BEST PRACTICE RESTORATION:
BUILDING THE EVIDENCE-BASE FOR RESTORING EUCALYPT WOODLANDS OF SOUTHERN AUSTRALIA

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

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ABSTRACT

Approximately a third of the Earth's surface is degraded. The enormous scale of degradation has stimulated multilateral agreements with ambitious restoration targets (e.g. The Bonn Challenge aspires to restore 350 million ha by 2030). Humankind has greater awareness than ever before of the factors contributing to landscape degradation, and has developed sophisticated practices to assist in its repair. The principal management intervention used to combat the biodiversity declines associated with land degradation is restoration. However, unprecedented environmental challenges from climate change, rapid biodiversity loss, and human population pressures add to the complexity of achieving sustainable restoration outcomes.

There are valid concerns that sub-optimal restoration interventions are jeopardizing outcomes, which brings into question our capacity to reach global targets. To establish a strategic approach for improving restoration practice and to promote resilient outcomes, I reviewed current restoration practices and found that the management of plant genetic resources and inconsistent monitoring of projects are key impediments to optimal restoration outcomes. I found a suitable mechanism for investigating these knowledge gaps, through embedded experiments, and subsequently established them in restoration projects.

I addressed the plant genetic resource knowledge gaps by testing *in situ* the relationship between plant fitness and seed origin for six Myrtaceae species. I investigated plant fitness in three empirical studies that included five common garden experiments, from provenances spanning 2.5 degrees of latitude (*ca.* 460 km) in southern Australian eucalypt woodlands, and found sub-optimal plant performance was common. Furthermore, signals of maladaptation occurred in two of my three empirical studies. I determined that the Myrtaceae species I studied persisted in a range of climatic conditions by combining specific adaptations to aridity and acclimating to new environmental conditions via phenotypic plasticity. I confirmed that this response was strongly directional (e.g. arid to mesic), and the genetic diversity harboured in non-local provenances could be harnessed to counteract plant fitness concerns (e.g. adaptation lags due to climate or lack of connectivity due habitat fragmentation), and ultimately help to achieve more sustainable outcomes.

I then explored the utility of high throughput 16S amplicon sequencing (e.g. metabarcoding soil eDNA) as an assessment tool to assist in monitoring restoration performance. I used metabarcoding of soil eDNA to assess a chronosequence of restoration and found that the process of restoration (i.e. revegetation of the native plant community) strongly impacted soil bacteria, an important functional component of the ecosystem. I observed dramatic changes of the bacterial community after eight years of revegetation, where the bacterial communities in younger sites were more similar to cleared degraded land and older restoration sites were more similar to remnant native stands. This work has identified evidence of community flux and functional recovery following restoration that would remain unrecognised through orthodox monitoring.

The synthesis of this work supports the use of evidence-based approaches to iteratively improve restoration practices. Science-practice synergies will come from harvesting the knowledge of these approaches and networking the results more broadly is the most efficient mechanism to achieve best-practice restoration and resilient project outcomes.

DECLARATION

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, 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.

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Nicholas James Court Gellie

Sign Date: 23rd January 2017

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My appreciation is also given to my co-supervisor, Nikki Thurgate the person who encouraged me to pursue this scholarship and therefore is also directly responsible for making it possible. Your guidance, good humour and pragmatism has been valuable throughout my candidature. Martin Breed the third member of my supervisory panel has been an exceptional influence on this body of work. His determination for professional excellence and unabridged mentorship has bolstered my resolve to produce a thesis that I am very proud of completing. I am truly grateful to you for your wise advice, and will reflect with fond memory of the wonderful experiences we have had in the last few years. I can't wait to see what the next chapter will be.

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experience and stimulating important debate that has added to the content of my thesis. I have also been fortunate to work closely with an extended research group at TERN and have immense gratitude for the expertise they have extended to me. During my candidature I was privileged to be exposed to a long list of existing academic collaborators that have substantially contributed to my professional development. The collegial nature of these collaborations and the new friendships forged is testimony to a solidarity in the restoration research community that I am now eager to be a part of.

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CHAPTER 1: INTRODUCTION

Global restoration

The scale of the problem

> *“**In a recent global assessment, roughly a third (i.e. 29%) of all arable land was considered degraded (Nkonya et al. 2016)**. Previous estimates of twice this figure (e.g. 66% Bot et al. 2000; Gibbs and Salmon 2015) reflect the considerable variation in how degradation is defined, used and assessed (Hobbs 2016). However, if this recent conservative estimate by Nkonya et al. (2016) was to be consolidated into one geopolitical boundary, a landmass which we might hypothetically call the ‘Federated States of Degradia’ (Figure 1), this federation would exceed the size of Russia (approx. 2 billion ha). Degradia’s population would include more than 3 billion of the world’s poorest and most vulnerable people (Nkonya et al. 2016) who would be restricted in their economic capacity to deal with the consequences of unsustainable land management on biodiversity and ecosystem services.”*
>
> *(Chapter 2; Gellie et al. 2017 pending revision, Frontiers in Ecology and the Environment)*

The scale of the input

The scientific community has greater awareness than ever before of the factors contributing to landscape degradation and as a result we have developed sophisticated practices to assist in its restoration. To address degradation an unprecedented mobilisation of global resources is now devoted to ecological restoration (e.g. the restoration industry is reported to turnover more than $USD 2 trillion annually; Cunningham *et al*. 2008). However, these enormous investments are being rolled out on a stage with unprecedented environmental challenges, rapid loss of biodiversity, and human population pressures that are all contributing to the complexity of achieving sustainable restoration outcomes.

