DE	ΔAIC*c*	*w*AIC	*k*
Seed production (Seed)				
Seed ~ NND	53.39		0.26	2
Seed ~ NN5A	50.02	1.82	0.11	2
Seed ~ NND + NN5A	54.17	2.13	0.09	3
Seed ~ NND + t_m	53.93	2.27	0.08	3
Seed ~ NND + r_p	53.93	2.27	0.08	3
Seed ~ t_m- t_s + NND	53.52	2.50	0.07	3
Seed ~ NN5A + t_m	52.03	3.32	0.05	3
Germination rate (G)				
G ~ NND	22.01		0.16	2
G ~ NND + r_p	26.51	1.02	0.10	3
G ~ t_m- t_s + NND	26.50	1.03	0.10	3
G ~ t_m- t_s + NND + r_p	32.78	1.52	0.07	4

G ~ NND + NN5A	23.36	2.11	0.06	3
G ~ t_m- t_s + NND + t_m + r_p	38.87	2.14	0.05	5
G ~ NN5A	14.96	2.25	0.05	2
G ~ NND + t_m	22.92	2.26	0.05	3
G ~ NND + t_m + r_p	29.51	2.75	0.04	4
G ~ NN5A + r_p	20.37	3.11	0.03	3
G ~ t_m- t_s + NND + t_m	28.40	3.16	0.03	4
G ~ t_m- t_s + NN5A	19.81	3.29	0.03	3
G ~ t_m- t_s + NND + NN5A	27.76	3.39	0.03	4
G ~ NND + NN5A + r_p	27.27	3.57	0.03	4

Table 5: Evidence for the relative importance of spatial and genetic factors to reproductive output measures. The index of the relative importance of predictor variable *i* (AIC_i) is the sum of Akaike weights (*w*AIC) over all models that include predictor *i*. This importance weight gives evidence for how strong the support is for each predictor variable, regardless of whether the predictor is in the best-fitting model or not.

Response variable	Predictor variable	AIC*i*
Seeds production	NND	0.77
	NN5A	0.42
	t_m	0.23
	t_m- t_s	0.20
	r_p	0.23
Germination rate	NND	0.78
	NN5A	0.33
	t_m	0.27
	t_m- t_s	0.39
	r_p	0.41

The distance to the nearest neighbour was the strongest predictor of outcrossing rate (t_m), explaining approximately 21% of the variation. Outcrossing rate was negatively correlated with the two spatial variables. The two spatial variables were not better than the null model (~1) at predicting bi-parental inbreeding or correlated paternity (Table 6; Appendix 2).

Table 6. Genetic output patterns investigated with general linear models. NND: nearest neighbour distance (m), NN5A: bounding area of the five nearest neighbours (ha), t_m: outcrossing rate, t_m- t_s: bi-parental inbreeding and r_p: correlated paternity. % DE: percent deviance explained by the model; ΔAIC_c: indicator of difference between model Akaike's Information Criterion corrected for small samples sizes (AIC_c) and the minimum AIC_c in the model set; *w*AIC: weight that show the relative likelihood of model *j*; *k*, the number of parameters; only models with a ΔAIC_c less than the null model (~ 1) are shown. Refer to Appendix 2 for full table.

Model	% DE	ΔAIC*c*	*w*AIC	*k*
Outcrossing rate (t_m)				
t_m ~ NND	20.83		0.54	2
t_m ~ NND + NN5A	22.21	2.11	0.19	3
t_m ~ NN5A	14.05	2.14	0.19	2
t_m ~ 1	0.00	3.72	0.08	1
Biparent inbreeding (t_m - t_s)				
t_m - t_s ~ 1	0.00		0.58	1
Correlated paternity (r_p)				
r_p ~ 1	0.00		0.57	1

Pollen flow

In total, 158 seedlings (45% of 354) were directly assigned to one of the 125 potential pollen donors in CERVUS (Table 2). Of these, 72 were assigned at the 95% confidence level and 86 at the 80% level, with the remaining 196 seedlings unassigned. Of the seedlings directly assigned, 44 (28%) were the result of self-pollination, 54 (34%) were sired by one of the five nearest conspecifics, and 28 (18%) resulted from nearest neighbour matings. When all seedlings were considered (n=354), 72% of successful pollen came from beyond the five nearest neighbours. Of the 125 potential pollen donors sampled, 47 of these were found to have sired at least one seedling (not including selfs), and the number of seedlings assigned to a single father ranged from 1 to 11 (mean 2.4 ± 0.05 SEM).

Offspring that were directly assigned as resulting from selfing were least common for aggregated trees (5 out of 64), followed by intermediate trees (11 out of 33) and dispersed trees (28 out of 61; Fig. 3). Pollen flow distances were highly variable, with outcrossing occurring between trees separated by 4 to 1900 m but were most frequent at 10-20 m (Table 2, Fig. 2). Of the outcrossed seedlings, the mean distance of pollen flow was 240 m (± 21 SEM) and the median distance was 53 m, with approximately 60% of successful pollen coming from within 100 m, and 75% from within 200 m. The mean outcross pollen flow distance for dispersed trees was 294 m (± 60 m SEM, median = 163 m), for intermediate trees 157 m (± 80 m SEM, median = 31 m) and 244 m (± 57 m SEM, median = 52 m) for aggregated trees.

We detected 10 long distance pollination events (ca 1000 - 2000 m). These occurred between trees in aggregated patches 6 of the 10 times, and between aggregated and dispersed or intermediate trees in the remaining 4 cases. Six mother trees received pollen from trees more than 1000 m away and one aggregated individual was the pollen donor for five of these long distance events. When these long distance events were excluded, the mean pollen flow distance for dispersed trees was 165 m (± 4.8 SEM, median = 142), 85 m (± 4.7 SEM, median = 29) for intermediate trees and 75 m (± 1.8 SEM, median = 38) for aggregated trees.

We also detected pollen flow from the remnant cohort into the revegetation area, with two seedlings directly assigned to a remnant tree (pollen flow distances of

163 m and 218 m). Although not directly assigned, 32 other seedlings (ca 8%) had alleles that were only found in the remnant individuals.

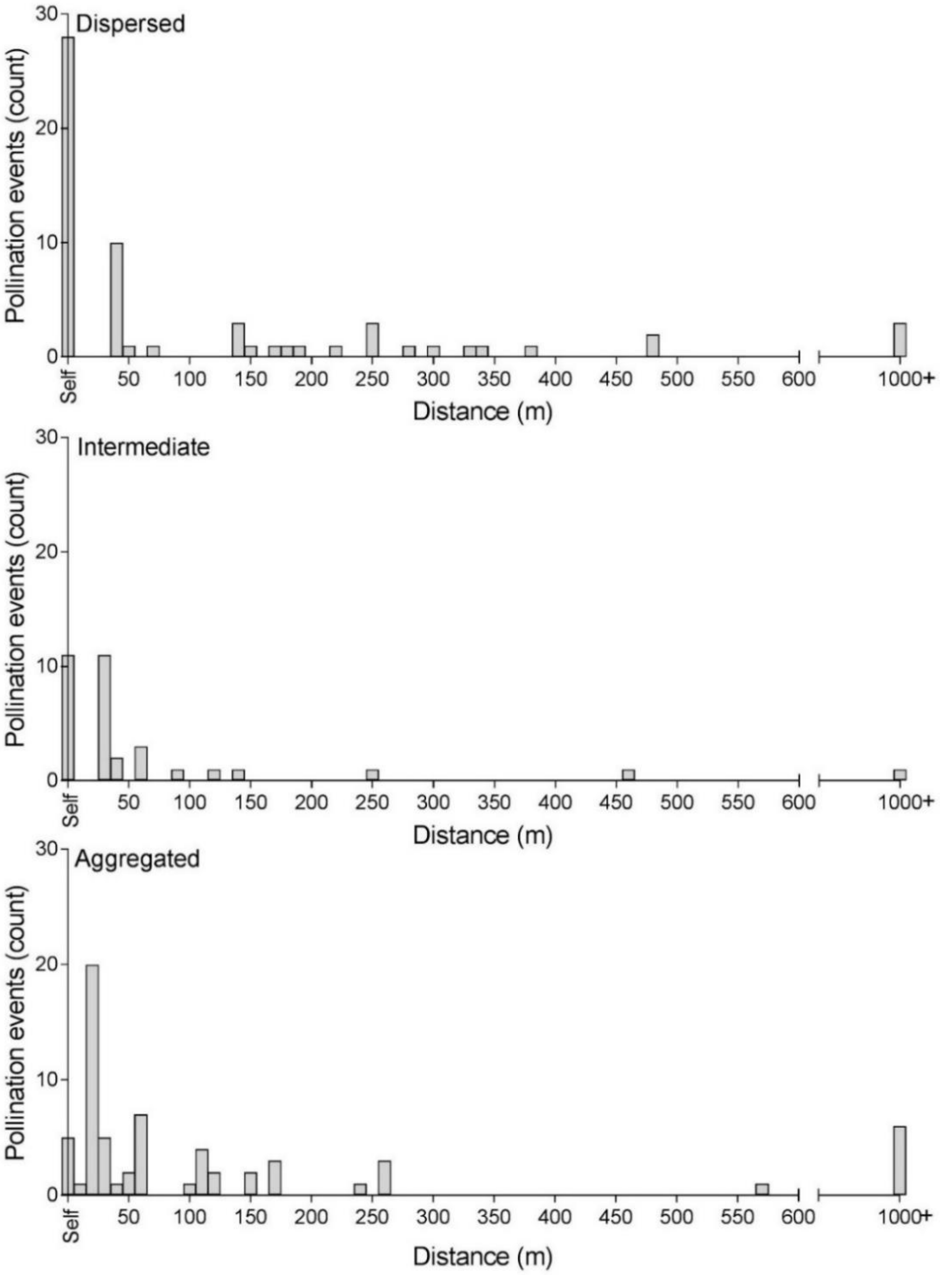


Figure 3. Number of pollination events for each distance category (10 m increments) for the seedlings directly assigned in the three arrangement classes. Records at 1000 + are the 10 long distance events (ca 1000-2000 m). In total, 64 of 154 seedlings were assigned for aggregated trees, 33 of 78 assigned for intermediate trees and 61 of 122 assigned for dispersed trees. The 196 seedlings that were unassigned are not represented.

Discussion

Revegetation activities can result in dispersed arrangements of species (McCallum *et al.* 2018a), and this has the potential to influence reproduction in these restored communities. However, little is known about the role of planting arrangement on plant reproduction and fitness. In the revegetated *Eucalyptus leucoxylon* population studied, seed production, germination and outcrossing were positively associated with greater spatial aggregation. However, extensive pollen flow was observed across this population (up to ca 2000 m), facilitating strong outcrossing even in spatially isolated individuals, and genetically linking remnant and revegetated areas. Genetic diversity was similar between the remnant and revegetated cohorts, and across generations, with the genetic diversity of seed being independent of the spatial aggregation of the mother tree. These results suggest that robust pollination systems have been established in the Monarto Woodlands, even though plantings were undertaken with little consideration of fine-scale arrangements. However, spatial aggregation increased the quantity of outcrossed seed produced and facilitated pollen flow between patches, and these outcomes may be important for maintaining genetic diversity across subsequent generations. Therefore, opportunities exist to improve revegetation outcomes by planting in clusters.

Seed production and outcrossing rate were positively associated with spatial aggregation in *E. leucoxylon* and pollen flow occurred most frequently between trees spaced 10-20 m apart. However, despite these trends, seed crops were rarely dominated by neighbouring trees and not all nearest neighbours contributed pollen to the seed crops of mother trees.

Gene flow in bird pollinated species often departs from nearest neighbour matings (reviewed by Krauss *et al.* 2017). Birds may respond more to flowering intensity than the distance between plants, bypassing closely spaced individuals for those with more flowers, while, pollen carry-over can also contribute to matings between more widely spaced individuals (Paton & Ford 1977; Krauss *et al.* 2009). Variable flowering times within plant populations and aggressive interactions between birds can also result in departures from nearest neighbour matings (Krauss *et al.* 2009, 2017).

Eucalyptus leucoxylon is predominantly bird pollinated (Ottewell *et al.* 2009; Zilko *et al.* 2017), and individuals within populations often flower at different times and with different intensities (Paton 2008; Merigot & Paton 2018). Consequently, it is likely a combination of bird pollination and variable flowering times contributed to the extensive pollen flow and departures from nearest neighbour matings observed here for revegetated *E. leucoxylon*.

Extensive pollen flow and pollen carry-over, coupled with the lack of spatial genetic structure often seen in revegetated sites can result in high multiple paternities and lower levels of bi-parental inbreeding compared to natural sites (Ritchie & Krauss 2012; Frick *et al.* 2014). In the *E. leucoxylon* studied, the number of pollen donors ranged from 1 to 15 across families, but on average 4-5 unrelated trees contributed pollen to each family and there were no differences in correlated paternity or bi-parental inbreeding with level of aggregation. Consequently, the quality and diversity of seed produced was similar between trees, regardless of the degree of spatial aggregation of the mother tree. Taken together, these results indicate that robust pollination systems have been established in the Monarto Woodlands, despite evidence of some pollination limitation (Chapter 4) and even though plantings were undertaken with little consideration of spatial arrangement.

The genetic diversity of seed sources can also affect the sustainability of revegetated populations, and there are concerns that older revegetated sites may have insufficient genetic diversity to support genetic and demographic processes (Byrne *et al.* 2011; Broadhurst 2013). This was not the case for the *E. leucoxylon* studied with similar levels of genetic diversity observed across remnant and revegetated cohorts and across generations. In addition, genetic connectivity between remnant and revegetated areas was re-established, with up to 8% of seedlings resulting from matings between remnant and revegetated trees. Therefore, it is unlikely that seed sourcing practices will limit the on-going persistence of this revegetated *E. leucoxylon* population.

Conclusions

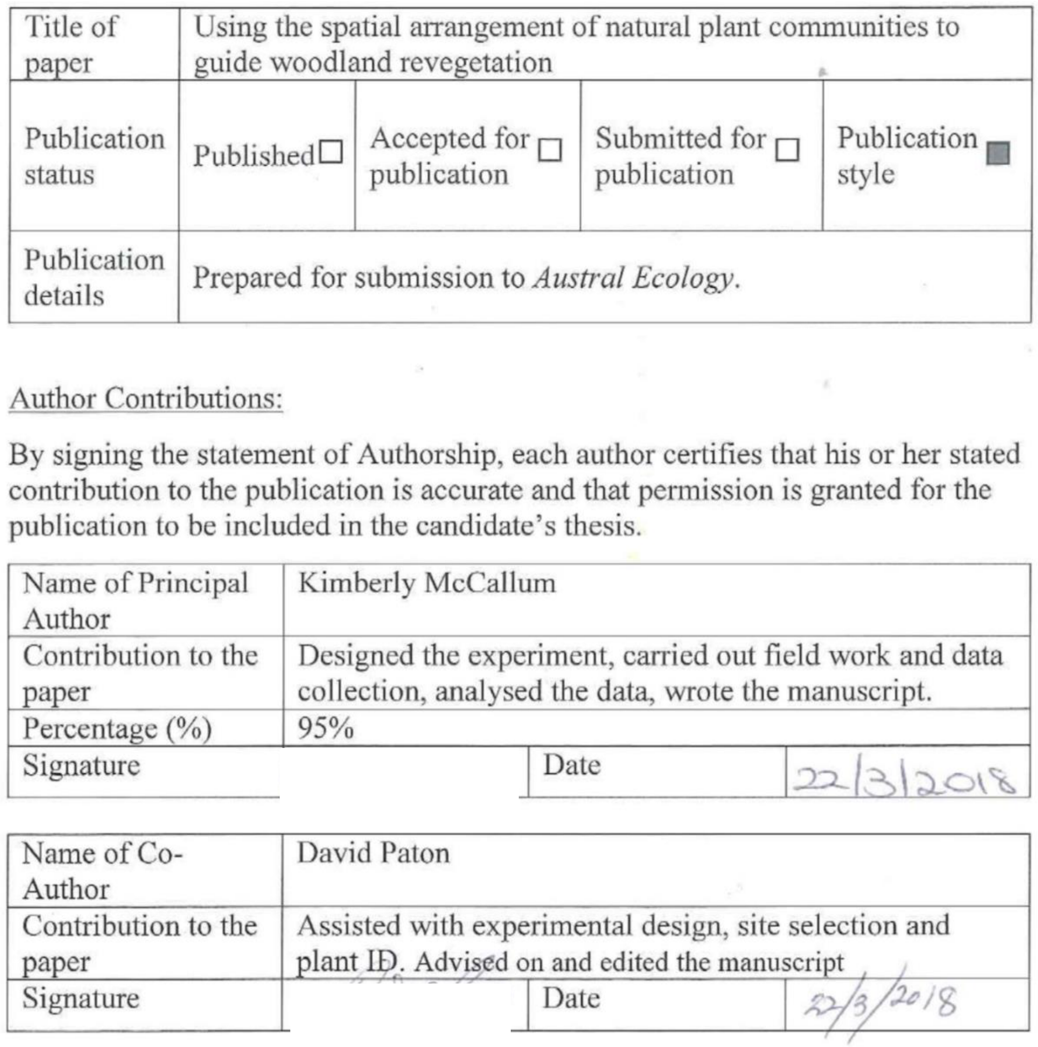
In the *E. leucoxylon* population studied, extensive pollen flow helped overcome the negative effects of dispersed planting arrangements, allowing seed of a similar quality to be produced by trees with different levels of spatial aggregation. However, there were still quantifiable benefits to being aggregated, with aggregated trees maintaining higher outcrossing rates (80-100%), producing more seeds per fruit and contributing more to long-distance pollen flow (1000-2000 m). Therefore, opportunities exist to improve the reproductive performance of revegetated populations through greater consideration of planting arrangements.

Acknowledgements

This research was supported by funding from the Australian Wildlife Society, BioR, Field Naturalists Society of South Australia, Holsworth Wildlife Research Endowment and the Nature Foundation of South Australia awarded to KPM, and Australian Research Council funding (DP150103414 to AJL and MFB; DE150100542 to MFB). KPM was supported by an Australian Government Research Training Program Scholarship and the Noel and Vivian Lothian Scholarship. We thank Geoff Brooks for allowing us to access vegetation with the Monarto Zoological Park and the staff at AGRF, Adelaide Node, particularly Nicole Burtt, for genetics laboratory support.

Chapter 6. Natural plant arrangements

Statement of authorship



Title of paper	Using the spatial arrangement of natural plant communities to guide woodland revegetation			
Publication status	Published ☐	Accepted for publication ☐	Submitted for publication ☐	Publication style ☒
Publication details	Prepared for submission to *Austral Ecology*.			

Author Contributions:

By signing the statement of Authorship, each author certifies that his or her stated contribution to the publication is accurate and that permission is granted for the publication to be included in the candidate's thesis.

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Contribution to the paper	Designed the experiment, carried out field work and data collection, analysed the data, wrote the manuscript.		
Percentage (%)	95%		
Signature		Date	22/3/2018

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Contribution to the paper	Assisted with experimental design, site selection and plant ID. Advised on and edited the manuscript		
Signature		Date	22/3/2018

Using the spatial arrangement of natural plant communities to guide woodland revegetation

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Running head: Re-creating natural arrangements

Author contributions: All authors conceived the ideas and developed the experimental design. KPM carried out the field surveys, data collection and analysis. KPM wrote the first draft of the manuscript and all authors contributed to revisions.

Abstract

Reproducing plant arrangements similar to those of natural communities has been promoted as a way of improving the ecological value of revegetation, but for this to occur, we need to better understand the spatial patterns of natural vegetation. To help achieve this, the spatial arrangement of *Eucalyptus leucoxylon* dominated woodlands were documented at five locations, spanning a distance of 300 km across the Mt Lofty and Southern Flinders Ranges, South Australia. The GPS position of all trees and shrubs were mapped across 12 hectares of native vegetation. Spatial arrangement was highly variable both within and between sites, but despite this variability conspecific aggregation and patchiness were evident at all locations. Aggregation was strongest at distances < 10 m and was generally stronger in shrubs than trees, and in rare species (low relative abundance), than more abundant species. Consequently, for *Eucalyptus* woodland revegetation projects seeking to re-create more natural plant arrangements, designs should focus on producing patchy systems with aggregated arrangements of species (particularly for rare species and shrubs), interspersed with areas of open space. On-ground trials are now required to determine how this information can effectively be incorporated into revegetation practices.