The enormous scale of land degradation has stimulated multilateral agreements with ambitious restoration targets (e.g. the Convention on Biological Diversity (CBD) and the Aichi Target 15, and the aims of the United Nations Framework Convention on Climate Change (UNFCCC) for Reducing Emissions from

Deforestation and Forest Degradation (REDD+)). More recently commitments during COP21 to *Initiative 20x20* (e.g. the restoration of 20 million hectares of land in Latin America and the Caribbean by 2020) have confirmed restorations growing importance in policy on the world stage.

Current restoration practices

> ***"Ecosystem restoration is increasingly relied upon to combat the global declines in biodiversity, ecosystem services, and land quality*** *(IPBES 2014; Suding et al. 2015). The recent formation of the Intergovernmental Platform on Biodiversity and Ecosystem Services (IPBES) draws attention to the key role restoration has in combatting these global issues. Aspirational goals have been set and agreed to, such as restoring 350 million ha by 2030 at the Sept 2014 UN Climate Summit in New York, requiring substantial investment (Menz et al. 2013). These impressive ambitions will rely on effective restoration practices being employed and the addition of integrated policy support (Suding et al. 2015)."*
>
> *(Chapter 6; Gellie et al. 2017, Molecular Ecology)*

Despite enormous commitments (e.g. the Bonn Challenge and Initiative 20x20) restoration practices operate on a spectrum from near total success to complete failure (Suding 2011), primarily because restoring degraded landscapes is a complicated activity where biotic and abiotic factors can substantially influence outcomes (Bucharova *et al.* 2016). Resolving questions about which species will achieve the desired outcomes (i.e. the species mix), how best to prepare and apply them to the site (e.g. breaking dormancy, herbicides or bio controls), mechanisms of delivery (tube stock, direct seeding, natural regeneration) and the genetic source of the seed to be used often remain unanswered due to time and economic constraints of projects. Therefore, evidence based restoration supported by effective monitoring of performance is required to improve current practice (e.g. the 20 year Banksia woodland post sand mining restoration of the Swan coastal plain provides a good example of industry collaboration and best-practice (Steven *et al*. 2016)).

> ***"A recent meta-analysis of 221 restoration projects found highly variable and, in some cases, suboptimal outcomes*** *(Crouzeilles et al. 2016), corroborating previous studies (Benayas et al. 2009; Moreno-Mateos et al. 2012; Wortley et al. 2013) and policy reviews (Baker & Eckerberg 2016), which together provide clear evidence of deficiencies in current restoration practices."*
>
> *(Chapter 6; Gellie et al. 2017, Molecular Ecology)*

Restoration input

> ***"Seed is the fundamental component of restoration plantings, and choosing the origin of the seed is an early commitment in the restoration process that has important consequences*** *(Hufford and Mazer, 2003; McKay et al., 2005; Broadhurst et al., 2008). Historically, the preferential use of local seed – local provenancing – has been encouraged to optimise restoration outcomes (Hufford and Mazer, 2003; McKay et al., 2005). A local provenancing strategy is assumed to maximise success by preserving local adaptation, but what constitutes a 'local provenance' is not easily defined, so provenancing often defaults to arbitrary spatial boundaries (McKay et al., 2005; Jones, 2013). In addition, assuming a local advantage does not acknowledge the impact of important drivers of ecosystem change on local adaptation (e.g. climate change, habitat fragmentation), which may ultimately limit future restoration success (Godefroid et al., 2011; Breed et al., 2013)."*
>
> *(Chapter 3; Gellie et al. 2016, Biological Conservation)*

Fragmented landscapes can disrupt the gene flow and mating systems of plants, significantly impacting progeny fitness relative to those individuals found in continuous habitats (Breed *et al.* 2015). Furthermore, contemporary climate change is adversely influencing local adaptation in long lived plants (Christmas *et al.* 2015), via changes to selection over short periods of time (Bellard *et al.* 2011), disrupting the adaptive landscape in which plants live. Seed sourcing and provenance strategies that maximise long-term population fitness are vital in order to gain climate change resilience (Breed *et al.* 2013). Though maintaining, or perhaps enhancing, genetic diversity, while conserving local adaptation can be an extremely challenging task (Vander-Mijnsbrugge *et al*. 2010), making it imperative to broaden the scientific foundation supporting this aspect of restoration practice.

__“There has been a recent push to advance the experimental evidence of local adaptation for species commonly used in restoration, as this information will help build the empirical foundation of seed collecting for restoration__ (Breed et al. 2013; Jones 2013; Thomas et al.2014). Developing experimental evidence of local adaptation is a topic of great concern to land managers today due to the risks and uncertainty caused by climate change and its potential impact on restoration success (Harris et al. 2006; Chazdon 2008; Havens et al. 2015).”

(Appendix E; Breed, Gellie and Lowe 2016, Restoration Ecology)

An embedded provenance experiment at *Scotia Sanctuary* near the South Australian-New South Wales border. **Photo credit: Nick Gellie**

Alternative input

*“**Applying principles of evolutionary biology to restoration can improve restoration outcomes** (Mijangos et al. 2015) by, for example, helping to overcome genetic quality issues of seed sourced from fragmented populations or seed that is unable to adapt quickly enough to climate change (Sgrò et al. 2011; Carroll et al. 2014). The genetic consequences of habitat fragmentation are well studied, and fragmented tree populations generally experience increased inbreeding and decreased pollen diversity (Eckert et al. 2010; Breed et al. 2015b; Lowe et al. 2015). Thus, avoiding highly fragmented populations will reduce negative fitness consequences and maintain the adaptive potential of seed used for restoration (Lowe et al. 2005, 2015; Breed et al. 2012).*

Seed sourcing alternatives have been proposed to mitigate the impacts of climate change but require more sophistication than simply avoiding isolated trees. Alternative strategies include translocating genetic material from non-local seed sources (e.g. predictive provenancing) or mixing multiple sources that only sometimes include ‘local’ seed (e.g. climate-adjusted, composite, admixture provenancing; Breed et al. 2013). However these strategies are not universally accepted as they suffer from a lack of empirical studies (Gibson et al. 2016) and the perception of risk of malaptation from translocation (Bucharova 2016).

The theoretical pros and cons of such alternative seed sourcing strategies are well documented (Broadhurst et al. 2008; Crowe and Parker 2008; Sgrò et al. 2011; Breed et al. 2013; Prober et al. 2015). In short, the perceived risks of these climate-ready alternatives include increased risks of outbreeding depression and disruption of local adaptation. With risks of outbreeding depression often overstated, particularly for outcrossing and common species (Frankham et al. 2011), it is reasoned that if local adaptation is already being eroded by climate change, these genetic risks are worth taking (Hoffmann and Sgro 2011; Aitken and Bemmels 2015; Gellie et al. 2016). Despite this ongoing debate, both empirical and theoretical evidence generally supports the need for changes to seed sourcing practices to help reduce the impacts of climate change (Aitken et al. 2008; Broadhurst et al. 2008; O’Neill et al. 2008; Breed et al. 2013; Breed et al. 2016b), and a clear way forward to help provenance decision-making is to test provenance performance in situ during restoration (Gellie et al. 2017).”

(Chapter 5: Gellie et al. 2017, submitted to Evolutionary Applications)

Monitoring output

The inconsistency of restoration monitoring, poor consensus on evaluation criteria, and a lack of access to past monitoring data has been suggested as a major hurdle to meeting restoration targets (Suding 2011). Too often simplistic non descriptive metrics (e.g. survival) are the only criteria to judge success. Additionally, failing to identify the aspects of current practice that are impeding or indeed facilitating success will hamper follow up management (Burton 2014; Collen & Nicholson 2014) and their omission means that their results do not participate in the effectiveness of subsequent projects (Chapter 2, see Fig 2). Restoration begins the process of regaining ecological function to degraded lands with trowels and seedlings as the front line weapons used to implement restoration strategies. However, the capacity to scale up current practices to new challenges (e.g. the Bonn Challenge), integrate new technology (e.g. eDNA monitoring Gellie *et al.* 2017), and learn from past faults will require closer scrutiny of the methods we presently employ followed by evidence-based iterative improvements to practice.

> *"**Effective monitoring provides the evidence needed to make adaptive management interventions if, for example, restoration goals are being missed** (Collen & Nicholson 2014). With consistent monitoring, the restoration end-users and practitioners can demonstrate their achievements relative to investor or policy goals. Traditionally, monitoring involves field-based visual surveys of ecological communities (e.g. taxonomic inventories) (Butchart et al. 2010), which rely on expert observers. These approaches are time consuming, expensive and often not standardised across projects (Thomsen & Willerslev 2015) or between observers (Vittoz & Guisan 2007). Consequently, many restoration projects go unmonitored, or where monitoring is conducted, it remains limited in scope and utility (Ruiz-Jaen & Aide 2005). These limitations can hamper the ability of follow-up management to achieve desired restoration goals (Burton 2014; Collen & Nicholson 2014)."*
>
> *(Chapter 6; Gellie et al. 2017, Molecular Ecology)*

Thesis aims and objectives

The primary aim of this thesis is to develop strategic approaches to improve restoration practices and promote resilient restoration plantings.

To achieve this primary aim, a series of secondary objectives required investigation:

First, a critical review of restoration interventions was undertaken to understand the deficiencies of, identify the knowledge gaps in, and recognize the impediments to current practice from these interventions.