Keywords

Ecological restoration, *Eucalyptus* woodland, plant spatial pattern, restoration planting, spatially designed revegetation

Introduction

The establishment of resilient, self-sustaining and biodiverse ecosystems is necessary if revegetation is to live up to its full potential, but plantings often fail to completely fulfil these objectives (Miller *et al.* 2017). As a result, revegetation may need to move away from simple tree and shrub plantings (Paton & O’Connor 2010), and towards more ecologically informed designs (Jonson 2010; McCallum *et al.* 2018b).

In natural systems, the spatial arrangement of plants influence the majority of ecological processes that occur, from those at the individual plant level to those at the ecosystem level (Dale 1999; Miller *et al.* 2010). For example, arrangements can influence processes such as plant survival and growth, plant-plant interactions (facilitation, competition), pollination, seed dispersal, herbivory, water and nutrient cycling and habitat value (Legendre & Fortin 1989; Miller *et al.* 2010; Garcĩa-Menese & Ramsay 2012). Therefore, it is likely that the arrangement of plants within revegetated sites will also play a fundamental role in these restored systems (McCallum *et al.* 2018b).

However, spatial arrangements are rarely considered an important feature of revegetation, with plantings generally occurring in an *ad hoc* manner (Miller *et al.* 2010). As a result, random, haphazard or linear arrangements of species are common in planted systems, which contrast the aggregated patterns that dominate natural vegetation (Perry *et al.* 2008; Jonson 2010; McCallum *et al.* 2018a, b).

In natural systems, arrangements often develop over multiple generations in response to various environmental (e.g. climate, soil type, slope), disturbance (e.g. fire, floods), morphological and ecological variables (e.g. seed dispersal, competition) and are a crucial feature of these systems (Miller *et al*. 2010; Perry *et al*. 2013; Young *et al*. 2017). Consequently, planting in a more natural manner has been promoted to assist the functional development of revegetated systems and improve the ecological value of restoration plantings (Harrington 1990; Sluis 2002; Zhang *et al.* 2011; McCallum *et al.* 2018a).

Millions of hectares of good quality revegetation is needed to restore the temperate eucalypt woodland systems of Australia (Freudenberger 2018), but revegetation is limited by a lack of information on the spatial and temporal patterns of these woodlands (Lovett *et al.* 2008). Here we describe the natural spatial arrangement of species within *Eucalyptus* dominated woodlands in South Australia and highlight key components of these arrangements that can potentially be incorporated into revegetation design.

The specific aims of the research are (1) Describe the spatial arrangement of woody plants (trees, shrubs) within *Eucalyptus leucoxylon* dominated woodlands, including nearest neighbour distances, local abundance, aggregation, community composition and amount of open space; and (2) Determine if arrangements change between sites and across environmental gradients.

Methods

Location and site selection

Surveys were undertaken from November 2014 to May 2015 in *E. leucoxylon* dominated woodlands at five locations within the Mount Lofty and Southern Flinders Ranges, South Australia, extending over a distance of approximately 300 km. This region has a Mediterranean climate, with hot, dry summers and wet, cooler winters. Mean annual temperature and rainfall varies across the region, with increases in rainfall and decreases in temperature tending to occur from north to south (Bardsley 2006). The survey area is only a portion of the total distribution of *E. leucoxylon* (Fig. 1).

Eucalyptus leucoxylon woodlands are generally characterised by widely spaced trees, with a mid- and understorey of shrubs and grasses (Bonifacio *et al.* 2016; Jellinek & Te 2016). These woodlands have been extensively cleared and *E. leucoxylon* now exists predominantly as scattered trees or in fragments of varying sizes (Paton *et al.* 1999; Ottewell *et al.* 2005).

Surveys were conducted in Dutchmans Stern Conservation Park, Mt Remarkable National Park, Spring Gully Conservation Park, Para Wirra Conservation Park and Belair National Park (Fig. 1). From this point forward, the five locations will be referred to as Dutchmans Stern, Mt Remarkable, Spring Gully, Para Wirra and Belair.

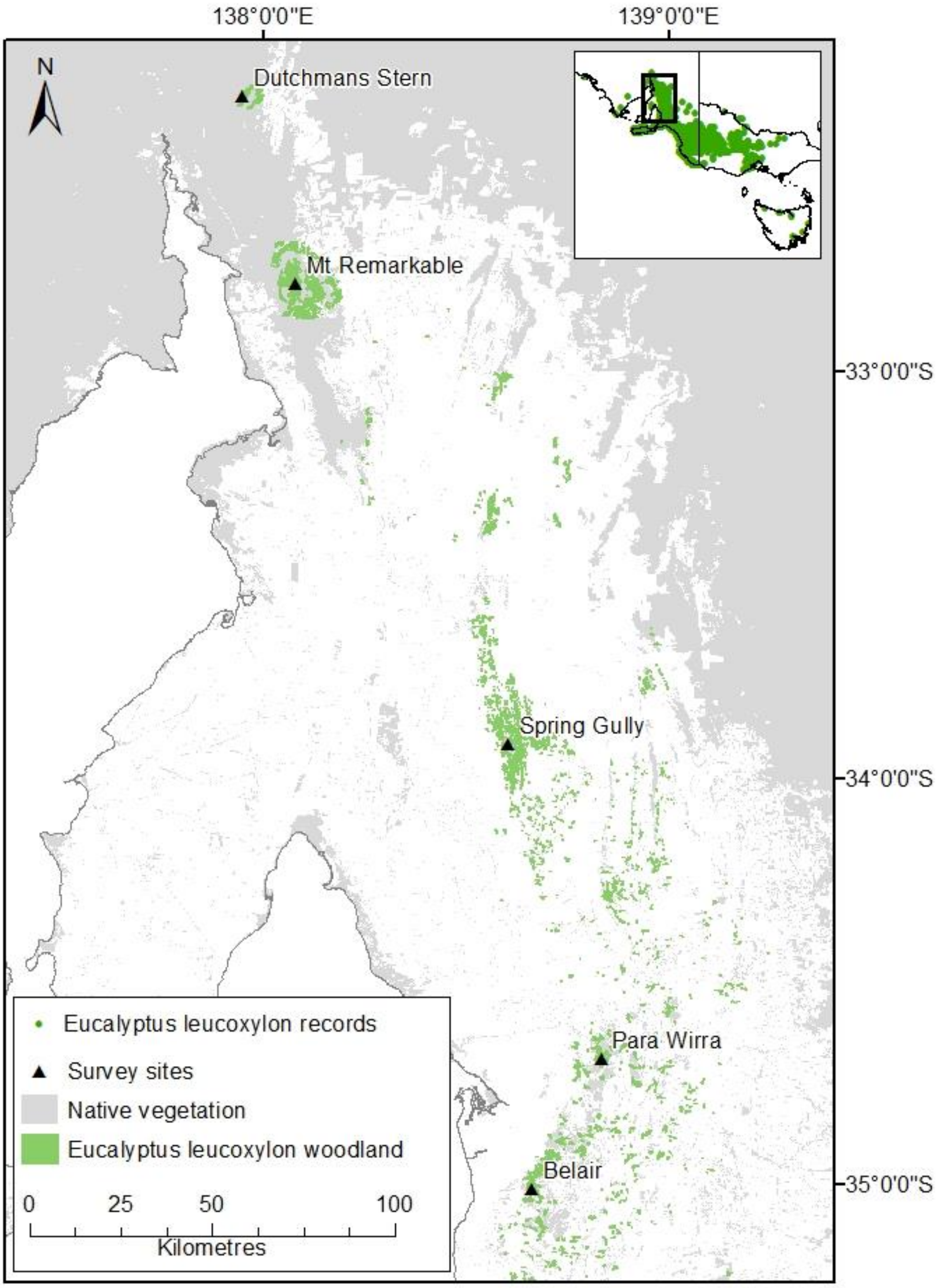


Figure 1. The location of the five survey sites within South Australia, showing the distribution and extent of native vegetation cover and *Eucalyptus leucoxylon* dominated woodlands. Inset, location of all *E. leucoxylon* records [www.ala.org.au, online accessed 12-June-2014]. Data sources: Department of Environment, Water and Natural Resources, South Australia; Atlas of Living Australia.

Field Survey

Surveys were undertaken in 100 m by 100 m (1 ha), 50 m by 50 m (0.25 ha) or 25 m by 25 m (0.0625 ha) quadrats. At least three quadrats and 1.5 ha of vegetation were surveyed at each location. Quadrat size was determined by local site conditions including terrain and vegetation density. The largest possible quadrat was used at each survey location to maximise the amount of data collected. The location of quadrats were selected based on the presence of *E. leucoxylon*, with a preference for areas that had ≥ 20 mature *E. leucoxylon.* In total, thirty plots were surveyed across the five locations - comprised of eight 100 m x 100 m plots, fourteen 50 m x 50 m plots and eight 25 m x 25 m plots (Table 1).

Quadrats were orientated north-south-east-west, and within each quadrat the location of every tree and shrub species was recorded with a handheld GPS (Garmin GPS 62). The accuracy of the GPS varied depending on site topography, and was generally < 5 m. The GPS position was recorded at the stem of each individual, with all woody plants recorded regardless of size. Canopy width (distance across the canopy (m)) was estimated for every individual and trunk diameter at breast height (DBH) was measured for all *Eucalyptus* individuals, except seedlings.

Table 1. Location, number and size of quadrats surveyed in *Eucalyptus leucoxylon* dominated woodlands, South Australia. Plot dimensions (m) in brackets. Sites are arranged in a north to south direction down the table (Refer to Appendix 3 for further information on field surveys).

Location	1 ha (100 x 100)	0.25 ha (50 x 50)	0.0625 ha (25 x 25)	Total Area (ha)
Dutchmans Stern	5			5
Mt Remarkable	1	1	5	1.5625
Spring Gully	1	5	1	2.3
Para Wirra	1	2		1.5
Belair		6	2	1.625

Analysis

Analysis was undertaken in ArcGIS ArcMap v 10.3.1 (ESRI) and the Excel add-in, SpPack (Perry 2004).

Stem densities and canopy cover

Stem density was determined for each of the five survey sites by dividing the sum of the quadrat areas by the total number of stems recorded. Percent canopy cover was estimated in ArcMap using buffers based on the canopy radius (= half canopy width) of each plant surveyed. To control for areas of overlap between different canopies, buffers were merged (combining the multiple input datasets) into a single output dataset.

Nearest neighbour distance (NND) and local density

Nearest neighbour analysis was completed in SpPack using the 1st order nearest neighbour function to determine the mean distance between conspecifics (mean for each species). First order analysis characterises spatial pattern at a single length scale (i.e. distance to nearest neighbour; Perry 2004). Nearest neighbour distances (NND) were only calculated for species that had 20 or more individuals within a quadrat. Results were double-checked manually, and any measures influenced by edge effects were removed. Edge effects can influence the data when individuals are close to the quadrat edge, so the distance to the nearest neighbour within the survey area may be larger than the true value.

The minimum distance between plants could not fall below 1 m because this was the smallest scale of measurement of the GPS. As a result, local abundance was also calculated for each species (> 20 individuals/quadrat) as a way to describe more closely spaced arrangements. Local abundance was determined by the number of conspecifics within a 10 m radius and was obtained using the buffer tool in ArcMap. Only individuals with a 10 m buffer completely within the survey quadrat were analysed.

Aggregation and species associations

To determine the percent of neighbouring pairs that were conspecific for each site, the 1st order nearest neighbour function was used to determine the species type of the nearest neighbour for each individual. Analysis was completed for all individuals, regardless of the number of each species within the quadrat.

The 1st order nearest neighbour function and the neighbourhood density function (NDF) were used to assess aggregation. The 1st order nearest neighbour function was used to calculate the Clark-Evans R-Score as a basic measure of conspecific aggregation (Clark & Evans 1954) and was only calculated for species that had 20 or more individuals in at least one quadrat. The Clark-Evans R-Score is based on the ratio of the observed mean nearest neighbour distance to the distance expected under complete spatial randomness. A value $R>1$ suggests regularity, while $R<1$ suggests aggregation (Clark & Evans 1954; Perry 2004). The Clark-Evans R-Scores were used to assess aggregation as a function of species relative abundance (percent of each species (number of stems per species/total number of stems) per quadrat). Linear regressions were used to determine the strength and significance of correlations between abundance and aggregation for under-, mid- and overstorey classes, using GraphPad Prism 7.

The NDF was used to get an indication of spatial arrangement over a range of distances. NDF (also known as the pair-correlation function, or o-ring function) is a non-cumulative measure that isolates specific distance classes and determines the mean number of neighbours within each class (Condit *et al.* 2000; Perry 2004). NDF was used to determine if species had random, aggregated or regular arrangements and the spatial scale at which these patterns occurred. The NDF was completed for species that had 20 or more individuals in at least one of the eight, 1 ha quadrats. NDF analysis was completed using 2 m increments and confidence intervals were based on 499 replicates. Edge-weighted area correction was used to account for edge effects (Perry 2004). Mean results are presented for under-, mid- and overstorey species.

The multiple neighbourhood density function (M-NDF), was used to assess spatial associations between species. M-NDF was completed at the plot level, using species with 20 or more individuals. Data were grouped according to species and edge-weighted area correction was used (Perry 2004). Outputs were used to determine positive, neutral or negative associations between species and the percent of each type of association at each of the five sites.

Environmental gradients

Climate layers were sourced from WorldClim (http://www.worldclim.org/bioclim) for tile 410, which covers southern Australia with a resolution of 1 km^2. WorldClim data for Australia are based on Bureau of Meteorology (BOM) records (Hijmans *et al.* 2005). Mean annual temperature and rainfall data were extracted from the WorldClim data for the five survey sites. If quadrats occurred within different 1 km^2 grid cells, mean values were calculated for that site. Linear regressions in GraphPad Prism were used to assess strength and significance of correlations between percent canopy cover, nearest neighbour distance, aggregation and segregation with mean annual temperature and mean annual rainfall.

Results

Composition and stem density

In total, woody perennial species were mapped across 11.99 ha, with the GPS position of >18,000 plants recorded. Fifty-five different species were recorded, including ten *Eucalyptus* species, five non-eucalypt tree species (*Allocasuarina verticillata, Callitris* spp, *Exocarpus* spp) and 36 shrub species (Table 2). Four weed species were recorded (Olive (*Olea europaea*), Boxthorn (*Lycium ferocissimum*), Prickly Pear (*Opuntia stricta*) and Topped Lavender (*Lavandula stoechas*). *Hakea laurina* was recorded growing outside of its natural range. Species composition and relative abundance varied both within and between sites (Table 2, Fig. 2).

Table 2. List of all native species recorded during the surveys. No.: total number of plants recorded; Canopy layer: if the species forms the under (ground cover, small shrub), mid (medium or large shrub) or overstorey (tree); D: Dutchmans Stern, M: Mt Remarkable, S: Spring Gully, P: Para Wirra and B: Belair. Open circle: species recorded but not enough individuals (< 20) to calculate arrangement measures, closed circle: species recorded, and arrangement calculated. NND: mean distance in metres to the nearest conspecific (minimum to maximum) and local abundance: mean number of conspecifics within a 10 m radius (minimum to maximum). Aggregation: Clark-Evans R-Score (SD), where R occurs along a gradient, with 0 being highly aggregated, 1 random and >1 regular. Where there were insufficient individuals to calculate arrangement values, no values are provided. Arrangement values are the mean values across all sites in which the species was recorded.

Species	No.	Canopy Layer	D	M	S	P	B	NND (range)	Local abundance (range)	Aggregation
Acacia acinacea	258	Under				●	o	1.0 (1-2)	95 (27-172)	0.29
Acacia argyrophylla	97	Mid			●			1.3 (1-7)	56 (2-71)	0.23
Acacia calamifolia	12	Mid		o						
Acacia continua	44	Mid			o	●		4.2 (1-44)	9 (1-15)	0.38
Acacia paradoxa	152	Mid			●	o	●	1.6 (1-7)	38 (3-62)	0.47
Acacia pycnantha	3066	Mid	o	●	●	●	●	1.4 (1-16)	42 (1-110)	0.65 (0.23)
Acacia quornensis	488	Mid	●					2.4 (1-23)	15 (1-43)	0.51 (0.13)
Acrotriche serrulata	219	Under				●		1.6 (1-26)	19 (1-43)	0.45 (0.07)
Allocasuarina verticillata	184	Mid	●	o	o	o	o	1.9 (1-16)	15 (1-30)	0.55 (0.20)
Astroloma conostephioides	3946	Under		o	●	●	●	1.1 (1-8)	56 (4-184)	0.59 (0.14)
Astroloma humifusum	88	Under		o	o	●	o	2.0 (1-14)	4 (1-9)	0.19
Bursaria spinosa	120	Mid	●	o	o		o	3.1 (1-15)	8 (1-18)	0.41 (0.09)
Callitris glaucophylla	14	Over	o	o						
Callitris gracilis	84	Over			o	●	●	2.7 (1-15)	5 (1-11)	0.54 (0.07)
Calytrix tetragona	301	Mid	o	o		●	●	1.2 (1-7)	55 (2-111)	0.34 (0.27)
Cassinia laevis	1136	Mid	●	●				1.9 (1-20)	24 (1-89)	0.54 (0.12)
Cratystylis conocephala	8	Under	o							
Dianella revoluta	13	Mid			o					
Dodonaea viscosa	2952	Mid	●	●		●	●	1.4 (1-28)	51 (1-144)	0.58 (0.24)
Einadia nutans	1	Under		o						
Enchylaena tomentosa	112	Under	●	o				1.6 (1-18)	32 (1-66)	0.26
Eucalyptus camaldulensis	91	Over	o	●			o	3.6 (1-17)	6 (1-12)	0.58

Species	No.	Canopy Layer	D	M	S	P	B	NND (range)	Local abundance (range)	Aggregation
Eucalyptus fasciculosa	88	Over				●		5.0 (1-22)	3 (1-8)	0.72 (0.12)
Eucalyptus goniocalyx	47	Over				●		5.6 (1-19)	3 (1-6)	0.70
Eucalyptus leucoxylon	1470	Over	●	●	●	●	●	2.7 (1-26)	14 (1-49)	0.82 (0.14)
Eucalyptus macrorhyncha	431	Over			●			2.0 (1-16)	22 (1-50)	0.58 (0.13)
Eucalyptus microcarpa	20	Over		o		o	o			
Eucalyptus odorata	22	Over		●				9.8 (1-35)	2 (1-3)	0.90
Eucalyptus polybractea	2	Over	o							
Eucalyptus porosa	212	Over	●					2.6 (1-16)	9 (1-23)	0.70
Exocarpus aphyllus	1	Over			o					
Exocarpus cupressiformis	5	Over	o							
Goodenia ovata	73	Mid				o	●	3.0 (1-16)	7 (1-12)	0.73
Hakea laurina	5	Mid					o			
Hakea rostrata	141	Mid				●		1.5 (1-11)	36 (1-70)	0.33
Hibbertia crinita	243	Mid		●		●		1.2 (1-15)	13 (1-37)	0.47 (0.33)
Juncus sp.	80	Mid					●	1.2 (1-5)	25 (7-41)	0.42
Leptospermum myrsinoides	61	Mid				●		1.1 (1-2)	27 (16-43)	0.12
Maireana brevifolia	26	Mid	o	o						
Myoporum montanum	2	Mid	o	o						
Olearia decurrens	30	Mid	o	o						
Pimelea microcephala	2	Mid	o	o						
Pteridium esculentum	83	Mid					●	1.0 (1-2)	42 (35-54)	0.36
Ptilotus obovatus	11	Mid	o							
Pultenaea largiflorens	372	Mid			●	●		1.4 (1-21)	25 (1-72)	0.49 (0.29)
Rhagodia parabolica	78	Mid	o	●				1.5 (1-4)	7 (1-32)	0.76
Senna artemisioides	20	Mid	o							
Spyridium parvifolium	125	Mid			o	●		1.7 (1-26)	9 (1-21)	0.34 (0.34)
Xanthorrhoea quadrangulata	297	Mid	o	●	o			1.1 (1-3)	69 (1-114)	0.79 (0.33)
Xanthorrhoea semiplana	418	Mid				●		1.2 (1-8)	41 (2-87)	0.47

Understorey species accounted for 25% of plants recorded (515 ± 720 SD stems/ha), midstorey species 55% (1080 ± 590 SD stems/ha) and overstorey species 20% (340 ± 180 SD stems/ha), but the proportions of the three vegetation classes varied between the sites (Fig. 2). Stem densities were lowest at Dutchmans Stern, the most northerly site, with 540 stems per hectare, compared to Para Wirra which had the highest density with 3634 stems per hectare (Fig. 2). Patchiness and open space were common at all sites, with percent canopy cover ranging from 51% (± 7% SD) at Spring Gully to 14% (± 7% SD) at Dutchmans Stern (Fig. 2).