Second, to test the outcomes of the first objective I gathered empirical evidence on the relationships between plant fitness (i.e. the principal metric of restoration success) and provenance distance from planting sites (i.e. the range of genetic resources available to practitioners). This investigation was undertaken for core restoration species of southern Australian eucalypt woodlands. The following specific questions were addressed using provenance trials:

- Are there fitness and functional trait differences between provenances of our study species?
- If present, are these differences consistent with local adaptation?

Third, this thesis examines efficient and complimentary ways to monitor restoration planting outcomes (i.e. a proxy for restoration success) specifically with a focus on functional return of the restoration community. This required developing and integrating genomic techniques (i.e. eDNA metabarcoding) into current practice, and evaluating the utility of eDNA metabarcoding in restoration assessment.

Thesis structure

The body of the thesis comprises five papers that have been submitted, accepted or are pending submission. These chapters are presented in the format of the journal or as the published version, and are preceded by a title page and statements of authorship. Supplementary information is also presented at the end of each chapter where relevant.

This chapter (Chapter 1) covers the aims and objectives of my work and provides background to the topic area. In this chapter I frame the thesis composition, beginning with a review of restoration practice (Chapter 2), followed by empirical papers that present a body of evidence testing local-provenancing and alternate seed sourcing strategies along with their suitability for restoration under climate change (Chapters 3-5). The last data chapter (Chapter 6) develops a new genomic monitoring tool for restoration. The final chapter (Chapter 7) synthesises the evidence presented in the thesis, summarises how best to apply this body of work to meet the challenges currently facing the restoration industry, and identifies future areas of priority research.

Chapter 2 is a manuscript pending revision for *Frontiers in Ecology and the Environment* that documents the knowledge gaps in current restoration practices with an overview of suitable mechanisms that could be used to reconcile these gaps. This paper proposes a systematic approach to restoration that incorporates embedded experiments, and networking results as a mechanism to improve outcomes. Examples are given where embedded empirical experiments in allied disciplines (e.g. forestry and urban design) have contributed significantly to adaptive management. I identify that generating provenance data is a fundamental component required for improving restoration practice as it provides the evidence base for restoration seed collection decision-making, particularly under climate change. Provenance data then becomes the focal theme of Chapters 3-5 of this thesis. The manuscript from Chapter 2 was also catalytic in producing an infographic and a 5 minute cartoon entitled “The Federated States of Degradia” (see Appendix A), which will be used to promote the paper and the ideas it contains more widely through social media.

Chapter 3 is a paper published in *Biological Conservation* and is the first case study of the thesis implementing the recommendations from Chapter 2. A robust provenance experiment was embedded at a site that is currently undergoing restoration in the Mount Lofty Ranges of South Australia. Approximately 1500 *Eucalyptus leucoxylon* plants were grown from seed collected at three different sources that were 5 km, 20 km and 45 km from the restoration site - where the local provenance was most mesic and aridity increased with distance from the restoration site. The expectation was that if the local plants were locally adapted then they would outperform plants from more distant sources. However, this expectation was not found. Rather, local plants had the highest death rates, grew the slowest, and showed increased invertebrate attack and greater signals of stress - all signs of local maladaptation. By contrast, the plants from more arid sources survived and grew the best. These results clearly give support for implementing a seed provenance strategy that accounts for climate change by mixing seed in an arid-to-mesic direction. In addition, the embedded experiment approach advocated in Chapter 2 provided an effective way to facilitate adaptive management options for restoration stakeholders based on the empirical evidence.

Chapter 4 is a paper prepared for submission and provides a second restoration embedded experiment case study. The common garden experiment undertaken in Chapter 3 is expanded to include a reciprocal transplant experiment of the common mallee species *Eucalyptus gracilis*. This study also explores the importance of revisiting and gathering data from past embedded experiments. It included a suite of functional traits, not often used in provenance trials, which helped enrich the study by providing insight into plant resource acquisition strategies. In this chapter, two common garden experiments established at either end of an aridity gradient (e.g. 300 km apart; 238 vs. 386 mm mean annual rainfall) in 2010 were revisited. The planting site was found to be a strong predictor of survival, which was lower for all provenances at the more arid site. Plant height and reproductive activity displayed adaptive differentiation, where the local provenance performed best at both sites. All functional traits had significant provenance effects (indicating a strong genetic influence), that varied according to expectations of aridity adaptation. These findings suggest that *E. gracilis* can occur across a range of climatic conditions by combining specific adaptations to aridity and acclimating to environmental change via phenotypic plasticity.

Chapter 5 is a paper prepared for submission and provides a third empirical case study implementing the embedded experiments recommendation from Chapter 2. The single species common garden experiment of Chapter 3 is expanded to include a reciprocal transplant experiment of four common restoration species. Furthermore, the work of Chapter 4 is advanced by increasing the ecogeographic range from which donor provenances were selected. This study resulted in two common gardens of four provenances of four species *(ca.* 4,000 plants) spanning a 250 km aridity gradient running the length of Yorke Peninsula in South Australia. The local provenance failed to perform best in all but 3 of the 96 garden-species- provenance- trait combinations. The more arid provenances outperformed other provenances in 5 of these 96 combinations. Although additional work is required to fully explore the potential risks of translocation in this system, arid to mesic transfer appears to be a ‘no regrets’ management strategy to increase climate resilience of restoration plantings on the Yorke Peninsula for these species.

Chapter 6 is a manuscript published in *Molecular Ecology* and provides a significant extension to current monitoring practice. This draws on the conclusions of Chapter 2 and focuses attention on simplifying restoration success metrics. This chapter uses the idea that monitoring biodiversity recovery should include the assessment of microbial diversity – the soil microbiome – because of the numerous important functional roles microbes have in ecosystems. A novel method for identifying, quantifying and describing change in the soil microbiota during the restoration process is pioneered by this work. This work demonstrates that metabarcoding soil eDNA is an effective way of monitoring the flux in bacterial communities, and that identifying this change has significant scope for improving the efficacy of restoration interventions. The method proposed also opens up an opportunity for economically viable and retrospective assessments of past restoration efforts. As proposed in Chapter 2, finding a reliable tool for retrospective assessment of restoration performance could significantly contribute to greater restoration knowledge, especially if that knowledge is communicated and networked more widely. With further development, this genomics tool has great scope to assess site viability, monitor restoration interventions, and potentially to prescribe follow-up treatments - all having the potential to improve restoration outcomes.

Chapter 7 is a synthesis of the preceding chapters and considers the contribution that this body of work has made to the field of restoration ecology. I list my contribution to the National Standards of Restoration Practice, facilitating adaptive management options for our partners, networking provenance results, creating infrastructure, disseminating strategic guidelines for climate resilience, and monitoring innovation all as direct consequences of my thesis. I conclude by putting forward recommendations for future directions of scholarship to bridge the existing knowledge gaps that have been identified in this thesis.

CHAPTER 2: NETWORKED AND EMBEDDED EXPERIMENTS

Networked and embedded scientific experiments in restoration will improve outcomes

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Name of Principal Author (Candidate)	Nicholas Gellie		
Contribution to the Paper	Formulated the ideas, designed and composed the figures, wrote initial draft and subsequent revisions		
Overall percentage (%)	70		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	24/01/2017

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Andrew Lowe (corresponding author and principal supervisor)		
Contribution to the Paper	Formulated the ideas, commented and edited subsequent manuscript drafts		
Signature		Date	24/01/2017

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Gellie N. J. C. (candidate)

Formulated the ideas, undertook the literature review and wrote manuscript

I hereby certify that this statement of contribution is accurate

Signed ________________ Date: 23rd January 2017

Breed M. F.

Formulated the ideas, designed and composed figures, commented and edited subsequent manuscript drafts.

I hereby certify that this statement of contribution is accurate and consent to the inclusion of this manuscript in Nick Gellie's PhD thesis

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Involved in developing ideas and contributed to revisions of the manuscript.

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Lowe A. J.

Formulated the ideas, commented and edited subsequent manuscript drafts

I hereby certify that this statement of contribution is accurate and consent to the inclusion of this manuscript in Nick Gellie's PhD thesis

Signed __ Date: 23rd January 2017

Abstract

The world has vast areas of degraded land. In response, ambitious targets have been set to restore degraded ecosystems, including the New York Declaration on Forests target of 350 million ha of restoration by 2030. The achievement of these goals requires a marked improvement to restoration's evidence base, which could be realised by systematically embedding scientific experiments within restoration programs. We illustrate the operational feasibility of this principle with examples of successful transdisciplinary research involving embedded experiments in associated fields. Furthermore, we propose networking these embedded experiments globally and brokering the knowledge gained to catalyse innovation and improve restoration practices. To unify a networked approach to research infrastructure, we call on restoration stakeholders to develop acceptable industry standards of experimental design. Finally, we suggest how to effectively use this principle to meet biome-scale demands of the coming decades.

In a nutshell

- Approximately one third of the Earth's arable land is degraded as a result of unsustainable land management.
- With varying degrees of efficacy and no historical precedence of scale, restoration projects are now being undertaken to improve vast tracts of degraded land.
- The repeated nature of these projects provides an exceptional opportunity to address knowledge gaps more formally and improve restoration practices.
- We propose that deliberately embedding scientific experiments into restoration projects and networking this knowledge globally will establish a much-needed evidence base to improve outcomes

In a recent global assessment, roughly a third (i.e. 29%) of all arable land was considered degraded (Nkonya *et al.* 2016). Previous estimates of twice this figure (e.g. 66% Bot *et al.* 2000; Gibbs and Salmon 2015) reflect the considerable variation in how degradation is defined, used and assessed (Hobbs 2016). However, if this recent conservative estimate by Nkonya *et al.* (2016) was to be consolidated into one geopolitical boundary, a landmass which we might hypothetically call the 'Federated States of Degradia' (Figure 1), this federation would exceed the size of Russia (approx. 2 billion ha). Degradia's population would include more than 3 billion of the world's poorest and most vulnerable people (Nkonya *et al.* 2016) who would be restricted in their economic capacity to deal with the consequences of unsustainable land management on biodiversity and ecosystem services.