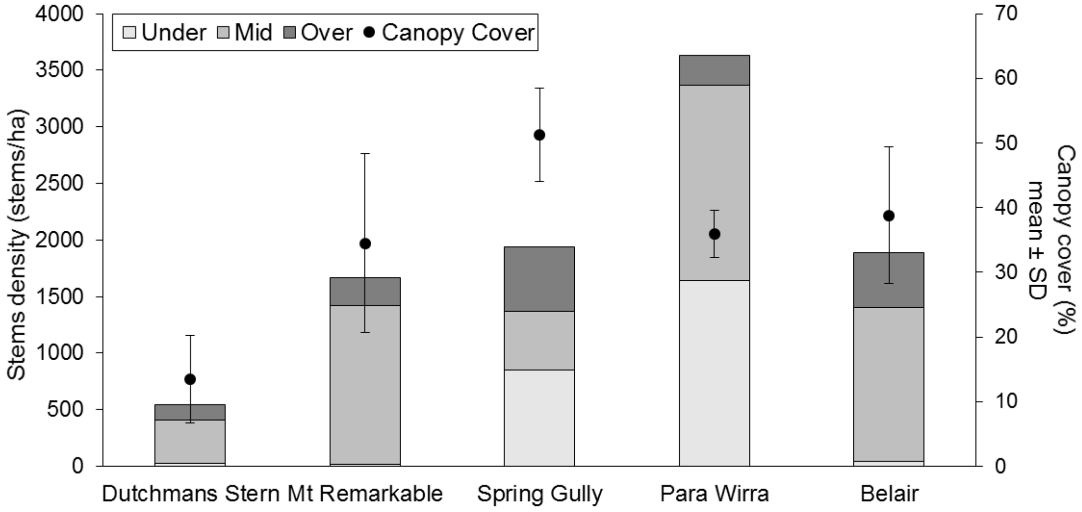


Figure 2. Stem density (stems per hectare) and canopy cover (%) for the five survey sites in *Eucalyptus leucoxylon* dominated woodlands, South Australia. The different shading represents the three different vegetation classes (under-, mid- and overstorey). Canopy cover (%) is based on the canopy width of each individual surveyed.

Nearest neighbour distance (NND) and local abundance

The distance between conspecifics (NND) and the local abundance (conspecifics within 10 m radius) of species was highly variable, with the mean NND ranging from 1.0 m for *Acacia acinacea* to 9.8 m for *Eucalyptus odorata* (Table 2). On average, understorey species were spaced 1.5 m (± 0.4 SD) apart and had 41 (± 36 SD) conspecifics within 10 m, midstorey were spaced 1.7 m (± 0.8 SD) apart with 29 (± 19 SD) conspecifics within 10 m and overstorey species were spaced 4.3 m (± 2.6 SD) apart with 8 (± 7 SD) conspecifics within 10 m.

The mean distance between conspecific eucalypts increased with increasing plant size classes, while local abundance decreased with increasing plant size (Table 3).

Table 3. Nearest neighbour distance (NND) and local abundance (number of conspecifics within 10 m) of the *Eucalyptus* species surveyed within *E. leucoxylon* dominated woodlands, South Australia, with increasing diameter breast height (DBH) size classes. Where insufficient large trees (> 30 cm DBH) were present, no summary statistics are given in the table. Mean values are shown for each measure with the range in parentheses.

	All		DBH ≥ 10 cm		DBH ≥ 20 cm		DBH ≥ 30 cm	
Species	NND	Local abundance	NND	Local abundance	NND	Local abundance	NND	Local abundance
Eucalyptus camaldulensis	3.6 (1-17)	6 (1-12)	3.9 (1-17)	5 (1-10)	5.1 (1-18)	3 (1-6)	9.9 (1-22)	2 (1-3)
Eucalyptus fasciculosa	5.0 (1-22)	3 (1-8)	6.2 (1-29)	3 (1-6)	11.3 (1-31)	1 (1-2)		
Eucalyptus goniocalyx	5.6 (1-19)	3 (1-6)	6.2 (1-19)	3 (1-6)	10.3 (1-23)	2 (1-4)		
Eucalyptus leucoxylon	2.7 (1-26)	14 (1-49)	4.2 (1-24)	12 (1-48)	5.9 (1- 24)	4 (1-11)	11.7 (1- 36)	2 (1-6)
Eucalyptus macrorhyncha	2.0 (1-16)	22 (1-50)	3.0 (1-21)	12 (1-25)	7.0 (1-26)	3 (1-7)		
Eucalyptus odorata	9.8 (1-35)	2 (1-3)	9.5 (1-35)	2 (1-3)	12.2 (1-37)	1 (1-3)		
Eucalyptus porosa	2.6 (1-16)	9 (1-23)	3.2 (1-16)	9 (2-22)	12.1 (1-22)	2 (1-3)		

Aggregation

Aggregation was common across the survey sites, with species often occurring in conspecific clusters of varying sizes, interspersed with areas of open space (Fig. 3). Based on the Clark-Evans measure of aggregation (1st order), understorey species were the most aggregated, with a Clark-Evans R-score of 0.36 (± 0.16 SD), followed by midstorey species with 0.47 (± 0.17 SD) and overstorey species 0.68 (± 0.16 SD; Table 2). Understorey species were significantly aggregated in all instances, midstorey species in 94% of cases and overstorey species in 79% of cases (overall significant aggregation in 90% of cases).

In total, 70% (± 7% SD) of nearest neighbour combinations were conspecific. Conspecific neighbours occurred least frequently at Dutchmans Stern with 58% of nearest neighbours being conspecifics but were similar in the four other sites ranging from 71% to 75%. Conspecific neighbours were most common for midstorey species, with 73% (± 8% SD), followed by understorey 72% (± 25% SD) and overstorey 39% (± 11% SD). Overstorey species often had an under- or midstorey nearest neighbour (Fig. 3), and when only overstorey species were analysed (all individuals considered, regardless of size), 76% (± 8% SD) had a conspecific neighbour.

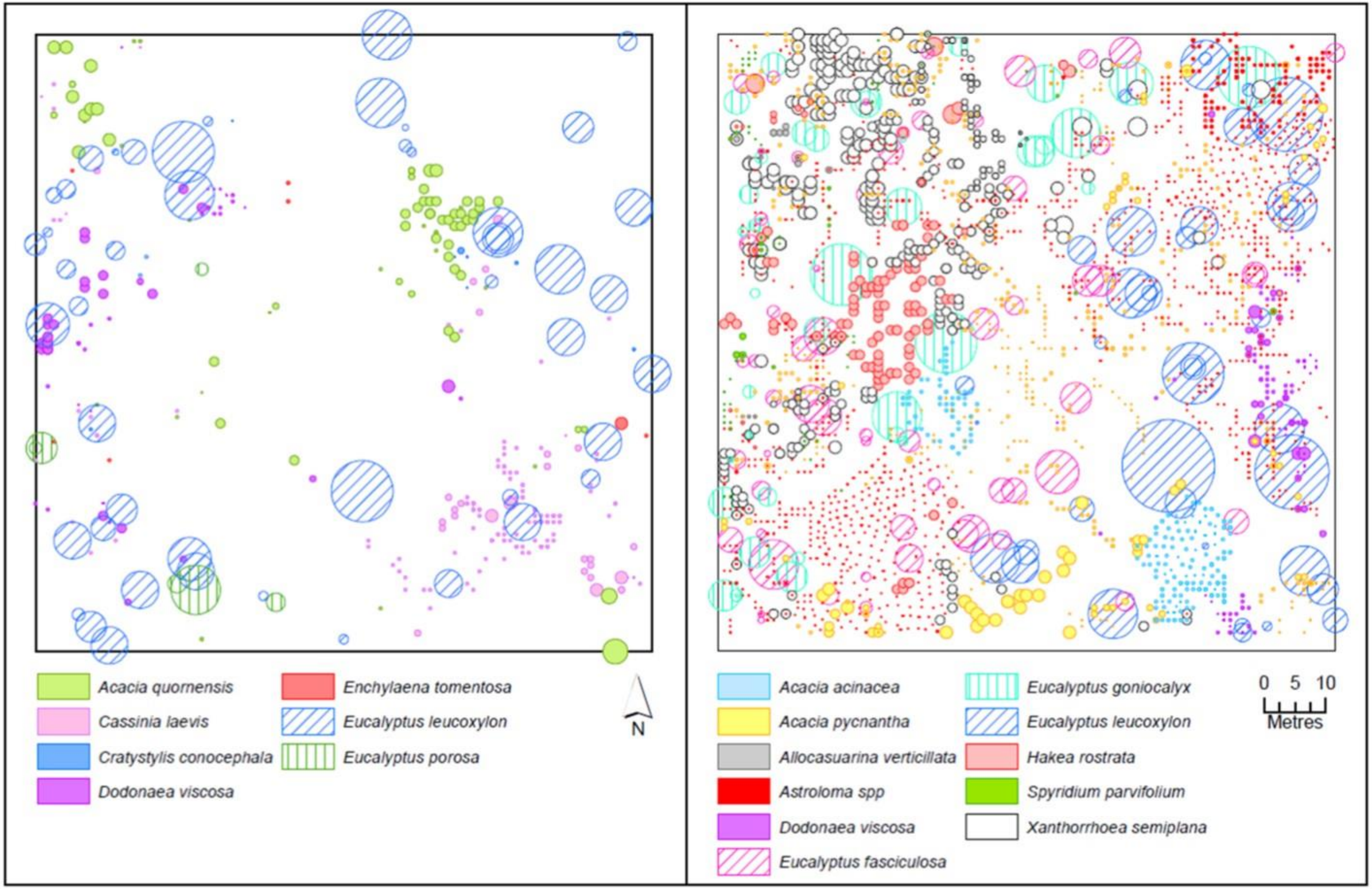

Acacia quornensis
Cassinia laevis
Cratystylis conocephala
Dodonaea viscosa
Enchylaena tomentosa
Eucalyptus leucoxylon
Eucalyptus porosa
N
Acacia acinacea
Acacia pycnantha
Allocasuarina verticillata
Astroloma spp
Dodonaea viscosa
Eucalyptus fasciculosa
Eucalyptus goniocalyx
Eucalyptus leucoxylon
Hakea rostrata
Spyridium parvifolium
Xanthorrhoea semiplana
0 5 10
Metres

Figure 3. Example of 1 ha survey plots from Dutchmans Stern (left) and Para Wirra (Right), representing the sparsest and densest survey sites. Species with five or more individuals within the 1 ha quadrat (100 m x 100 m) are shown. Only a sample of 10 species are shown in the Para Wirra plot (refer to Appendix 3 for a larger map with all species). Each colour represents a different species and the sizes of the circles are proportional to the canopy width of each individual surveyed. *Eucalyptus* species are represented by hatched circles and all other species by solid circles.

The degree of aggregation was influenced by relative abundance for under- and midstorey species (more aggregated at lower abundances), but not for overstorey species (Fig. 4; Linear regression; Under - $R^2 = 0.53$, $P<0.01$; Mid - $R^2 =0.40$, $P<0.0001$; Over - $R^2 = 0.001$, $P>0.5$).

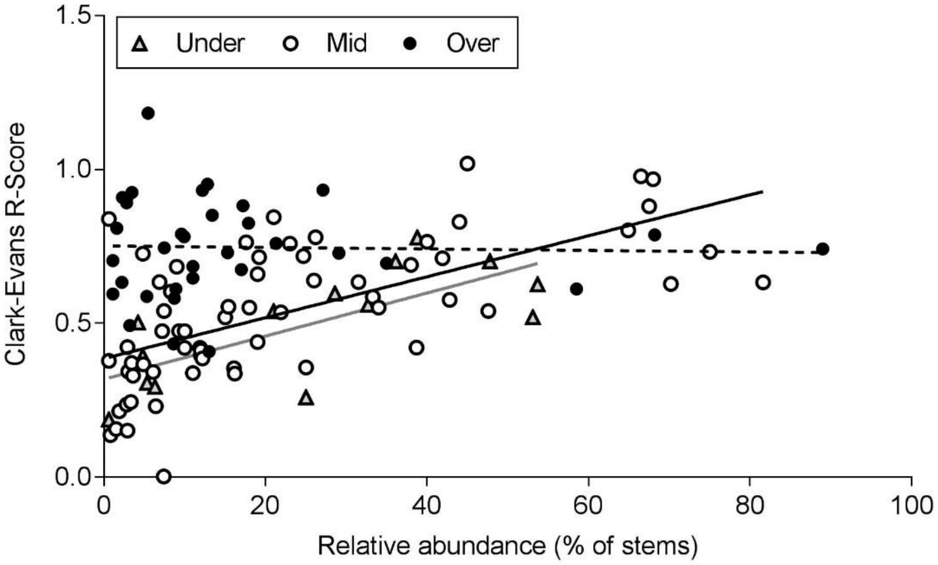


Figure 4. Degree of aggregation (Clark-Evans R-score) as a function of the relative abundance of a species for under-, mid- and overstorey species. Abundance is based on the number of individuals of each species per quadrat (only results from species with ≥20 individuals shown), relative to total number of individuals per quadrat. Clark-Evans R-Scores: 0 is highly aggregated, 1 is random and > 1 represents a dispersed arrangement. Lines show linear regression for each vegetation type: Over – dashed line, Mid – black line and Under – grey line (solid lines represent a slope that is significantly different from 0, at P=0.05).

Aggregation was also evident when the neighbourhood density function (NDF) was used. Aggregation was most pronounced at distances <10 m and was observed over similar distances in the three classes (Fig. 5). Aggregation was strongest in the understorey species, followed by midstorey and then overstorey. Aggregation was observed in 88% of cases using NDF, with random arrangements occurring in the remaining 12% of cases. The only genus to display random arrangements was *Eucalyptus*.

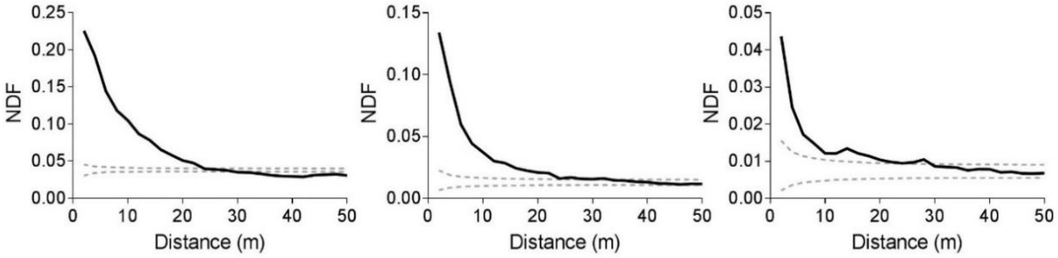


Figure 5. Neighbourhood density function (NDF) with 2 m distance increments. Results are shown for species recorded in the 1 ha plots only (mean results for 8 plots). Mean results for understorey (left), midstorey (middle) and overstorey (right) are shown. The black line shows the mean NDF at 2 m distance classes. The grey dashed lines show the upper and lower intervals expected under complete spatial randomness. Above the upper line represents aggregation, in between the lines represents random arrangements and below the lines show dispersed arrangements. Note the different Y-axis scales for each graph.

When the multiple neighbourhood density function (M-NDF) was used to assess associations between species, only neutral or negative associations were evident. Negative associations were observed in 48% of cases across the five sites. The percent of negative associations ranged from 62% at Dutchmans Stern to 32% at Spring Gully.

Environmental trends

The percent of canopy cover decreased and the distance between plants increased as temperature increased, and rainfall decreased (Fig. 6). Temperature was a better predictor of canopy cover than rainfall (Linear regression, temperature – $R^2 = 0.92$, $P<0.01$; rainfall – $R^2 = 0.54$, $P>0.1$). Rainfall was a significant predictor of NND for overstorey species (Linear regression, temperature $R^2 = 0.74$, $P>0.05$; rainfall $R^2 = 0.79$, $P<0.05$), temperature was significantly correlated with NND for understorey species ($R^2 = 0.97$, $P<0.05$), but not for midstorey species ($R^2 = 0.53$, $P>0.1$). Rainfall was not a significant predictor of NND for under- or midstorey species (Linear regression, $R^2 = 0.70$ and 0.54 respectively, $P>0.1$).

Trends with aggregation and climatic gradients varied between canopy layers, but none of these trends were significant (Linear regression, R^2 0.02-0.5, $P>0.1$; Fig. 6). The percent of conspecific neighbours decreased with increasing temperature and decreasing rainfall (Linear regression, temperature – $R^2 = 0.86$, $P<0.05$; rainfall – $R^2 = 0.78$, $P<0.05$), and the percent of negative associations increased with increasing temperature and decreasing rainfall, but not significantly (Linear regression, temperature – $R^2 = 0.65$, $P=0.1$; rainfall – $R^2 = 0.65$, $P=0.1$).

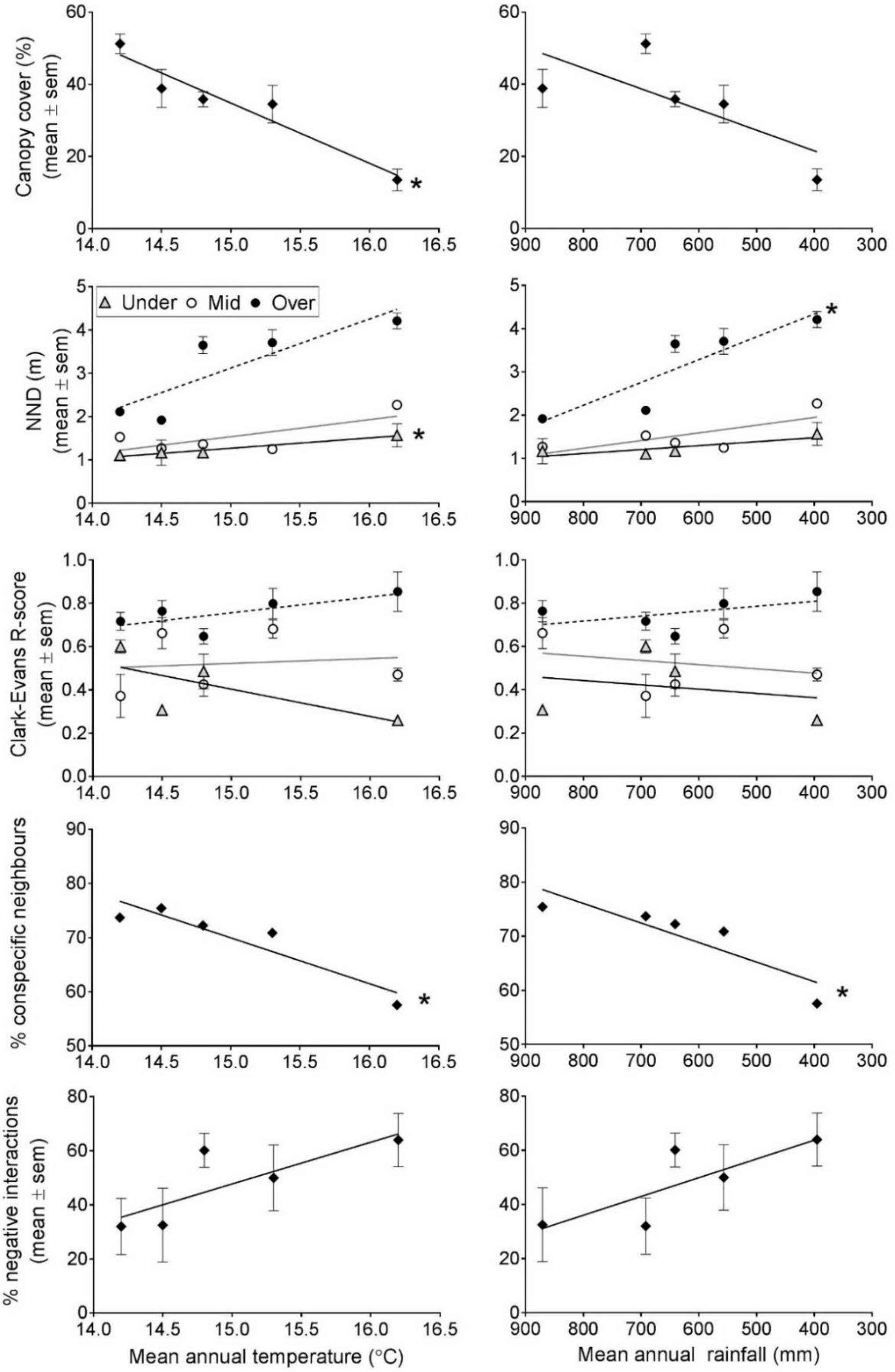


Figure 6. Spatial arrangement trends across the mean annual temperature (°C) and rainfall (mm) gradient of the five sites. From top to bottom - percent canopy cover, nearest neighbour distance (NND), Clark-Evans R-score (measure of aggregation, where R = 0 is highly aggregated, R = 1 random and R>1 regular), percent conspecific neighbours (%) and percent of negative associations between species (%). * shows trends with slopes that are significantly different from 0 at the P=0.05 level.

Discussion

The spatial arrangement of the *Eucalyptus leucoxylon* woodlands studied were highly variable, with stem densities, species composition, relative abundances and the amount of open space varying both within and between the five sites. Despite this variability, the majority of species were aggregated (88-90%, depending on analysis method), and it was common for individuals to have a nearest neighbour of the same species. Aggregation was strongest at distances < 10 m and in general, mid- and understorey species were more aggregated than overstorey species, and uncommon species (low relative abundance) were more likely to be aggregated than more abundant species. Complete admixture of different species was rare and there was no evidence of positive associations between species. Consequently, if *Eucalyptus* woodland revegetation projects seek to reproduce more natural plant arrangements, designs should consider arrangements of plants at fine-scales (< 10 m) and focus on constructing patchy systems with aggregated arrangements of species (using a range of planting densities and cluster sizes), interspersed with areas of open space.

Natural plant arrangements

In the *E. leucoxylon* woodlands studied, the majority of species displayed aggregated arrangements, and this is common in a range of natural systems (e.g. Condit *et al.* 2000; Perry *et al.* 2008). Aggregation can result from limited seed dispersal, habitat heterogeneity, disturbance and plant-plant interactions (Dale 1999; Miller *et al.* 2010). Aggregation is often stronger in smaller plants (Phillips & MacMahon 1981), and this was the case for the woodlands studied, with shrubs tending to be more aggregated than trees. In addition, species that had a lower relative abundance were generally more aggregated than more abundant species and this trend has also been observed in other systems (Condit *et al.* 2000; Davis *et al.* 2005).

There were no significant correlations between the degree of aggregation and climate (mean annual temperature and rainfall), which suggests that aggregation most likely results from finer scale variables, such as habitat heterogeneity and limited seed dispersal (Phillips & MacMahon 1981; Gardner *et al.* 2008). Similarly, the proportions of under-, mid- and overstorey species varied widely within and between sites, which suggests that local changes (e.g. topography, soil type) influenced the relative abundance of species more than climate (Phillips & MacMahon 1981; Perry *et al.* 2008), even though climate governs the overall distribution of species (Guerin *et al.* 2013). In contrast, the distance between conspecifics and the amount of canopy cover were significantly correlated with climate and this may result from stronger competition between plants when resources (e.g. water) are limited (Holmgren *et al.* 1997).

Dutchmans Stern, the site with the lowest annual rainfall and highest mean annual temperature had the most negative associations between species (62%) and individuals were less likely to have a neighbour of the same species (58%). Consequently, it is likely that competition both within and between species played a large role in structuring the vegetation at this site. In contrast, negative interactions between species at the wettest site (Spring Gully, 32%), were almost half that recorded at the driest site and individuals were also more likely to have a conspecific neighbour (75%). There was no evidence of positive associations between species and as a result, species occurred in conspecific clusters, spatially separated from other species or in clusters that overlapped to some degree with clusters of other species, rather than in multi-species clumps.

Using spatial survey data to guide revegetation design

In the woodlands studied, complete admixture of different *Eucalyptus* species was rare, and it was common for one species of eucalypt to dominate an area, before grading into another species. Consequently, revegetation designs may be most effective if sites are first divided into broader areas tailored to the eucalypt species being used and then supplemented with clusters of other species and areas of open space. This is already occurring to an extent, with some revegetated sites divided into defined plant assemblages based on soil type, hydrology and topography (e.g. Jonson 2010; Jellinek & Te 2016). However, once these zones are defined, guidelines only specify the number of plants/species/hectare to be planted (Jellinek & Te 2016), and this may limit the ecological value of these plantings (McCallum *et al.* accepted).

Individuals often had a conspecific nearest neighbour, particularly under- and midstorey species, but the distance between conspecifics and the local abundance of conspecifics was highly variable. Therefore, planting conspecifics next to each other (particularly for shrubs), with a range of nearest neighbour distances and local abundances, will help create more heterogeneous systems, similar to those in natural systems. Furthermore, the spacing between individuals, particularly for overstorey species can be tailored to the climatic conditions at the planting site, with larger spacing between conspecifics at more arid sites.

Variable distances between conspecifics may be particularly important for eucalypts because closely spaced arrangements can limit lateral growth and reproduction (Williams *et al.* 2006; Alcorn *et al.* 2007). The mean NND for *E. leucoxylon* was approximately 3 m, but ranged from 1-26 m, with individuals often having one or two neighbours close by, rather than equally spaced conspecifics in all directions. In addition, the mean NND values calculated were based on all individuals surveyed, including saplings and seedlings. In natural systems it is unlikely that all these individuals will make it to maturity, so the spacing between larger trees can help guide the timing and extent of thinning. For example, in *E. leucoxylon* the mean distance between all individuals was approximately 3 m, 4 m for individuals with a DBH ≥ 10 cm, 6 m for individuals with a DBH ≥ 20 cm and 12 m for trees with a DBH ≥ 30 cm.

The woodlands surveyed also contained trees and shrubs of various sizes and age classes (Fig. 3). Therefore, reproducing the variability seen in natural systems may require revegetation designs to consider temporal as well as spatial patterns. Multiple or staged plantings and disturbance (e.g. thinning, fire) may assist in the development of revegetated sites with multiple cohorts and greater structural diversity (Schneemann & McElhinny 2012; Stanturf *et al.* 2014).

On-going research

Aggregated arrangements can start to develop naturally in revegetated sites as a result of mortality and recruitment (Miller *et al.* 2010; Zhao *et al.* 2012), but initial planting arrangements can persist for decades (Jonson 2010) and during this time they can affect the ecological processes occurring within revegetated communities (Zhao *et al.* 2012; McCallum *et al.* 2018a). Therefore, re-creating more natural plant arrangements where aggregation and patchiness are common, has the potential to improve the development and function of revegetated sites (Sluis 2002; McCallum *et al.* 2018a). Planting in this way may facilitate pollination, plant reproduction and recruitment, improve habitat value and limit weed invasion (McCallum *et al.* 2018b). Monitoring of sites planted in a 'more natural' way will be necessary to assess the ecological outcomes of this approach and to determine if the ecological benefits of spatially designed revegetation outweigh the additional costs required to manipulate planting or seeding methods during the establishment phase of revegetation.

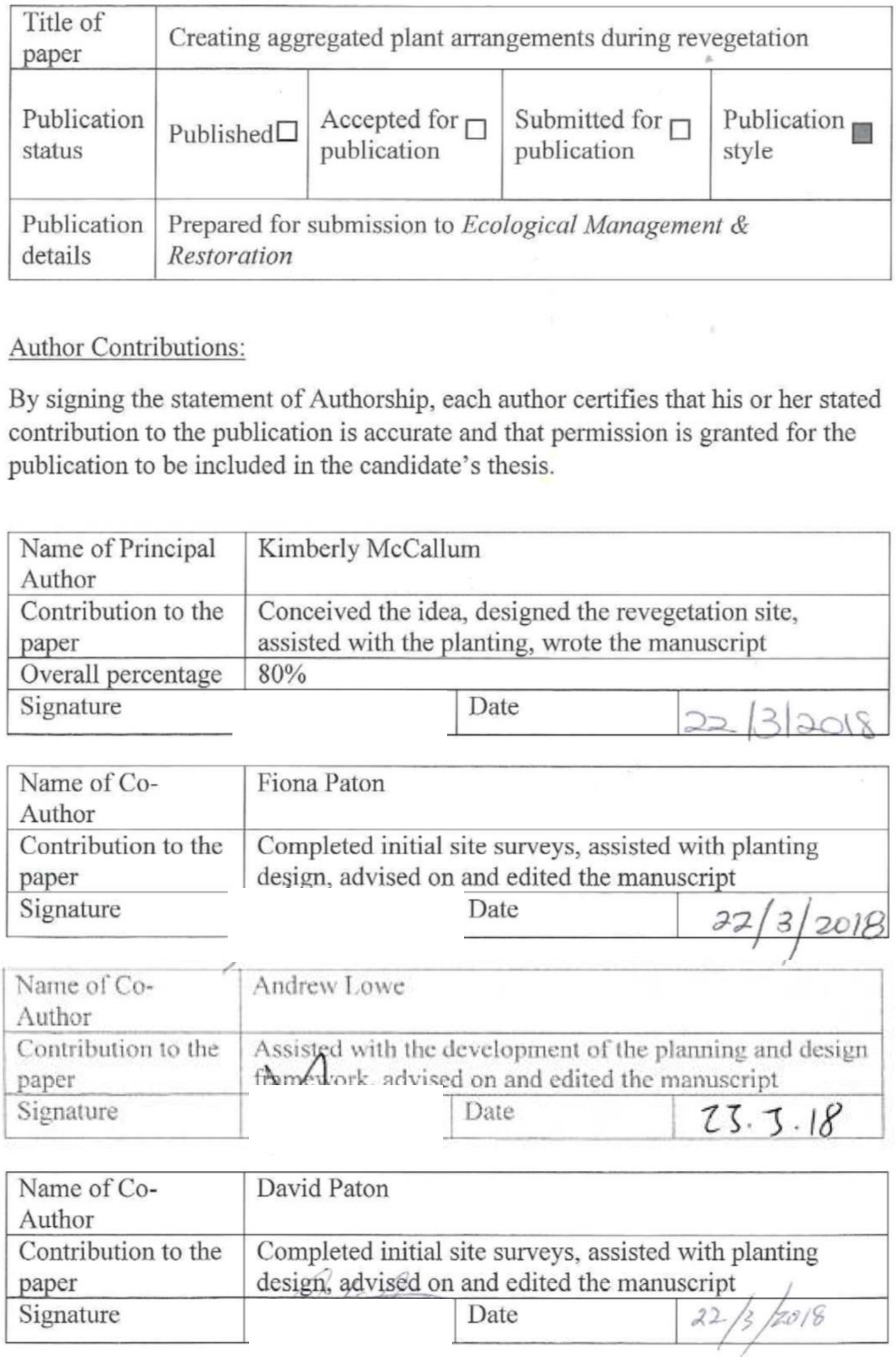
To further improve revegetation design, knowledge of environmental variation will also be important since manipulating spatial arrangements without considering local site conditions may not achieve the desired benefits. The work presented here could be usefully extended to match plant arrangements to the local environmental conditions (e.g. soil type, aspect, slope), which would allow planting designs to be tailored to the local conditions. In addition, woodland revegetation should be designed to create the most productive and biodiverse habitats possible, rather than just attempting to replicate remnant vegetation (Hunt & Paton 2018). This may be particularly important if conditions within revegetated sites differ from those in nearby remnants due to extensive degradation. In this case, species may need to be carefully selected based on traits that best suit the environmental conditions (Padilla *et al.* 2009; Laughlin 2014), and this may result in novel or hybrid ecosystems (Hobbs *et al.* 2009).

Acknowledgements

This research was supported by the Nature Foundation of South Australia. KPM was supported by the Noel and Vivian Lothian Scholarship and an Australian Government Research Training Program Scholarship provided through the University of Adelaide. Thanks to Chelsea McCallum for helping with the field surveys and Blair Kavanagh for sharing her knowledge of GIS and revegetation design. We thank the following rangers: Donna Ferschl (Dutchmans Stern Conservation Park and Mt Remarkable National Park), Kate McNicol (Spring Gully Conservation Park), Steve Taylor and Meryl Jenkins (Para Wirra Recreation Park) and Jennifer Pitman (Belair National Park). Thanks also to Greg Guerin for suggesting analysis options, and Andrew Lowe and Martin Breed for providing feedback on the manuscript.

Chapter 7. Revegetation design

Statement of authorship



Title of paper	Creating aggregated plant arrangements during revegetation			
Publication status	Published ☐	Accepted for publication ☐	Submitted for publication ☐	Publication style ■
Publication details	Prepared for submission to *Ecological Management & Restoration*			

Author Contributions:

By signing the statement of Authorship, each author certifies that his or her stated contribution to the publication is accurate and that permission is granted for the publication to be included in the candidate's thesis.

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Contribution to the paper	Conceived the idea, designed the revegetation site, assisted with the planting, wrote the manuscript		
Overall percentage	80%		
Signature		Date	22/3/2018

Name of Co-Author	Fiona Paton		
Contribution to the paper	Completed initial site surveys, assisted with planting design, advised on and edited the manuscript		
Signature		Date	22/3/2018

Name of Co-Author	Andrew Lowe		
Contribution to the paper	Assisted with the development of the planning and design framework, advised on and edited the manuscript		
Signature		Date	23.3.18

Name of Co-Author	David Paton		
Contribution to the paper	Completed initial site surveys, assisted with planting design, advised on and edited the manuscript		
Signature		Date	22/3/2018

Spatially designed revegetation – manipulating the arrangement of seedlings during woodland revegetation

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Running head: revegetation design

Author contributions: All authors conceived the ideas. KPM and AJL developed the planning and design framework. FLP and DCP completed preliminary site assessments. KPM and FLP designed the revegetation site with assistance from DCP. KPM, FLP and DCP assisted with the planting. KPM wrote the first draft of the manuscript and all authors contributed to revisions.

Abstract

Initial planting arrangements can persist for decades in revegetated woodlands, and this may limit the ecological value of these sites. Mimicking the arrangements found in natural systems, where aggregation and heterogeneity are common, has been promoted as a way of increasing the ecological value of revegetated systems; but this rarely occurs, with revegetation designs often based simply on the number of individuals of each species to be planted per hectare. Here we describe a range of potential methods for manipulating the position of plants within revegetated sites, using both tubestock planting and direct seeding, with the aim of creating patchy systems with aggregated arrangements of species. Following this, we trial one of these methods - tubestock planting into individual holes. We used GIS to plan the position of approximately 2400 tubestock seedlings from 21 species, across a 12-ha revegetation area. The design was focussed on creating a heterogeneous site with conspecific patches, areas of open space and variation in density and plant spacing. This method was successful in moving away from haphazard plantings towards more aggregated arrays, but final planting positions varied from the design due to on-ground implementation constraints. On-going monitoring is now required to assess the development and ecological function of this site to help inform future revegetation activities in the region.

Keywords

Ecological restoration, eucalypt woodland, plant spatial pattern, revegetation design, tubestock

Introduction

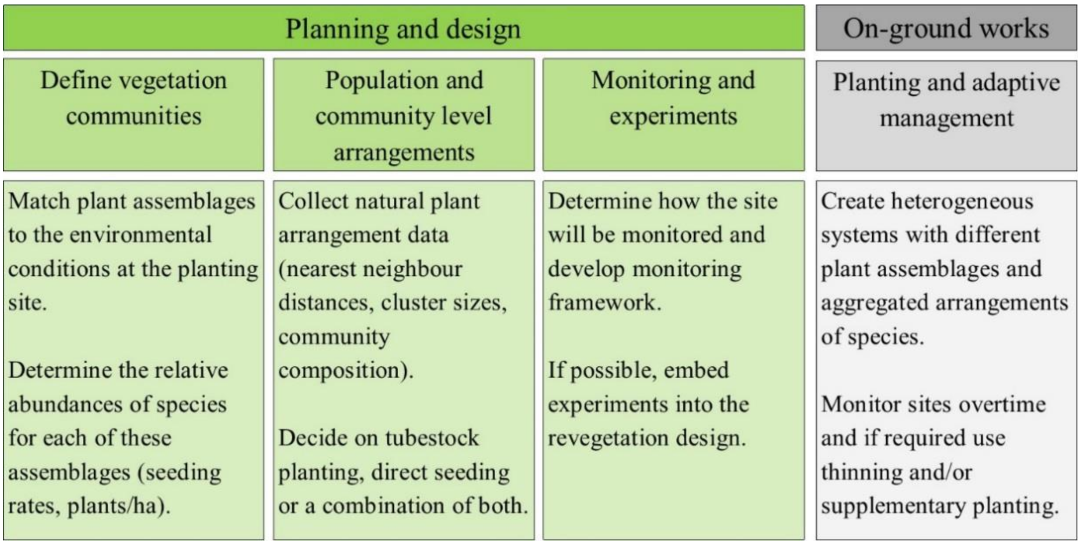
Revegetation is widely used to restore degraded land, especially where there has been extensive clearing (Wortley *et al.* 2013). Accordingly, tubestock planting and direct seeding are key restoration activities in many areas around the world (Godefroid *et al.* 2011; Broadhurst *et al.* 2017b), but the outcomes of these activities can be highly variable (Close & Davidson 2002; Commander *et al.* 2013). As a result, there have been calls for more ecologically informed revegetation and new approaches to restoration plantings (Jonson 2010).

Restoration ecology is a rapidly growing field but specific design principles for plantings are often lacking (Hobbs 1993). As a result, planting is generally done in an *ad hoc* way, with designs based on the number of plants per species per hectare to be planted (Dorrough *et al.* 2008; Jellinek & Te 2016). Planting in this way may underestimate the complexity of natural systems because little consideration is given to fine-scale population and community level patterns (Bartha *et al.* 2004; Miller *et al.* 2010). The arrangement of plants (both within populations and communities) influences the majority of ecological processes that occur within natural systems (Dale 1999), so it is likely they will also play a fundamental role in revegetation.

Consequently, opportunities exists to improve the ecological value of revegetation by manipulating planting arrangements (McCallum *et al.* 2018b). Here we set up a framework for planning, designing and undertaking revegetation which considers natural heterogeneity and fine-scale patchiness. We then describe a range of potential methods for controlling the spatial arrangement of plants during revegetation, using both tubestock planting and direct seeding. Following this, we trial one of these methods, tubestock planting into individual holes, at Frahn's Farm, South Australia, using GIS to plan the position of every tubestock seedling.

Revegetation planning, design and implementation framework

Revegetation may be most effective if planned over a range of scales, from the position of plant communities within the landscape, to the position of individual plants within those communities (McCallum *et al.* 2018b). Although, undertaking revegetation in this way will require a greater investment of time and resources into planning (Fig. 1), it has the potential to create more heterogeneous, self-sustaining and resilient systems that will require less management in the long-term (Jonson 2010). The value of revegetation works can be further enhanced if experiments are incorporated into the planting design (and documented) as data generated from these experiments can help inform future plantings (Breed *et al.* 2018; Gellie *et al.* 2018).



Planning and design			On-ground works
Define vegetation communities	Population and community level arrangements	Monitoring and experiments	Planting and adaptive management
Match plant assemblages to the environmental conditions at the planting site. Determine the relative abundances of species for each of these assemblages (seeding rates, plants/ha).	Collect natural plant arrangement data (nearest neighbour distances, cluster sizes, community composition). Decide on tubestock planting, direct seeding or a combination of both.	Determine how the site will be monitored and develop monitoring framework. If possible, embed experiments into the revegetation design.	Create heterogeneous systems with different plant assemblages and aggregated arrangements of species. Monitor sites overtime and if required use thinning and/or supplementary planting.

Figure 1. Revegetation planning and design framework.

Define vegetation communities

In natural systems, environmental variables such as soil type or topography drive the formation of specific vegetation associations, so this information can be used to select the most appropriate combination of species for revegetation (e.g. Jonson 2010; Jellinek & Te 2016). This process has been undertaken at the Peniup property in the Gondwana Link, Western Australia (Jonson 2010). At this site, a detailed planning process was undertaken across the 2400 ha area, resulting in the property being divided into nine zones based on soil type, past crop yields, clearing history, elevation and topography. Vegetation assemblages were then tailored to each of these zones, with the species used and ratio of those species varying between the nine zones. Estimates of canopy sizes at maturity were used to determine preferred seeding rates for each section (Jonson 2010).