Recognizing the scale and impact of this degradation has stimulated multilateral agreements with ambitious restoration targets, such as the Convention on Biological Diversity (CBD) Aichi Target 15, the aims of the United Nations Framework Convention on Climate Change (UNFCCC) for Reducing Emissions from Deforestation and Forest Degradation (REDD+), and more recently the Rio+20 land degradation neutrality goal.

In an attempt to provide a pragmatic means to achieve these targets, The Bonn Challenge (e.g. restoration of 150 million ha of degraded land by 2020) was tabled in 2011 by the Global Partnership on Forest & Landscape Restoration (GPFLR). This target was extended to 350 million ha by 2030 at the September 2014 United Nations Climate Summit in New York. In China alone 40 million ha have been designated for restoration by 2020, an area roughly the size of California (Xu 2011), and a further 100 million ha were committed to by the African Restoration Initiative (AFR100) announced in December 2015 at the Paris Climate Conference (COP21).

Setting large global restoration targets has affirmed the growing significance of restoration practice in environmental policy (Suding *et al.* 2015). However to direct restoration practices, policymakers require relevant tools to evaluate restoration against sustainable goals. To this end, in 2016 the United Nations Environmental Program's International Resource Panel secretariat put forward recommendations on how to decouple economic growth from environmental degradation (UNEP 2016). Furthermore, the

Intergovernmental Platform on Biodiversity and Ecosystem Services (IPBES) is undertaking a thematic assessment on land degradation and restoration. Therefore, scaling-up restoration projects will require evidence-based, cost effective interventions that are consistent with UNEP and IPBES recommendations and include a social-ecological context to restoration interventions.

Defining clear policy objectives is not a straightforward exercise. For example, even the term 'forest' is dynamic (Chazdon *et al*. 2016; Bastin *et al.* 2017), but we will focus on seed-based plantings associated with forest restoration because these interventions are central to GPFLR and restoration initiatives contributing to the Bonn Challenge and AFR100. Although forest restoration is only one of many restoration interventions in one of many biomes (Holl and Aide 2011), our belief is that with minor modification to address the key knowledge gaps (Figure 1b), any of these interventions could support long-term embedded experiments. Therefore, we volunteer an extension of the knowledge gaps identified in Perring *et al.* (2015) and highlight them as a framework to embed experiments in restoration (Figure 1b; WebTable 1).

In this article we propose that to achieve the spatial ambitions forecasted in the aforementioned multilateral agreements, restoration projects should become active scientific laboratories (Figure 2). By reviewing examples of allied industry programs and global networks that have taken this approach, we demonstrate that the model of incorporating experimental components into restoration projects could help answer unresolved questions in the field. We explore a biome-wide application of embedded experiments as a demonstration of how to establish synergies and create a much-needed evidence base for restoration science to maximize restoration success. Networking this principal at a global scale would then amplify project benefits, by helping deliver the evidence required for adaptive restoration at scale and linking scientists, land managers and decision-makers to ensure the most efficient use of limited resources.

Embedded restoration experiments

Proceeding with restoration that lacks the capacity to respond to emerging goals, because of limited evidence, will compromise the ability of the restoration sector to address the major challenges it faces during the new era of broad-scale restoration. For example, active forest restoration through replanting has tended to default to approaches that are driven by pragmatism, the efficacy of which may be unproven or not cost-efficient at larger scales (Murcia *et al.* 2014).

Robert Cabin (2007) introduced the term ‘intelligent tinkering’, not only in homage to Aldo Leopold but also to refer to a practitioner driven mechanism of improving restoration through trial and error. Many restoration projects have these tinkering components (e.g. site preparation, species mixes, timing of plant-out) but reporting can be erratic, is seldom enforced, and is often biased towards positive outcomes (Suding 2011). Without replication, formal scientific standards, and suitable curation, *ad hoc* research and intelligent tinkering may silo learning and knowledge locally. Unfortunately, these important restoration skills based on trial and error could then be lost to a new generation of practitioners if retiring restoration elders do not factor in the succession and dissemination of this knowledge.

Principal planning decisions about seed resources can affect the rate at which restoration plantings become established (Godefroid *et al.* 2011), and undermine the magnitude or diversity of ecosystem services provided (Benayas *et al.* 2009). Yet decisions about seed collection (Broadhurst *et al.* 2016) and species selection (Xu 2011) often default to a ‘local is best’ convention without first determining the suitability of the material. Simple embedded experiments (e.g. Gellie *et al.* 2016) can be used to address these gaps, as they remove the need for the risk averse circumspection of default strategies, and provide controlled infrastructure to use in further assessments.

Consistent monitoring is also recognised as a key gap that requires critical review to optimize the return of ecosystem functions to degraded land (Godefroid *et al.* 2011; Suding *et al.* 2015). To achieve functional return and therefore resilience, the restoration sector will need to recognize and incorporate important economic, social, cultural and political values and needs across both human and ecological spectra (Chazdon 2008). Projects from forestry and restoration (Panel 1) highlight the success of embedding experiments.