Population and community level arrangements

In natural systems, population and community level arrangements develop from a range of interacting environmental and ecological factors (Dale 1999; Alados *et al.* 2007; Alados *et al.* 2009; Gaston & Garcia-Vinas 2013). These factors influence plant spacing, degree of aggregation, community level arrangements and the relative abundances of species (McCallum *et al.* 2018b). Knowledge of natural plant arrangements can be used to guide revegetation designs and these data can be collected by recording the GPS position and species identity of plants within natural communities (Appendix 3).

Revegetation activities, such as tubestock planting or direct seeding determine the spatial arrangement of plants within revegetated sites, so the ability to re-create more natural plant arrangements will be dependent on the planting techniques used (Fig. 2).

Tubestock are generally planted into ripped rows or into individual holes (Close & Davidson 2002; Munro & Lindenmayer 2011), and this will allow more natural plant arrangements to be reproduced because the position of every seedling can be controlled. The position of these holes can be tailored to the plant spacing, cluster sizes and local densities desired. Spacing trees and shrubs at irregular distances will help create more heterogeneous systems (Bennett *et al.* 2000). If planting into ripped rows, conspecifics can be planted into neighbouring positions within the same row and in adjacent rows to create conspecific clusters (Fig. 2). Varying the spacing between rows and the spacing between plants within those rows will help create heterogeneous systems.

The arrangement of plants within revegetated populations and communities will be more difficult to control using direct seeding, but there are examples of aggregated arrangements being produced in grassland systems by hand seeding in patches (e.g. Wassmuth *et al.* 2009; Seahra *et al.* 2016). These techniques need to be further developed so they can be applied to woodland systems and large-scale revegetation (Yurkonis & McKenna 2014).

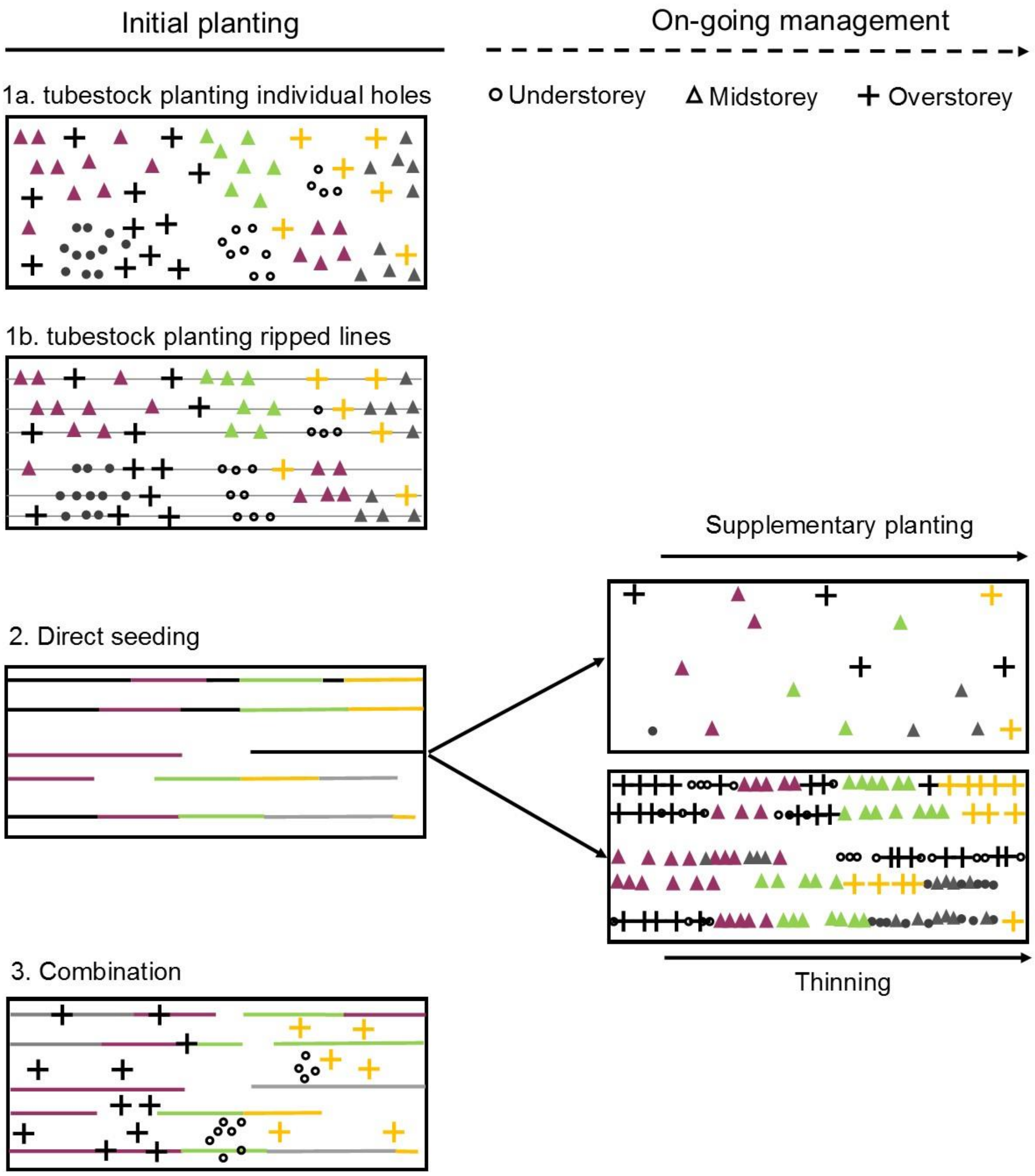


Figure 2. Potential methods for manipulating planting arrangements using tubestock planting, direct seeding or a combination of both to help establish aggregated arrangements of species during revegetation. Different colours represent different species (and/or different combinations of species for direct seeded sites), with different shapes representing plants from the different vegetation layers (under-, mid- and overstorey). Aggregated arrangements may be established during the initial planting, but if this does not occur, on-going management such as supplementary planting or thinning will be required. Supplementary planting can be used if there is poor survival after a planting and to increase conspecific aggregation or species diversity. Thinning can be used if dense stands establish, to remove species from areas with species mixtures to help establish conspecific clusters or to create areas of open space.

Direct seeding for large-scale revegetation is generally done by mechanical seeding, where seed is deposited (generally the same mix of seed applied at a constant rate) in rows or belts (Dalton 1993; Corr 2003; Jonson 2010). Alternatively, seed mixtures could be manipulated, so small areas are sown with a single species or with a selection of species (Fig. 2). Direct seeding often results in under- or overstocked patches (Close & Davidson 2002), so on-going management (thinning, supplementary planting), may be required to achieve the desired arrangements and densities (Fig. 2).

Alternatively, a combination of direct seeding and tubestock planting could be used, with common and easy to germinate species direct seeded, followed by tubestock planting in clusters of rare, difficult to germinate or less competitive species (e.g. Jonson 2010). Planting overstorey species, such as eucalypts as tubestock may also be beneficial because high density stands, which can influence tree growth and limit reproduction, can be avoided (Ward & Koch 1995; Williams *et al.* 2006; Alcorn *et al.* 2007).

Monitoring and experiments

The development of effective revegetation practices can be limited by little follow up monitoring and poor documentation of the techniques used (Ruiz-Jaén & Aide 2005a). Setting up monitoring plots within revegetated sites and recoding the GPS position of seedlings will allow survival, recruitment and changes in spatial arrangement to be assessed as the vegetation matures (Jonson 2010). However, one of the major challenges associated with measuring the success of woodland revegetation is the long time period between planting and the development of mature habitat, which can take decades or centuries (Mac Nally 2008; Vesk *et al.* 2008). Embedding well designed experiments into revegetation can help overcome this challenge (Breed *et al.* 2018; Gellie *et al.* 2018), and there are some successful examples of this occurring (e.g. Wassmuth *et al.* 2009; Perring *et al.* 2012; Gellie *et al.* 2016).

Frahn's Farm case study

Location

The revegetation site is located at Frahn's Farm, approximately 70 km southeast of Adelaide, South Australia (Fig. 3). Frahn's Farm is a 550–ha property on Crown Land, which was originally cleared for grazing. The site contains fragments of remnant vegetation and mature revegetation (planted in the 1970s). The cleared areas are being revegetated in stages, with works commencing in 2016. The revegetation is a joint project between BioR and Natural Resources SA Murray-Darling Basin (BioR 2018). In 2017, 12-ha of the site was set aside for revegetation, and in the following section we describe how the planning, design and planting activities were delivered at this planting stage.

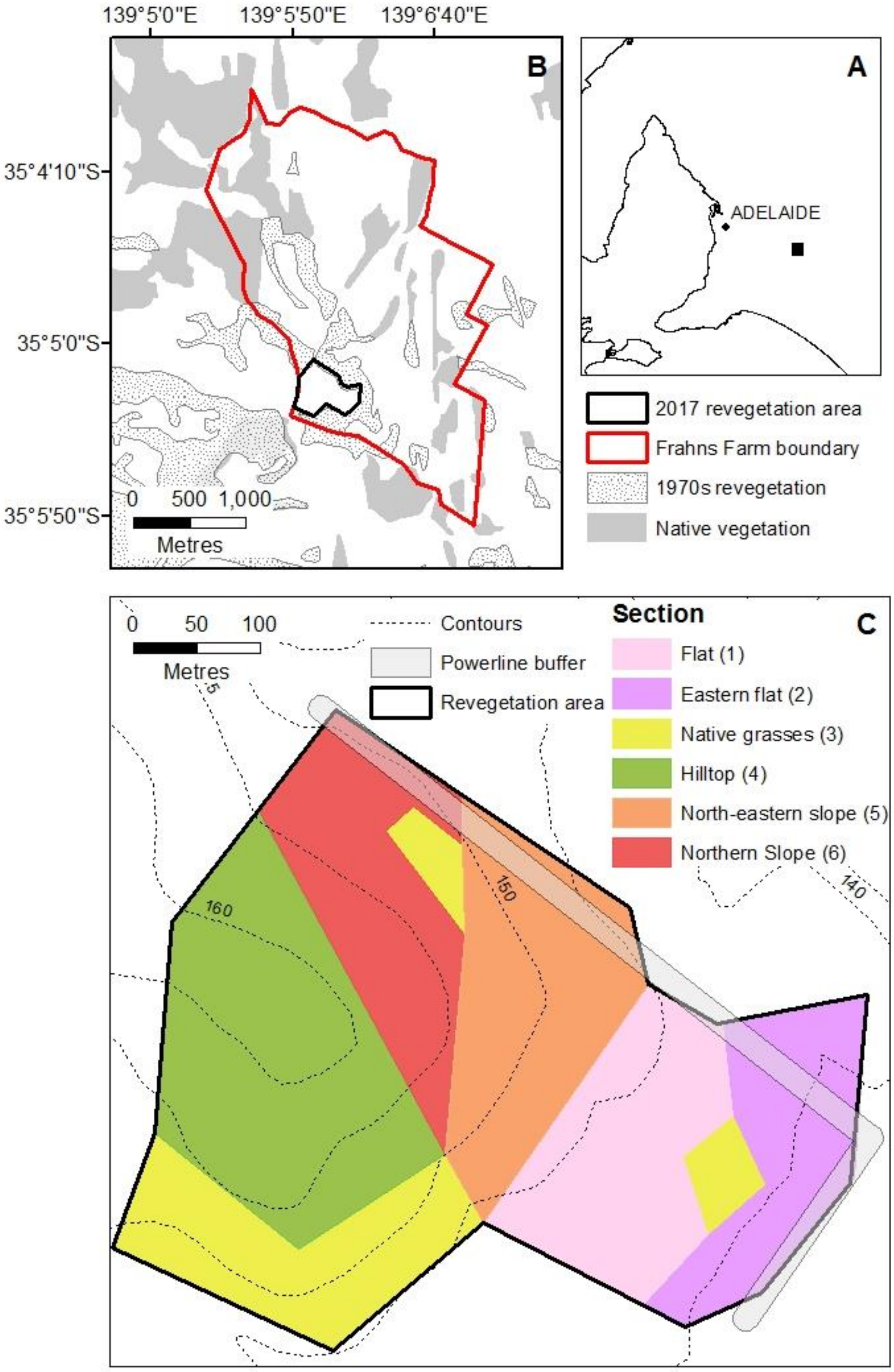


Figure 3. Location of Frahn’s Farm, South Australia (A), showing the position of the 2017 revegetation area. Frahn’s Farm contains fragments of native vegetation, as well as areas of revegetation that were planted in the 1970s (B). The 2017 planting area was broken into six planting zones based on topography and existing cover of native grasses (C). Data sources: Department of Environment, Water and Natural Resources; the Rural City of Murray Bridge.

Revegetation design

The area set aside for revegetation in 2017 was divided into six planting zones based on the topography of the site and the existing cover of native grasses (Fig. 3). Soil type was relatively consistent across the site, so in this instance, soil type was not used to define planting zones. Planting also occurred along the creek lines in gaps in the established 1970s revegetation. Woody species were selected for each of these zones based on knowledge of their natural distributions and habitat requirements. In total, 21 species (trees and shrubs) were selected for planting, with ca 2400 seedlings used (Table 1). Seed was sourced from populations in the Mt Lofty Ranges and Adelaide Plains.

The revegetation design was completed using ArcGIS program ArcMap version 10.3.1 (ESRI). The position of every seedling was planned by creating a shapefile, with a separate point feature for every individual. The predicted canopy diameter for each species at maturity was used to estimate canopy cover, allowing a heterogeneous system with areas of open space and a range of plant densities to be created (Fig. 4). Plant spacing was based on knowledge of natural plant arrangements (Chapter 6). Where species specific information was not available, plant spacing ranges were estimated from similar species (same genera, growth form or size).

A powerline is situated along the north-eastern boundary of the site, so no plantings were planned in the 10 m buffer around this line and only sparse plantings were planned for the native grass sections (Fig. 4).

Table 1. Species and number of seedlings used in the revegetation design. Zone refers to the six landscape zones detailed in Fig. 3, with zones 1 and 2 occurring on the flat areas, zone 3 occurring in patches of native grass, zone 4 on the hilltop and zones 5 and 6 occurring on the slopes. Canopy width (m) is the estimated size at maturity and was used to plot the canopy sizes shown in Fig. 4. Nearest neighbour distance is the maximum distance observed or expected in natural *Eucalyptus* woodlands. Where available the mean value or most frequently observed spacing range is provided in parentheses.

Species	Tubestock number	Zone	Canopy width (m)	Nearest neighbour distance range (m)
Acacia acinacea	35	1,4,5	2	≤ 5 (1)
Acacia argrophylla	40	1,4,5,6	1	≤ 10 (1.5)
Acacia pycnantha	130	1,2,3,4,5,6	2	≤ 20 (1-4)
Allocasuarina verticillata	335	1,2,3,4,5,6	2	≤ 20 (2-5)
Callitris gracillis	500	1,2,3,4,5,6	2	≤ 20 (1-10)
Eucalyptus dumosa	60	1,2,5,6	4	≤ 20
Eucalyptus fasciculosa	60	1,4,5	8	≤ 30
Eucalyptus gracilis	40	1,2,4,5	6	≤ 20
Eucalyptus incrassata	30	4,5,6	6	≤ 20
Eucalyptus leptophylla	60	1,2,4,5,6	6	≤ 20
Eucalyptus leucoxylon	450	1,2,5,6	8	≤ 30 (5)
Eucalyptus odorata	20	4	8	≤ 30 (7)
Eucalyptus porosa	30	4	6	≤ 20 (4)
Eucalyptus socialis	20	1,6	6	≤ 20
Lomandra effusa	60	4,6	1	≤ 3
Lomandra juncea	150	1,4,5,6	1	≤ 3
Melaleuca acuminata	170	1,2,5,6	1	≤ 10 (1-5)
Melaleuca lanceolata	94	1,2,5,6	2	≤ 15 (1-5)
Pittosporum angustifolium	15	1,5	2	≤ 15 (1-6)
Rhagodia crassifolia	5	1	1	≤ 4
Senna artemisioides	80	3,4,6	1	≤ 6 (2)

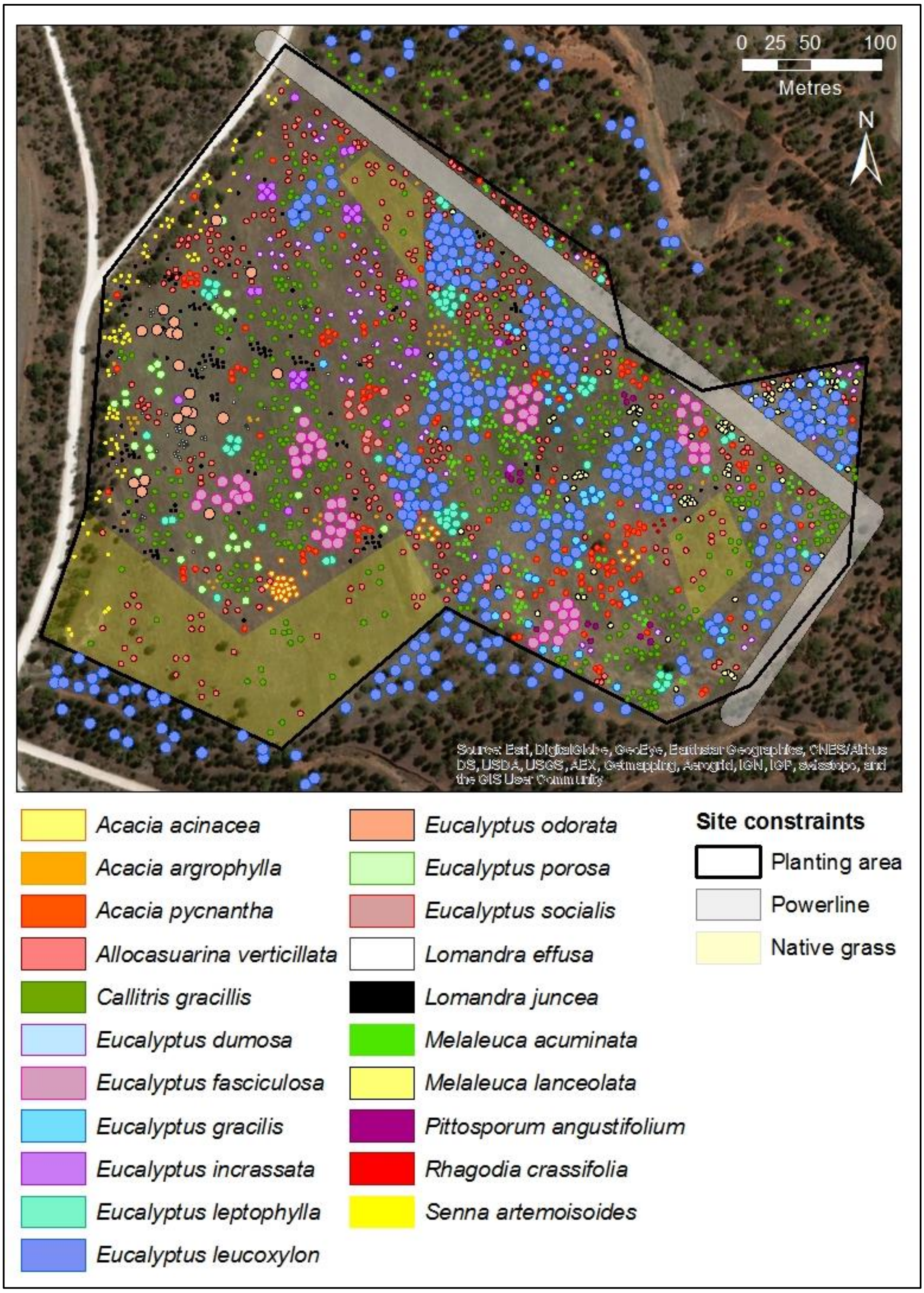


Figure 4. Frahn’s Farm revegetation design, showing the planting position of every tubestock individual. Each colour combination represents a different species. The size of each circle is proportional to estimated canopy width of adult individuals. Grey areas show the position of the powerline, under which planting did not occur. Yellow areas show where native grasses were already established, with minimal planting done into these areas. Some individuals were also planted into previously revegetated areas around creek lines. Imagery: Esri basemaps.

Experimental components

To allow further research into the role of planting arrangement, conspecifics were planted in different sized clusters, at a range of nearest neighbour distances and within different local assemblages of species. As species reach reproductive maturity, the influence of spatial arrangement on reproductive output can be assessed for a range of species, similar to that undertaken for *Eucalyptus* species (McCallum *et al.* 2018a). In addition, the *Eucalyptus leucoxylon* and *E. fasciculosa* seedlings planted form the basis of a long-term study looking at whether floral resources can be manipulated in revegetated systems, based on the flowering times of mother plants, with the aim of creating year-round floral resources (Merigot & Paton 2018).

On-ground works

The site was divided into 87, 50 m by 50 m (0.25 ha) grid cells to guide on-ground plantings (Fig. 5), and the corners of each of these cells was marked with a labelled wooden stake, so cells could be identified in the field. Contractors were hired to spot spray the area and dig individual holes for each tubestock. Spraying was undertaken 3-4 weeks before the planting day and the digging of holes was completed within the week of the main planting day. Contractors were supplied with maps detailing the position of every hole and the number of holes required for each planting grid cell. Tubestock were organised into trays based on the number and identity of seedlings required for each of the grid cells the day before the main planting event.

The planting day occurred on 18 June 2017, with approximately 100 volunteers assisting for 6 hours (10 am – 4 pm). At the start of the planting day, trained ecologists laid out the tubestock (a tubestock placed in/near each hole), for each of the grid cells according to the planting design (Fig. 5). Volunteers then followed planting tubestock, watering and putting up guards. A second planting day was required (ca 10 hours, 6 people) to complete the planting and guarding of all seedlings.

Outcomes

Much of the planting area consisted of hard, rocky soils, particularly around the hilltop (Fig. 6), so the position of holes varied from those mapped because of these constraints. Spot spraying was undertaken 2-3 weeks before holes were dug, so when the position of holes changed, the benefits of spraying were subsequently lost. Much of the site was covered by dry grass (Fig. 6) and this made it difficult to locate the holes, especially when the positions differed from those planned and sprayed. As a result, the focus during the on-ground plantings shifted towards planting individuals of the same species in adjacent holes, with cluster sizes similar to those mapped, but the distribution and exact location of clusters differed from the original design.

We estimated that it required an additional 2 - 2.5 minutes per seedling to complete a design of this nature, although it is not possible to determine the extra time required exactly. This included three days to complete the revegetation design and planning process (24 hrs, 1 person), one day to organise the seedlings (8 hrs, 2 people) and half a day extra to layout the seedlings (4 hrs, 4 people).

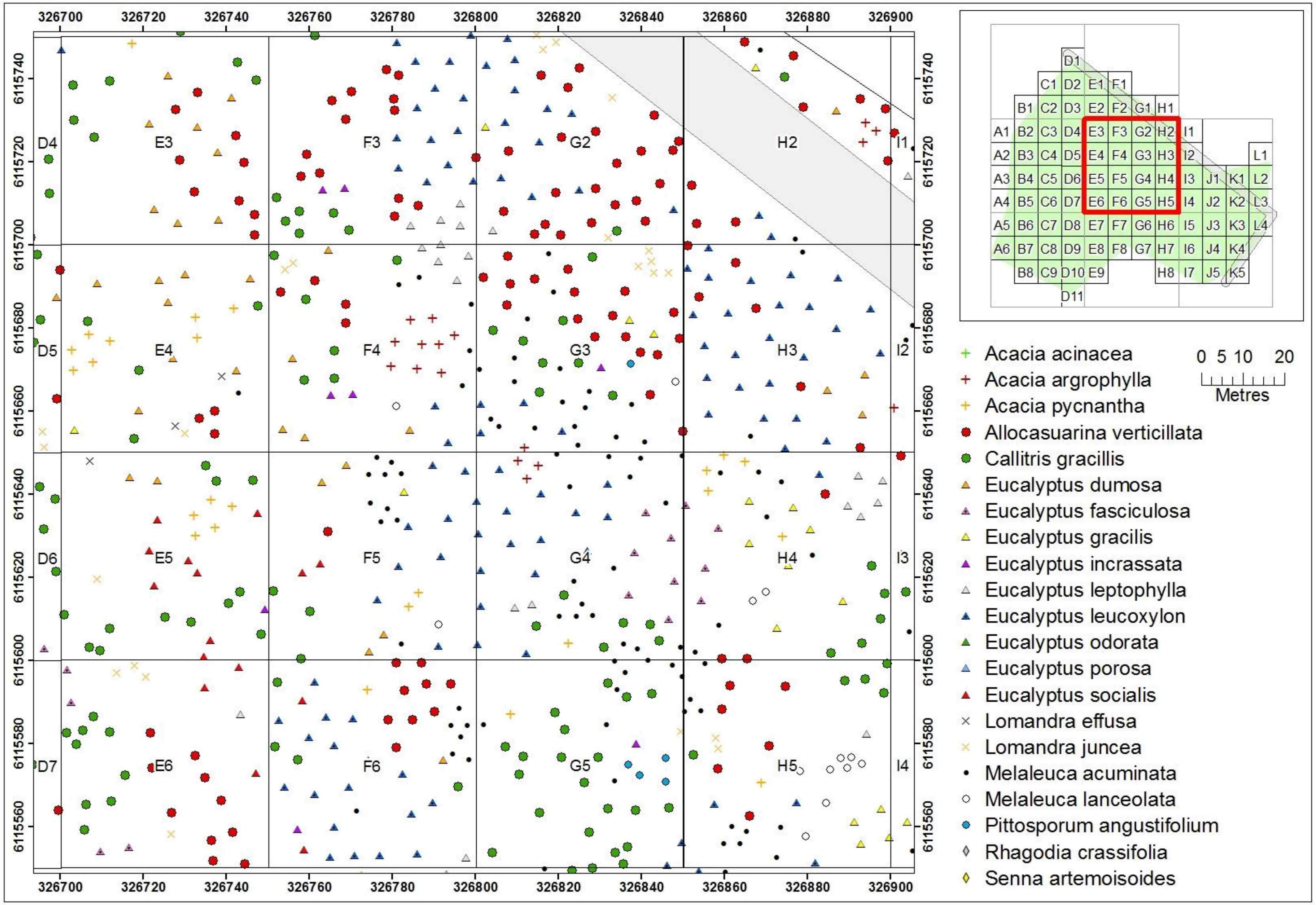


Figure 5. An example of the map used to position the tubestock seedlings. Black lines show the boundary of each 50 m by 50 m grid cell. Each coloured symbol represents a different species and labels (e.g. E3) represent the unique code for each grid cell.

In addition to this, extra time was needed by the contractors to prepare the site and dig the holes (total contractor time = 60 hours, we estimate that 20-30 hours of this time was required to create a patchy arrangement of holes).

Figure 6. The Frahn’s Farm 2017 revegetation area at the completion of planting. At this site, 2400 tubestock seedlings from 21 species were planted into individual holes. Site constraints including hard rocky soil and long, dry grass increased the effort required to revegetate the site. [Photo credit Kimberly McCallum, June 2017].

The main issue that hindered the execution of the design was locating the holes and matching the holes to the plan (especially when the position of holes differed from those mapped). The methods we used could be improved if holes are dug first, the GPS position of each hole recorded, and the planting design then tailored to the position of these holes. This method would also reduce the need to survey the area after planting. Alternatively, the simplest option may be to divide the revegetation site into planting zones, select the most suitable species and relative abundance of those species for each zone, and then create clustered arrangements by planting conspecific tubestock in clumps without a detailed planting map. However, this option would require a detailed survey during/after the planting to record the position of every seedling, either across the entire site or in specific monitoring plots. This survey data could be used to monitor plant growth, seed production, pollination and recruitment as a function of planting arrangement as the vegetation matures.

Several other small refinements can also be made to the technique used. We recommend the design should start by setting up the 50 m by 50 m grid cells and then positioning conspecific clusters within these cells, rather than overlaying the grids after the design has been finalised. At Frahn’s Farm, the numbers of the grid cells did not line up across the site due to the shape of the planting area (e.g. A1 borders B2, Fig. 5). Aligning the grid cells so that numbers line up across the grid (e.g. A1 borders B1), would make it easier to orientate in the field. In addition, if conditions permit, we recommend laying out the tubestock on the afternoon before the planting day and recording the GPS position and identity of seedlings as they are positioned.

Discussion and conclusions

We show that with a detailed planning process, it is possible to create more natural, aggregated arrangements during revegetation, albeit with some on-ground implementation constraints. A design of this nature required more effort, but it is a relatively small increase in comparison to the time and resources needed to complete a revegetation project (i.e. seed sourcing, seedling propagation, site preparation and planting, watering and guarding, volunteer recruitment). In addition, creating more natural plant arrangements has the potential to improve the resilience, sustainability and habitat value of revegetated sites and if this occurs, revegetated areas will require less work to maintain in the long-term. Similar trials are now required to determine if comparable outcomes can be obtained with direct seeded sites.

Frahn’s Farm is being revegetated in stages, with the 2017 planting the second of five stages. Therefore, the approach developed here can be refined during the subsequent plantings. Furthermore, experiments embedded into the Frahn’s Farm revegetation will allow the site to act as a long-term and large-scale experiment, helping to inform future revegetation works in the region.

Acknowledgements

We thank Succession Ecology for site preparation at Frahn’s Farm. Kylie Moritz (Natural Resources SAMDB) for helping plan and undertake the revegetation, all the volunteers who assisted on the planting day. Hayley Merigot for growing the *E. leucoxylon* and *E. fasisculosa* seedlings and providing feedback on the manuscript. Finally, thank you to the members of BioR and all the volunteers who gave up their time planning and undertaking the revegetation.

Chapter 8. General discussion

Globally, two-thirds of terrestrial ecosystems have been degraded and as a result huge targets have been set to restore areas of land (Suding 2011). One area that is a priority for restoration is the temperate *Eucalyptus* woodlands of southern Australia (Yates & Hobbs 1997). To manage biodiversity across this region it has been estimated that native vegetation cover needs to be increased to 30% (Smith *et al.* 2013), and to achieve this over 6 million hectares of good quality revegetation is required (Freudenberger 2018).

However, rebuilding ecosystems is challenging (McDonald *et al.* 2016b), and as a result many revegetation projects fall short of establishing systems with similar levels of ecosystem function to natural vegetation (Menz *et al.* 2013; McDonald *et al.* 2016b; Miller *et al.* 2017). It is therefore important that we learn as much as possible from established plantings so methods can continue to be improved (Broadhurst *et al.* 2017a). However, some evidence suggests that revegetation will be more successful if based on a knowledge of natural communities and ecological processes (Bennett *et al.* 2000; Prach *et al.* 2001; Bartha *et al.* 2004).

In natural plant communities, the spatial arrangement of plants influence a range of ecological processes, including plant growth and survival, competition, pollination, seed dispersal, herbivory and water infiltration (Dale 1999; Murrell *et al.* 2001; Stoll & Prati 2001; Bautista *et al.* 2007). Therefore, creating more natural plant arrangements during revegetation has been promoted as a way of improving the functional value of restoration plantings (Sluis 2002; McCallum *et al.* 2018a), but planting in this way rarely occurs and the outcomes of failing to do so are poorly understood. Considering this, the central question posed by my thesis was - “Can manipulating the spatial arrangement of plants improve revegetation outcomes?”

An active area of research is addressing the role of planting arrangement in revegetation, and many research gaps remain, particularly regarding pollination. In natural systems, closely spaced plants often receive higher quantities of outcrossed pollen and a greater diversity of pollen than more dispersed individuals, increasing seed production, seed viability and offspring fitness (Butcher *et al.* 2005; Breed *et al.* 2014). I hypothesised that similar trends would occur in revegetated systems and this would affect the reproductive performance of planted populations.

My experimental results support this hypothesis with the spacing between conspecifics and the degree of aggregation influencing seed production, pollination, plant mating patterns and pollen flow distances in the revegetated Monarto Woodlands. Reproductive output was highly variable, but despite this variability, aggregated *Eucalyptus* trees and/or those with a near neighbour (≤ 20 m) produced more seeds per fruit on average, than dispersed individuals (Chapters 3 & 4). Germination rates were similar between aggregated and dispersed trees, which indicates that outcrossing was possible for all trees, regardless of spatial arrangement. Paternity analysis confirmed extensive pollen flow (up to ca 2000 m), with all *E. leucoxylon* trees producing some outcrossed seed (27-100%; Chapter 5). However, despite such extensive pollen flow there was evidence of some pollination limitation (Chapter 4), which suggests that pollinators are able to disperse pollen widely but at times are insufficient to deliver full pollinator services for the plants in this system.

In light of my findings, greater consideration of planting arrangements in projects seeking to revegetate *Eucalyptus* woodlands, especially nearest neighbour distances and the degree of aggregation, can improve reproductive fitness in restored populations, but will only be worthwhile if revegetated systems attract and sustain adequate numbers of pollinators. Aggregation is common and large distances between conspecifics are rare in natural systems, so natural plant arrangements may be an effective guide for revegetation designs (Chapter 6). With a more detailed planning processes and a little extra effort during on-ground works I show that it is possible to manipulate the arrangements of species during revegetation (Chapter 7); on-going research is now required to assess the functional development of sites planted in this manner.

Practical recommendations

Seed production was highest in the eucalypt species studied when there was another conspecific within 20 m and, although extensive pollen flow was recorded for *E. leucoxylon*, pollen flow occurred most frequently between individuals spaced 10-20 m apart. In contrast, population abundance had little influence on seed production, so using a standard number of plants per hectare to guide revegetation may limit the reproductive performance of revegetated populations.

In *E. leucoxylon*, it was estimated that up to 15 fathers contributed to the seed crops of individual mother trees (Chapter 5), so clustering several individuals together with 10-20 m spacing can promote outcrossing and seed production in this species. It has been suggested that a density of 30-40 trees per hectare can help avoid inbreeding in eucalypts (Prober & Brown 1994), so it is likely that the best outcomes will be achieved if both the number and arrangement of individuals within a populations are considered.

Clustering seedlings from a range of different mother trees can randomise the spatial genetic structure of revegetated populations, minimise bi-parental inbreeding and increase the diversity of pollen received (Ritchie & Krauss 2012; Ritchie *et al.* 2017). Therefore, it is possible to control both the spatial arrangement and genetic structure of populations during revegetation with the aim of increasing reproductive fitness.

However, spatial aggregation will only be effective if there are enough pollinators in the system and those pollinators deliver an adequate quantity and diversity of pollen (Chapter 4). Consequently, revegetation designs should also consider the needs and foraging behaviour of pollinators (Dixon 2009; Catterall 2018). Pollinator diversity tends to be positively associated with the richness and abundance of the floral resources available (Hegland & Boeke 2006). Therefore, creating structurally diverse systems that include both a wide diversity of plant species and species that provide large quantities of nectar and/or pollen has the potential to attract and sustain pollinators in revegetated systems (Menz *et al.* 2011; Munro *et al.* 2011; Cusser & Goodell 2013; Gross 2017).

Temperate eucalypt woodlands are known for their structural complexity and high diversity, resulting from a patchy distribution of canopy trees and a wide variety of understorey shrubs (Hobbs & Cramer 2003). Therefore, re-creating aspects of natural communities may improve the habitat value for pollinators and facilitate cross-pollination. In the *E. leucoxylon* woodlands studied aggregation was common and admixture of different species was rare. Therefore, revegetation designs that create a patchy distribution of eucalypts, supplemented with conspecific clusters of mid- and understorey species should improve both the habitat value for pollinators and the reproductive fitness of the re-instated plant populations.

Limitations and further research

The research presented here was largely carried out in the Monarto Woodlands and this is a unique system because of the high diversity of species used (ca 250 sp.). Many older plantings only contain one or a few species of trees (Yates & Hobbs 1997; Paton 2000; Vesk *et al.* 2008), so it is unlikely that the results observed here will hold true in other revegetation of a similar age. However, creating diverse systems that include a range of trees, shrubs and ground covers is now widely promoted for revegetation (McDonald *et al.* 2016a; Cuneo *et al.* 2018). As a result, revegetation projects can include over 100 different species (e.g. Jonson 2010; Haby & Klein 2012), and this may result in conspecific plants occurring within a matrix of other species, similar to that of the Monarto revegetation. Consequently, the results presented here may be more applicable to recent (and future) revegetation, but may give little insight into the processes occurring within older, lower diversity plantings.

Assessment of seed production (seed number per fruit) was central to my thesis, with seed production data presented in chapters 3-5. These data sets were highly variable with mean seed number per fruit ranging from ca 0-50 seeds between study trees and varying by up to 30 seeds per fruit within individual trees across years. In general, these data was more variable for aggregated or closely spaced trees (ca 0-50 seeds/fruit), and decreased as arrangements became more dispersed (ca 0-5 seeds/fruit). Consequently, aggregation gave trees the chance of producing high numbers of seeds per fruit, but did not guarantee it; and this suggests that variables apart from spatial arrangement also play important roles in eucalypt reproduction.

In natural systems, reproduction in eucalypts has been shown to be more resistant to the impacts of habitat fragmentation than other species, due to large floral displays, long generation times, strong outcrossing and regular long-distance pollen flow (Byrne *et al.* 2008; Ottewell *et al.* 2009; Breed *et al.* 2015b). Reproduction in the eucalypts studied here was influenced by planting arrangement (to a degree), so it is likely that similar trends will also be evident in a range of other species and may be more pronounced in some of those species (particularly insect pollinated species). Further research is now required to determine if the trends observed here hold true for other species and systems.

In the Monarto Woodlands, conspecifics generally occurred within a matrix of other species, such that the nearest flowering neighbour was often a different species and this may have facilitated pollinator movements between species (Kunin 1993; Kunin & Iwasha 1996). Pollinator visits to specific plant species depend on the attractiveness and abundance of that species but also the abundance and attractiveness of co-flowering neighbours (Làzaro *et al.* 2009; Seifan *et al.* 2014). Consequently, co-flowering plant species may compete with or facilitate each other and influence each other’s pollinator visits (Thomson 1982; Làzaro *et al.* 2009). It is therefore important to extend this work to community level arrangements and undertake observations of pollinator foraging behaviour to better understand the role of planting arrangements on pollination.

The data presented in Chapter 4 highlights that seed production is influenced by differences in flowering intensity across years, so it is likely that plant mating patterns and patterns of pollen flow will also vary across years. Unfortunately, paternity analysis could only be undertaken on seed from one year (2015, low flowering intensity), so it is unclear whether the trends seen here are consistent across years. Potentially, pollen flow in 2015 may have been more extensive than other years because fewer trees flowered and those that did flower produced fewer flowers, most likely resulting in pollinators foraging over larger areas.

The high costs associated with genetic analysis limited the amount of analysis that could be undertaken, with genotyping only completed for ≤ 15 seedlings per mother (individual trees can produce thousands of seeds each year) and only a portion of potential fathers (ca 15% sampled). As a result, the genetic analysis only provides a potentially limited insight into the plant mating patterns and patterns of pollen flow within the revegetated *E. leucoxylon* population, which may vary substantially between individual trees and years.

Genetic analysis was undertaken on seedlings and not seed, therefore assessments of mating systems and patterns of pollen flow were based on the seeds that could be germinated and grown into seedlings. If inbred seed did not germinate or had poor survival, this could sway the results towards higher levels of outcrossing. In addition, there were many unsampled trees in the study area (only ca 15% of potential fathers sampled), and this may have resulted in seedlings being incorrectly assigned if an ungenotyped plant was the true sire. Therefore, actual patterns of pollen flow may differ to a degree from those presented here.

Mating systems and pollen dispersal are affected by the size, density and spatial genetic structure of populations (Sampson *et al.* 2016). Spatial genetic structure in natural populations generally results from limited seed or pollen dispersal, causing neighbouring individuals to be more similar genetically than individuals from further away (Vekemans & Hardy 2004; Sampson *et al.* 2016). Revegetation activities generally disrupt the spatial genetic structure of populations and this can have implications for mating within these populations by reducing bi-parental inbreeding and increasing pollen diversity (Ritchie & Krauss 2012; Ritchie *et al.* 2017). As a result, it is likely that mating between near neighbours will have different outcomes for individuals in natural and restored populations, but the extent of this is unclear. Further research in revegetated sites to assess how planting arrangement and a lack of spatial genetic structure influence plant reproduction is needed and if both these measures can be manipulated to improve plant reproductive fitness.