There will be substantial benefits to be gained by improving restoration practices. A report by The Global Commission on The Economy and Climate (Stern and Calderon 2014) estimated that achieving the expanded Bonn Challenge goal of 350 million ha of restored land by 2030 would contribute US$170 billion per year to global ecosystem services, agroforestry and carbon sequestration economies. The report also proclaims "land degradation can be addressed by well tested practices" (Stern and Calderon 2014), but omits to state a suitable vehicle for such testing. To accurately test the validity of current practice, research needs to be entrenched, rigorous and replicated within and across projects.

Stakeholders could share the cost of establishment, which does not need to be restrictive (see NutNet model in Panel 2 for an example), and the value in the additional cost of embedding experiments may be found in productivity gains. For example, costs (e.g. design, monitoring, and logistics) were offset against gains in an Australian woodland restoration project by identifying the source of sub-optimal plant performance (Gellie *et al.* 2016). Encouraging adaptive management approaches like the example we give that are based on embedded experiments could (and in our opinion should) be linked to the accreditation for payments for environmental services (PES) schemes involving restoration.

Restored landscapes are a valuable resource that can benefit society more widely. As restoration interventions mature, and with suitable governance, there is the potential for cost recovery of the experiments. Projects could supply seed resources, and embedded experiments could test how to sustainably harvest them for future restoration projects (Broadhurst *et al.* 2016). The potential use of timber and non-timber forest products (Shackleton and Shackleton 2004), and the indirect benefits of enhancing human wellbeing by restoration (Aronson *et al*. 2016), could be better quantified by experimentation. Investigating the dynamics of restoration interventions via experimentation would help quantify the provision of biodiversity and ecosystem services (Benayas *et al.* 2009), and improve engagement with policymakers using these frameworks.

Networking experiments

A network of knowledge based on the findings of restoration experiments will need to marry design with operational feasibility to overcome logistical hurdles. Integrating, interpreting and extrapolating the results of these experiments is a non-trivial task, but the restoration and research communities stand to gain much from improved cooperation with each other. A global network will provide a forum to broadcast barriers to success, test strategies to mitigate these barriers and other common problems (e.g. impacts of climate or desertification). Ideally a network would also provide a platform to broker the knowledge gained from embedded experiments. Embedding research into real-life applications across larger scales provides end-user integration across jurisdictional boundaries that better reflect biome or global trends, which many scientists want but all too often do not achieve. Panel 2 provides examples from research networks that have institutionalized experimental design into their programs, outlining an approach we now encourage for restoration.

Networking restoration experiments, as opposed to simply embedding trials for project specific interventions, encourages rapid learning through dissemination, and expands our capacity to test ecological theory with broadscale replication (Bradshaw 1987). A more defined knowledge network will also enable generalities of larger-scale biological processes to be explored (e.g. ecosystem services, niches, and functional groups; Montoya *et al.* 2012). Lessons in governance and relevance to global priorities learnt from Long Term Ecological Research (LTER) networks could be applied, even incorporated into a restoration research network (Barbosa *et al.* 2004). Extrapolating trends globally (as done in LTER facilities, see Panel 2) would move restoration from topical prescriptions to problems to a global synthesis of practice. Synergies in transdisciplinary aspects of restoration could be sought (e.g. in the areas of economics, governance and legal structures in social-ecological-systems) when compared across different markets (Chaves *et al.* 2015).

Another overlooked opportunity here is the potential for citizen science and community-based programs to contribute to the research effort. Such efforts build capacity for monitoring restoration outcomes among local communities, thus improving communication of project rationale and transparency. Options for incorporating strategies that promote key ecosystem services (e.g. air and water purification, local climate amelioration, soil stabilization, carbon sequestration, pollination services) could also be highlighted, and more efficiently communicated through community involvement in experiments (Benayas *et al.* 2009). Indeed, it is an essential principle of the management of common-pool resources that monitoring needs to be transparent, and at least to some degree, the responsibility of resource users (e.g. innovative data collection and curation methods can encourage broader participation, see https://portal.landpotential.org).

How can embedded and networked experiments be applied?

Applying embedded and networked experiments in restoration at larger scales will help to harvest the evidence needed to bridge key knowledge gaps (WebTable 1). The world's five Mediterranean climate regions form a global biome that has a disproportionately large concentration of biodiversity, but unsustainable land practices have led to acute degradation (Hoekstra *et al.* 2005). This biome is economically and biogeographically disjunct (i.e. five biodiversity hotspots on five continents, and countries with varying degrees of economic development; Myers *et al.* 2000). Sustained pressure from agriculture, urbanisation and deforestation (Cowling *et al.* 1996) has stimulated ecological research and restoration of biodiversity in the Mediterranean biome (Doblas-Miranda *et al.* 2015). We explore how restoration synergies could be achieved by networking embedded experiments across this biome to strategically address the six key knowledge gaps (identified in Figure 1b; WebTable 1);

1. Defining outcomes – simple manipulations of planting treatments (Jaunatre *et al.* 2014), species mixes targeted to ecosystem services (Perring *et al.* 2013), and changes in fire regime (Armesto *et al.* 2009) are key focal points to consider for the design of embedded experiments in this biome.
2. Social integration – using embedded experiments will engage communities and help facilitate participation (Benayas *et al.* 2009). Embedded experiments can also test the feasibility of seed production and propagation industries (Brancalion *et al.* 2012; Broadhurst et al. 2016).
3. Financing – testing species mixes allows investigation of carbon bio-sequestration (Standish and Hulvey 2014) and non-timber forest products (Shackleton and Shackleton 2004) as revenue streams. Networking the knowledge of a biome opens the opportunity

for wealthier member states to assist poorer states in developing embedded experiment infrastructure and improve restoration.