It was not possible to control the arrangement of trees used in this research because of the extended time period required for eucalypts to reach reproductive maturity. As a result, it was difficult to sample trees evenly across the nearest neighbour and aggregation gradients because isolated trees (> 50 m) were uncommon, and this is a concern because these isolated trees had a large influence on the trends observed. If similar research is undertaken on species with shorter generation times, the potential exists to control planting arrangements (i.e. different isolation distances, conspecific pairs, linear arrangements, different cluster sizes, seedlings from a range of mothers), and set up replicated experiments. Alternatively, experiments can be incorporated into revegetation designs, allowing plantings to act as long-term and large-scale experiments. This will require a greater investment of time and resources into the initial planting but will allow the development and function of revegetated areas with different spatial arrangements to be monitored and analysed overtime (Chapter 7).

In the natural woodlands studied it was common to see trees and shrubs of various sizes and age classes (Chapter 6). Recruitment is an important process in natural woodlands and it contributes to structural diversity and allows the on-going persistence of species (Hobbs & Cramer 2003; Gibson *et al.* 2012). In the Monarto Woodlands, eucalypt species have been flowering and setting fruit for at least 20 years, but recruitment is rare (Paton *et al*. unpubl. data), and a lack of recruitment has also be observed in other revegetated systems (Schneemann & McElhinny 2012; Neldner & Ngugi 2017).

Monarto was planted with little consideration of spatial arrangement, but trees still have the ability to produce good quality seed and that seed can germinate relatively quickly, so it is likely that other factors apart from planting arrangement also limit recruitment. Recruitment failure can be the main driver of species loss from communities (Gibson *et al.* 2012), so more research into the recruitment dynamics of revegetated woodlands is needed if self-sustaining systems are to be created. Many eucalypts and other Australian woodland species require some form of disturbance to either trigger germination or to create recruitment gaps (Yates *et al.* 1994; Schneemann & McElhinny 2012). Therefore, on-going management of revegetated sites, including disturbance (e.g. fire or scalping) may be required to facilitate regeneration (Schneemann & McElhinny 2012).

The natural plant arrangement surveys were completed across a range of sites within the Mt Lofty and Southern Flinders Ranges, but surveys were restricted to areas of intact vegetation within the reserve system. This region has been extensively cleared of native vegetation, particularly on the more fertile plains, so the vegetation surveyed may differ in composition and arrangement from the vegetation which would have once covered these areas – the areas that are now a focus for revegetation. Therefore, there may be some disconnect between the vegetation available to guide revegetation and the vegetation that would be most suitable for the areas in need of restoration.

The value of the natural arrangement survey data could be improved if additional analysis was undertaken linking vegetation patterns to local environmental conditions, such as small scale changes in soil type or slope. It is important that the drivers of natural spatial arrangements are understood otherwise there is a risk of forcing specific arrangements on species when they may not occur in that manner under the conditions present. In addition, disturbance is an important predictor of spatial arrangement in natural systems, so more research into the disturbance history of survey sites is needed to better understand the arrangements observed.

A potential limitation of this research is the focus on re-creating more natural plant arrangements because it is unclear whether natural arrangements are indeed optimal. The results observed here suggest that re-creating more natural arrangements should be more effective than continuing to plant in an *ad hoc* manner; but more research is needed to determine arrangements that maximise the functional outcomes for revegetation. In addition, on-going assessment is required to determine if the benefits of manipulating planting arrangements outweigh the additional costs of revegetating in this way.

Concluding remarks

Spatial arrangement is a key feature of natural plant communities and influences the majority of ecological processes that occur. In spite of this knowledge, the position of plants within revegetated sites are rarely considered an important characteristic of restoration plantings and this has the potential to influence how revegetated woodlands function and develop.

My work shows that planting arrangement can influence seed production, pollination, outcrossing rate and patterns of pollen flow in a revegetated eucalypt woodland, with the distance between conspecifics a key predictor of seed production and outcrossing rate. In contrast, population abundance had little influence on reproduction, so basing revegetation designs solely on the number of plants/species/hectare to be planted may limit the ecological value of revegetation projects.

Creating more natural plant arrangements during revegetation, where aggregation is common and intermixing of different species is rare, has the potential to improve the reproductive fitness of revegetated eucalypt populations. In the natural *Eucalyptus* woodlands studied, aggregation was most pronounced at distances < 10 m, so re-creating more natural arrangements will require a commitment to fine-scale planning.

The challenge is now to find simple and effective ways to manipulate planting arrangements during on-ground works and then monitor the functional development of these sites.

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Appendix 1. Supplementary material for Chapter 3

Table S1. P values for negative binomial generalized linear model for 2016 seed production data for the six eucalypt species. NS represents non-significant terms that were removed from the final model. P values are listed for all variables used in the final models. Superscript numbers rank the significant terms based on the amount of variance explained.

Species	Nearest neighbor	Abundance	DBH	Health	Fruit crop
E. caesia	$< 0.0001^{1}$	NS	NS	$<0.001^{2}$	NS
E. incrassata	$<0.1^{1}$	NS	NS	NS	$<0.1^{2}$
E. leucoxylon	$< 0.01^{1}$	NS	$< 0.05^{2}$	NS	NS
E. platypus	$< 0.001^{1}$	NS	$<0.05^{4}$	$<0.01^{3}$	$<0.0001^{2}$
E. stoatei	$< 0.001^{1}$	NS	NS	NS	$<0.05^{2}$
E. woodwardii	$<0.05^{1}$	NS	NS	NS	NS

Table S2. Evidence for the relative importance of different nearest neighbor distances (1st to 5th nearest neighbor) to seed number per fruit for the six eucalypt species studied and the four seasons of *E. leucoxylon* data. The index of the relative importance of predictor variable *i* (AIC_i) is the sum of Akaike weights (*w*AIC) over all models that include predictor *i*. This importance weight gives evidence for how strong the support is for each predictor variable, regardless of whether the predictor is in the best-fitting model or not. * denotes the strongest predictor for each data set.

Species	Year collected	AIC*i*				
		NN1	NN2	NN3	NN4	NN5
Eucalyptus caesia	2016	0.38	0.59*	0.54	0.45	0.28
Eucalyptus incrassata	2016	0.77*	0.34	0.32	0.27	0.19
Eucalyptus leucoxylon	2014	0.81*	0.23	0.22	0.36	0.30
Eucalyptus leucoxylon	2015	0.27	0.25	0.38*	0.28	0.26
Eucalyptus leucoxylon	2016	0.22	0.32	0.33	0.92*	0.88
Eucalyptus leucoxylon	2017	0.38	0.59*	0.54	0.45	0.28
Eucalyptus platypus	2016	0.68*	0.37	0.29	0.36	0.36
Eucalyptus stoatei	2016	0.95*	0.30	0.28	0.24	0.18
Eucalyptus woodwardii	2016	0.49	0.44	0.39	0.57*	0.34

Table S3. P values for negative binomial generalized linear models for *Eucalyptus leucoxylon* seed production data across the four sampling years. NS represents non-significant terms that were removed from the final model. P values are listed for all variables used in the final model for each species. Superscript numbers rank the significant terms based on the amount of variance explain.

Year Collected	Nearest neighbor	Abundance	DBH	Health	Fruit crop
2014	$<0.001^1$	NS	NS	NS	NS
2015	$<0.05^1$	NS	NS	NS	NS
2016	$< 0.01^1$	NS	$< 0.05^2$	NS	NS
2017	$<0.001^1$	NS	NS	NS	NS

Appendix 2. Supplementary material for Chapter 5

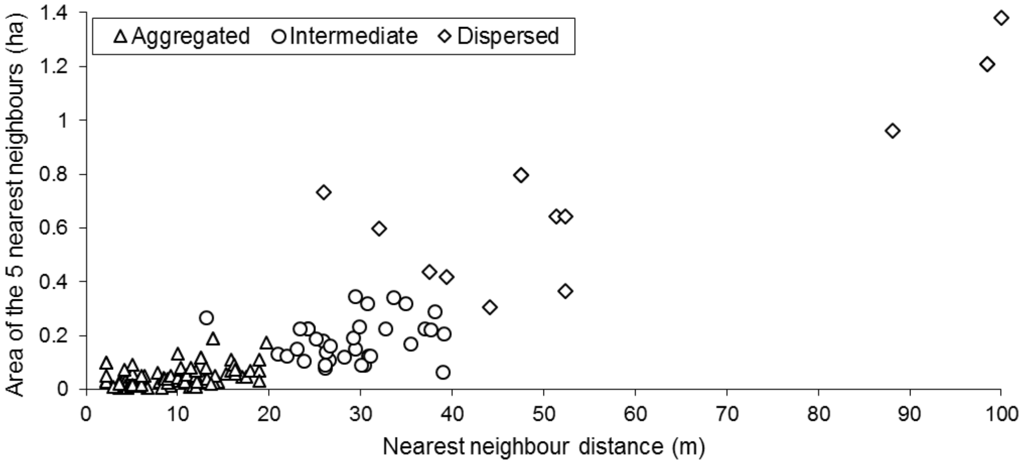

Figure S1. The originally sampled 148 *Eucalyptus leucoxylon* individuals and the allocation to the three aggregation classes based on the nearest neighbour distance (m) and the bounding area to the nearest five neighbours for each tree.

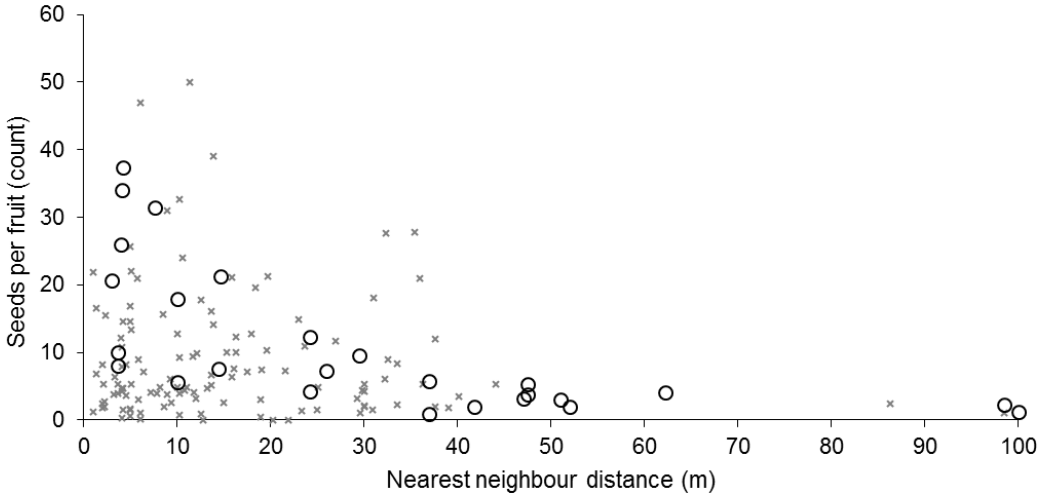

Figure S2. The originally sampled 148 *Eucalyptus leucoxylon* (x) and the subset of those that were selected for genetic analysis (o). Genetics trees were selected across the nearest neighbour distance and seed production (seeds per fruit) range available.

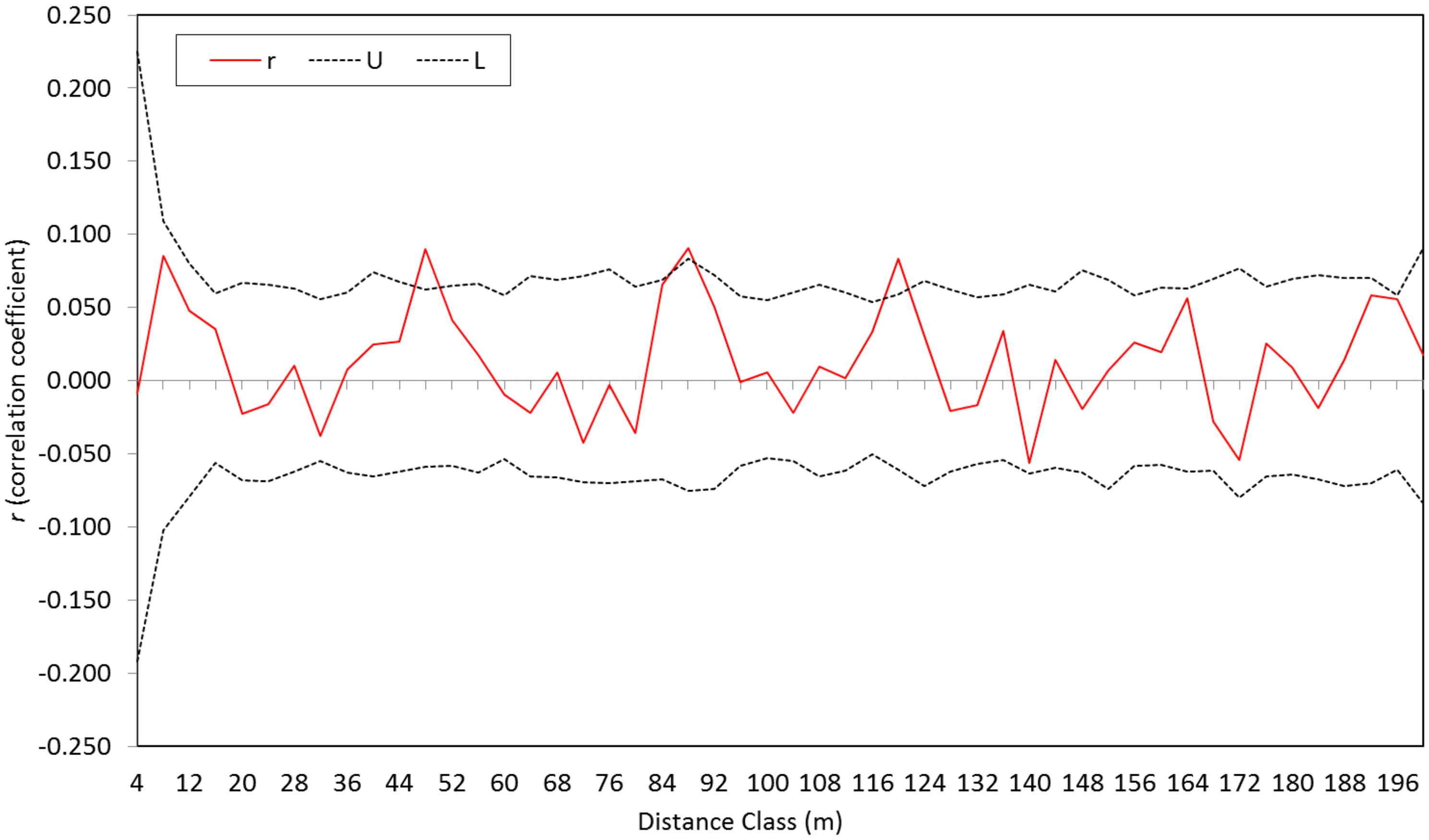

Figure S3. Spatial genetic structure (SGS) of sampled *Eucalyptus leucoxylon* within the revegetated area, using distance classes of 4 m with 50 classes in total. *r* is the calculated correlation coefficient (red line) and the dashed black lines are the 95% confidence intervals. The area in-between the confidence limits represents no significant genetic structure.

Table S4. Reproductive output patterns investigated with general linear models, using single predictor variables. Seed – seed number per fruit, G – percent of seeds germinated after 14 days, NND – nearest neighbour distance (m), NN5A – bounding area of the five nearest neighbours (ha), t_m – outcrossing rate. % DE - percent deviance explained by the model; ΔAIC_c - indicator of difference between model Akaike's Information Criterion corrected for small samples sizes (AIC_c) and the minimum AIC_c in the model set; *w*AIC, weight that show the relative likelihood of model *j*; *k*, the number of parameters; only models with a ΔAIC_c less than the null model (~ 1) are shown.

Model	% DE	ΔAIC*c*	*w*AIC	*k*
Seed production (Seed)				
Seed ~ NND	53.39		0.71	2
Seed ~ NN5A	50.02	1.82	0.29	2
Seed ~ t_m	16.50	15.16	0.00	2
Seed ~ 1	0.00	17.49	0.00	1
Germination (G)				
G ~ NND	22.01		0.60	2
G ~ NN5A	14.96	2.25	0.20	2
G ~ 1	0.00	4.11	0.08	1

Table S5. Reproductive output patterns investigated with general linear models. Seed: seed number per fruit, G: percent of seeds germinated after 14 days, NND: nearest neighbour distance (m), NN5A: bounding area of the five nearest neighbours (ha), t_m: outcrossing rate, t_m- t_s: bi-parental inbreeding and r_p: correlated paternity. % DE: percent deviance explained by the model; ΔAIC_c, indicator of difference between model Akaike's Information Criterion corrected for small samples sizes (AIC_c) and the minimum AIC_c in the model set; *w*AIC, weight that show the relative likelihood of model *j*; *k*, the number of parameters; only models with a ΔAIC_c greater than the null model (~1) are shown.

Model	% DE	ΔAIC*c*	*w*AIC	*k*
Seed production (Seed)				
Seed ~ NND	53.39		0.26	2
Seed ~ NN5A	50.02	1.82	0.11	2
Seed ~ NND + NN5A	54.17	2.13	0.09	3
Seed ~ NND + t_m	53.93	2.27	0.08	3
Seed ~ NND + r_p	53.93	2.27	0.08	3
Seed ~ t_m- t_s + NND	53.52	2.50	0.07	3
Seed ~ NN5A + t_m	52.03	3.32	0.05	3
Seed ~ NN5A + r_p	50.14	4.32	0.03	3
Seed ~ t_m- t_s + NN5A	50.09	4.35	0.03	3
Seed ~ NND + tm + r_p	54.98	4.48	0.03	4
Seed ~ NND + NN5A + t_m	54.88	4.54	0.03	4
Seed ~ NND + NN5A + r_p	54.54	4.74	0.02	4
Seed ~ t_m- t_s + NND + NN5A	54.28	4.89	0.02	4
Seed ~ t_m- t_s + NND + t_m	54.18	4.94	0.02	4
Seed ~ t_m- t_s + NND + r_p	54.15	4.96	0.02	4
Seed ~ NN5A + t_m + r_p	52.79	5.72	0.01	4

Seed ~ NND + NN5A + t_m + r_p	55.72	7.15	0.01	5
Seed ~ t_m- t_s + NND + t_m + r_p	55.57	7.24	0.01	5
Seed ~ t_m- t_s + NND + NN5A + tm	55.13	7.49	0.01	5
Seed ~ t_m- t_s + NND + NN5A + r_p	54.74	7.72	0.01	5
Seed ~ t_m- t_s + NN5A + t_m + r_p	53.39	8.48	0.00	5
Seed ~ t_m- t_s + NND + NN5A + t_m + r_p	56.29	10.23	0.00	6
Seed ~ t_m	16.50	15.16	0.00	2
Seed ~ t_m + r_p	20.69	16.39	0.00	3
Seed ~ 1	0.00	17.49	0.00	1
Germination rate (G)				
G ~ NND	22.01		0.16	2
G ~ NND + r_p	26.51	1.02	0.10	3
G ~ t_m- t_s + NND	26.50	1.03	0.10	3
G ~ t_m- t_s + NND + r_p	32.78	1.52	0.07	4
G ~ NND + NN5A	23.36	2.11	0.06	3
G ~ t_m- t_s + NND + t_m + r_p	38.87	2.14	0.05	5
G ~ NN5A	14.96	2.25	0.05	2
G ~ NND + t_m	22.92	2.26	0.05	3
G ~ NND + t_m + r_p	29.51	2.75	0.04	4
G ~ NN5A + r_p	20.37	3.11	0.03	3
G ~ t_m- t_s + NND + t_m	28.40	3.16	0.03	4
G ~ t_m- t_s + NN5A	19.81	3.29	0.03	3
G ~ t_m- t_s + NND + NN5A	27.76	3.39	0.03	4
G ~ NND + NN5A + r_p	27.27	3.57	0.03	4
G ~ 1	0.00	4.11	0.02	1

Table S6. Genetic output patterns investigated with general linear models, using single predictor variables. NND – nearest neighbour distance (m), NN5A – bounding area of the five nearest neighbours (ha), t_m – outcrossing rate, t_m- t_s – bi-parental inbreeding and r_p – correlated paternity. % DE, percent deviance explained by the model; ΔAIC_c, indicator of difference between model Akaike's Information Criterion corrected for small samples sizes (AIC_c) and the minimum AIC_c in the model set; *w*AIC, weight that show the relative likelihood of model *j*; *k*, the number of parameters; only models with a ΔAIC_c less than the null model (~ 1) are shown.

Model	% DE	$\Delta AICc$	*w*AIC	*k*
Outcrossing rate (t_m)				
t_m ~ NND	20.83		0.67	2
t_m ~ NN5A	14.05	2.14	0.23	2
t_m ~ 1	0.00	3.72	0.10	1
Bi-parental inbreeding (t_m- t_s)				
$t_{m} - t_s$ ~ 1	0.00		0.61	1
Correlated paternity (r_p)				
r_p ~ 1	0.00		0.61	1

Table S7. Evidence for the relative importance of spatial factors to genetic output measures. The index of the relative importance of predictor variable *i* (AIC_i) is the sum of Akaike weights (*w*AIC) over all models that include predictor *i*. This importance weight gives evidence for how strong the support is for each predictor variable, regardless of whether the predictor is in the best-fitting model or not.

Response variable	Predictor variable	AIC*i*
Outcrossing rate (t_m)	NND	0.73
	NN5A	0.37
Bi-parental inbreeding ($t_{m} - t_s$)	NND	0.24
	NN5A	0.23
Correlated paternity (r_p)	NND	0.25
	NN5A	0.24

Appendix 3. Supplementary material for Chapter 6

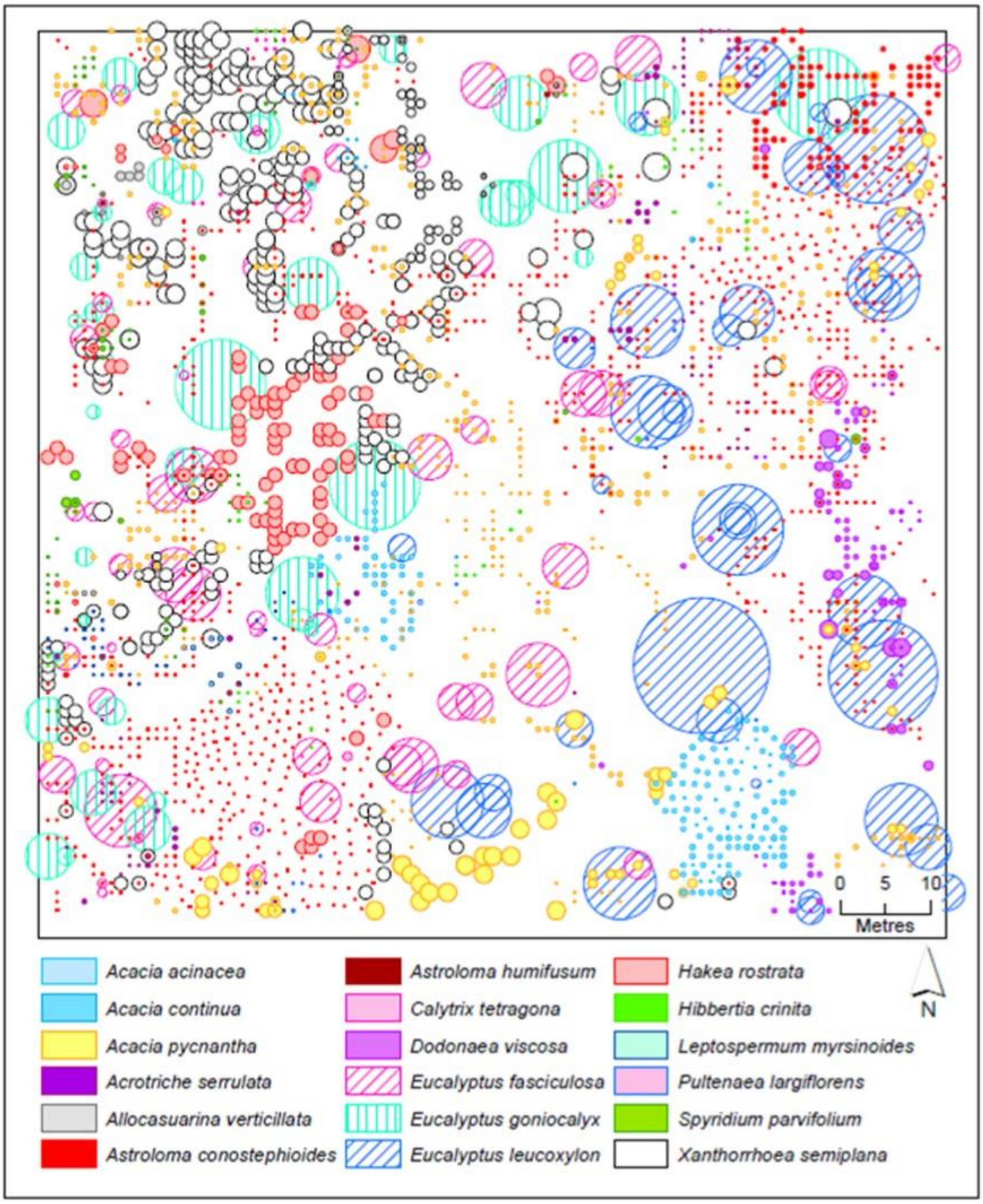

Figure S4. One ha survey plot from Para Wirra, showing species with five or more individuals. Each colour represents a different species and the sizes of the circles are proportional to the canopy width of each individual surveyed. *Eucalyptus* species are represented by hatched circles and all other species by solid circles.

Survey methods and plot size response

With a hand-held GPS, it took two people between 4 and 8 hours to complete a 1 ha quadrat at Dutchmans Stern (ca 400-800 stems), while at Para Wirra a 1 ha quadrat (ca 4000 stems) took 16 hours to complete.

To test for effects of quadrat size, smaller sub-plots (10 m x 10 m to 90 m x 90 m) were set up within all the eight, 1 ha plots. Nested sub-plots from the plot centre-point were used to test how the number of species detected changed with plot size. To calculate nearest neighbour distances, subplots were positioned around each species of interest (species with ≥ 20 individuals). Nearest neighbour distances were determined in SpPack as detailed above.

As plot size was increased from 10 m x 10 m (0.01 ha) to 100 m x 100 m (1 ha) the number of species recorded increased from 1.9 (± 1.4 SD) to 15.4 (± 4.1 SD). The largest increase in species number occurred from 10 m x 10 m to 50 m x 50 m (increase from 1.9 (± 1.4 SD) to 11.0 (± 3.4 SD); Fig. S5). The NND was similar for understorey species with increasing plot size. NND increased for midstorey species from 10 m x 10 m to 30 m x 30 m and continued to increase across the plot size range for overstorey species, but the greatest increase occurred from 10 m x 10 m to 30 m x 30 m.

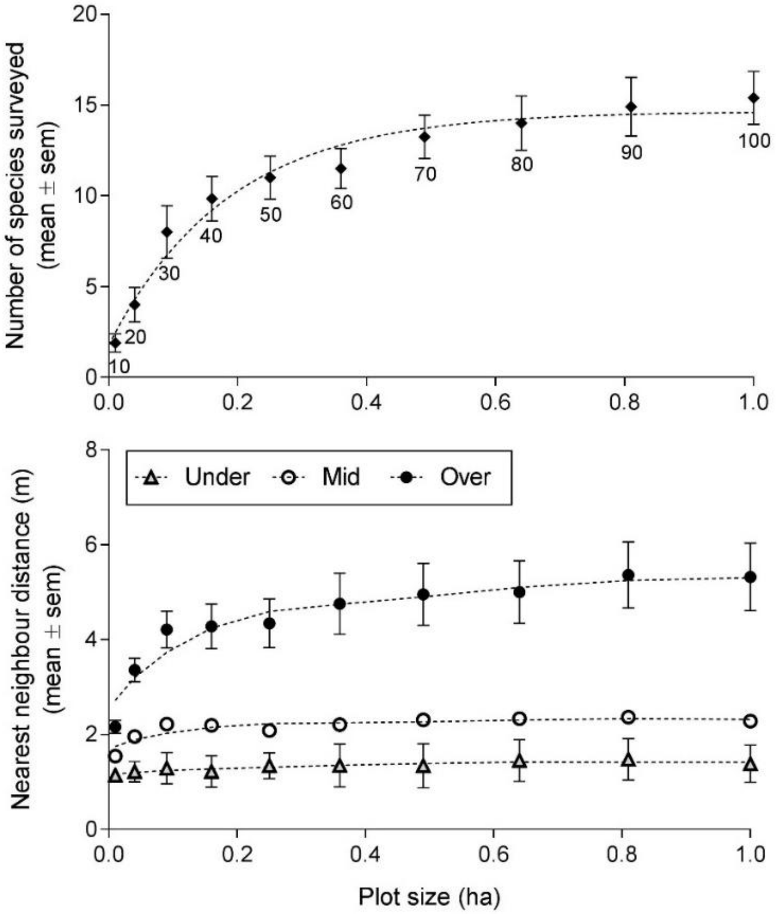

Figure S5. Number of species recorded and nearest neighbour distance (NND) with increasing plot size. Plot width (i.e. 10 = 10 m x 10 m) is also shown in the top figure. Species number is based on nested sub-plots from the plot centre-point for the eight, 1 ha plots. NND calculations are based on targeted subplots for each species with 20 or more individuals in the 1 ha plots.

Although more precise equipment is available (e.g. differential GPS), we show hand-held GPS units are effective in documenting woodland plant arrangements, so are a viable option for this type of research. Survey time can be reduced if smaller quadrats are used but surveying small areas can fail to capture species diversity, may underestimate nearest neighbour distances (particularly for overstorey species), and the data suffers more from edge effects.

Appendix 4. Publications

The following list contains other publications that I have been involved with during my postgraduate studies.

Baruch Z, Christmas M, Breed M, Guerin G, Caddy-Retalic S, McDonald J, Jardine D, Leitch E, Gellie N, Hill K, **McCallum K** and Lowe A (2017) Leaf trait associations with environmental variation in the wide-ranging shrub *Dodonaea viscosa* subsp. *angustissima* (Sapindaceae). *Austral Ecology* **42**: 553-561

McCallum K, Paton D, Breed F, and Lowe A (2018) Re-creating more natural plant arrangements can improve seed production in revegetated woodlands.
Pg 53-54 In:Proceedings of *Restore, Regenerate, Revegetate: A Conference on Restoring Ecological Processes, Ecosystems and Landscapes in a Changing World*. Held at the University of New England, Armidale, from 5–9 February 2017.

In:
Smith, R. (editor) (2018) Proceedings of *Restore, Regenerate, Revegetate: A Conference on Restoring Ecological Processes, Ecosystems and Landscapes in a Changing World*. Held at the University of New England, Armidale, from 5–9 February 2017 (Ecosystem Management, School of Environmental and Rural Science, University of New England, Armidale, New South Wales, Australia).

Available at: https://www.une.edu.au/about-une/academic-schools/school-of-environmental-and-rural-science/ersnews-and-events/restore-regenerate-revegetate-conference-2017

Re-creating More Natural Plant Arrangements Can Improve Seed Production in Revegetated Woodlands

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Abstract

The spatial arrangement of individual plants play a central role in how ecosystems function and influence processes such as growth, competition, pollination, seed dispersal, and water and nutrient cycling. Consequently, the spatial arrangement of plants within revegetated sites are likely to influence the functional outcomes of restoration plantings. There have been calls to base revegetation designs on natural plant arrangements; however this seldom occurs, and the outcomes of failing to do so are poorly understood. The aims of our research were to (1) describe and compare plant arrangements in remnant and revegetated woodlands, and (2) assess how planting arrangement influences the ecological processes occurring in the revegetated site, specifically seed production. We found that aggregation was common in the remnant community and nearest neighbour pairs were often conspecific. While, revegetated sites were generally more dispersed and had greater spatial admixture of species. In revegetated areas, aggregated trees, or those with a neighbouring *Eucalyptus leucoxylon*, produced more seeds per fruit than dispersed trees. Therefore, we recommend that mimicking natural plant community spatial arrangements, where aggregation is common and nearest neighbours are often conspecific, as a guide to planting design, is likely to increase seed production in projects seeking to revegetate woodlands.

Introduction

Revegetation aims to return functional ecosystems to cleared land, but planting designs are often *ad hoc* and ecological outcomes can be highly variable (Hobbs 1993; Sluis 2002). Questions have therefore been raised about the effectiveness of many revegetation attempts. Revegetation success is often measured by the area restored or number of seedlings planted rather than ecological function (Miller *et al.* 2010). Consequently, further research is needed to determine if revegetation simply returns plants to the landscape or whether it creates functional ecosystems (Hobbs 1993). In natural systems, plant arrangements influence processes such as growth, competition, pollination, seed dispersal and herbivory (Miller *et al.* 2010). Therefore, more detailed consideration of plant arrangements may improve ecosystem function in revegetated sites and a potential way to achieve this is to mimic the spatial arrangements of natural plant communities (Miller *et al.* 2010; Sluis 2002).

At this stage, however, plant spatial arrangements are often overlooked as an important feature of revegetated sites and the role that arrangement plays in revegetation is not well understood (Miller *et al.* 2010). Our research aimed to close this gap, by (1) describing the spatial arrangement of remnant and revegetated *Eucalyptus leucoxylon* (F. Muell) woodlands in the Monarto area, South Australia; and (2) exploring the role of planting arrangement on seed production in revegetated *E. leucoxylon*.

Methods

1) A remnant *Eucalyptus leucoxylon* woodland was surveyed (ca. 4 ha) in the Monarto area, South Australia. GPS position, size and species ID of all reproductive *Eucalyptus* were recorded. In the revegetated woodlands (1850 ha, 250 local and introduced species, established in the 1970s), only the GPS position of *E. leucoxylon* individuals were recorded (ca. 800 individuals across 85 ha). ArcGIS was used to map records and determine neighbour distances.

2) Fruit were collected from 75 revegetated *E. leucoxylon*, from four areas within the revegetation. Approximately 40 fruit (2013 flowering season) were collected per tree and the average number of seeds per fruit (seed production) determined for each tree. Seed production was assessed in trees with and without a near neighbour and in dispersed (0 *E. leucoxylon* within 30 m) and aggregated trees (5 *E. leucoxylon* within 30 m), using unpaired t-tests.

Results

Conspecific aggregation and open space were common in the remnant *Eucalyptus leucoxylon* woodland (Fig. 1). Approximately 90% of individuals had a neighbour of the same species and the fine-scale spatial admixture of the three different species was rare (Fig. 1). The distance between remnant *E. leucoxylon* ranged from < 1 to 24 m (mean 4.3 ± 4.7 m), with 95% of individuals having five conspecifics within 30 m. While, in the revegetated sites, *E. leucoxylon* occurred within a matrix of other species, with up to 50 different *Eucalyptus* species occurring together. *Eucalyptus leucoxylon* individuals were more dispersed in the revegetated area, with nearest neighbour distance ranging from 4 to 100 m, (average 18 ± 20 m). Furthermore, only 40% of individuals had an *E. leucoxylon* neighbour and only 50% had five *E. leucoxylon* within 30 m.

Planting arrangement influenced seed production in revegetated *Eucalyptus leucoxylon*. Individuals with a conspecific neighbour (≤6 m) tended to produce more seeds per fruit than those without a conspecific neighbour (conspecific neighbour: 9.6 ± 1.7 (n=30); no conspecific neighbour = 5.9 ± 1.1 (n=45)) (Fig 2A; unpaired t-test: t=1.9, P = 0.06). Aggregated *E. leucoxylon* (5 *E. leucoxylon* within 30 m) tended to produce more seeds per fruit than dispersed trees (0 *E. leucoxylon* within 30 m) (aggregated = 8.6 ± 1.4 (n=38); dispersed = 2.6 ± 1.0 (n=11)) (Fig. 2B; unpaired t-test: t=2.3, P = 0.03).

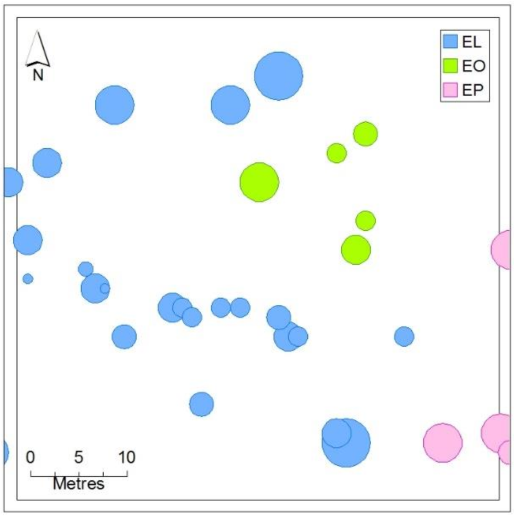

Fig. 1. A 50 m x 50 m survey area of remnant *Eucalyptus leucoxylon* woodland at Monarto, South Australia. The GPS position of all reproductive *Eucalyptus* species are shown, with each colour representing a different species. Circle size is proportional to canopy width. EL = *Eucalyptus leucoxylon*, EO = *Eucalyptus odorata* and EP = *Eucalyptus porosa.*

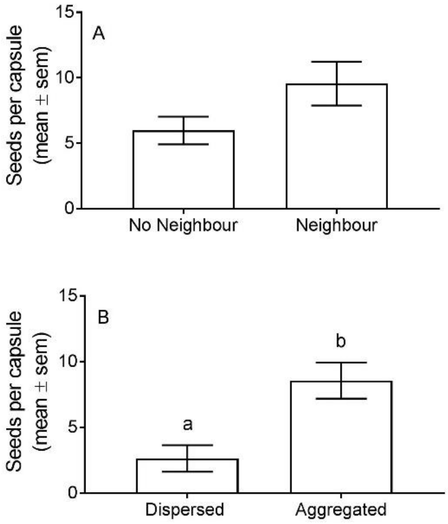

Fig. 2. Relationships between seed production and planting arrangement in revegetated *Eucalyptus leucoxylon* in the Monarto woodlands, South Australia. Top - with and without a conspecific neighbour (another *E. leucoxylon* in the next planting position) and bottom - dispersed (0 *E. leucoxylon* within 30 m) and aggregated (5 *E. leucoxylon* within 30 m).

Discussion

Conspecific aggregation was common and spatial mixing of different species was rare in the remnant woodland (Fig. 1). This contrasts to the random-to-dispersed arrangements observed in the revegetated sites. Revegetated *Eucalyptus leucoxylon* were more dispersed than the nearby remnants, with the average distance between conspecifics 18 m compared to 4 m in the remnant. The revegetated sites also had greater spatial admixture of different species, with *E. leucoxylon* individuals occurring randomly throughout the matrix of other species. As a result, only 40% of nearest neighbours were conspecific in revegetated sites, compared to 90% in the remnant.

The spatial distribution of *Eucalyptus leucoxylon* trees influenced seed production, with aggregated individuals and/or those with a neighbouring *E. leucoxylon* producing more seed than dispersed trees (Fig. 2A, B). Pollinators often move between neighbouring individuals (Hopper and Moran 1981). Therefore, it is likely that aggregated arrangements promoted pollinator movements (and pollen flow) between conspecifics. Greater numbers of pollinator visits tend to increase the amount and diversity of pollen received, which results in increased seed set (Breed *et al.* 2015; Ottewell *et al.* 2009). While, more dispersed or isolated trees often receive fewer pollinator visits and this can lower outcrossing rate. A reduction in seed set or seed quality as a result of lower outcrossing may limit natural regeneration in these dispersed individuals or populations (Ottewell *et al.* 2009).

Natural systems often have aggregated arrangements, with nearest neighbours of the same species and limited spatial admixture of different species (Fig. 1). The arrangements that were common in the remnant (conspecific neighbours, five neighbours within 30 m), were similar to those that improved seed production in the revegetated sites. Consequently, re-creating plant arrangements similar to those in natural systems can improve seed production in revegetated areas and in turn may help these sites to regenerate naturally and become self-sustaining. The position of individual plants can be easily controlled with tubestock plantings, but will be more difficult in broadcast seeded sites. Broadcast seeded sites may require additional hand seeding in patches or supplementary plantings over time to achieve aggregated arrangements (Jonson 2010).

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