4. Plant genetic resources – climate change presents a great threat to restoration success, and embedded experiments are already showing great promise in this important area (Breed *et al.* 2013; Gellie *et al*. 2016).
5. Technology adoption – genomics is opening up new opportunities for restoration monitoring and assessment (Williams *et al.* 2014). The evidence gained from embedded experiments will encourage consistent practices that allow before after control impact (BACI) experimental designs to investigate changes (Gellie *et al.* 2017).
6. Policy and governance – if restoration is approached as a potentially sustainable economic activity then this encourages oversight and efficiencies to be sought (see AFRP in Panel 2 where mandatory monitoring requirements have been pioneered in new legal frameworks; Chaves *et al.* 2015).

The Mediterranean biome shrubland vegetation identified as *matorral* in Chile is similar to *chapparal* in California, *maquis* in the Mediterranean basin, *fynbos* in South Africa, and open shrublands of southern Western Australia known as *kwongan*. These vegetation communities primarily exist in an agro-ecological mosaic that is degraded and challenging to restore. Site specific requirements may dictate that factors other than those listed above take priority (e.g. edaphic conditions, recruitment or controlling invasive species), which could be considered in the context of a split-plot design to test the effect of restoration interventions on them. However, consistently instating research infrastructure in the form of common gardens and reciprocal transplant experiments that explore plant origin and species mix at a bare minimum, would be a sound beginning. We have speculated on efficiencies that could be obtained from networking the knowledge gained by these types of embedded experiments, but they remain to be realised in this or in other biomes.

Conclusion

Few restoration projects successfully integrate well-designed experiments at the design phase. When such integration does occur, it is generally opportunistic rather than programmed and therefore the potential gains of cross-project learning are not achieved. Deliberately embedding experiments into restoration projects will help integrate knowledge exchange between researchers, land managers, and policymakers. We propose networking these embedded experiments globally and brokering the knowledge gained to catalyse innovation and improve restoration practices. To unify a networked approach to research infrastructure, we call on the restoration community to develop acceptable industry standards of experimental design. If, as postulated by Suding *et al.* (2015), “clarifying and informing policy is the common motivator”, and as professed in the New York Declaration on Forests, “restoration of degraded ecosystems can indeed be used as an auspicious solution to climate change”, surely exploring the efficacy of restoration through embedded experiments and networking the results is an investment that will pay generational dividends.

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WebTable 1.

Key knowledge gaps in restoration that still require addressing before large-scale projects can be effective, and how embedding scientific experiments within projects and networking this globally can benefit restoration outcomes (adapted from Perring *et al.* 2015).

Knowledge gap	Benefits of embedded experiments	Outcomes of a global networking of experiments	References
(1) Defining desirable restoration outcomes	Helps to identify barriers, set realistic restoration targets, and quantify co-benefits	Creates a global platform for assessment of, restoration interventions that can objectively address the viability of scaling up projects	Hobbs *et al.* 2006; Breed *et al.* 2016
(2) Integrating communities into restoration	Initiates restoration solutions that incorporate social environmental systems that empower communities	Global buy-in and roll out of interventions, where restoration knowledge leads to broadscale empowerment and sustainable restoration actions.	Chazdon 2008; Brancalion *et al.* 2012
(3) Finance and support of restoration actions	Links restoration interventions with economic outcomes in order to justify restoration financing and other support	Links topical restoration interventions with globally targeted ecosystem services. Provides fiscal sustainability for restoration by opening up alternative funding sources.	Chazdon 2008; Breed *et al.* 2016
(4) Sustainable and progressive management of plant genetic resources	Identifies the environmental impacts of seed harvesting and use for restoration and defines appropriate plant sources for restoration during global change	Improves resilience, sustainability and scalability of plant-based restoration interventions. Drives changes to policy and practice.	Xu 2011; Breed *et al.* 2013; Broadhurst *et al.* 2016
(5) Adopting emerging technologies	Provides new solutions to scale-up restoration fostering innovation	Enables cost savings and efficacy gains to translate to greater restoration impact	Williams *et al.* 2014; Zahawi *et al.* 2015
(6) Improving restoration policies and governance structures	Effective monitoring will improve accountability and lead to more sustainable practices.	Encourages good governance which helps to develop the legal and jurisdictional frameworks for restoration interventions. Provides a platform for knowledge brokering.	Suding *et al.* 2015; Richardson 2016

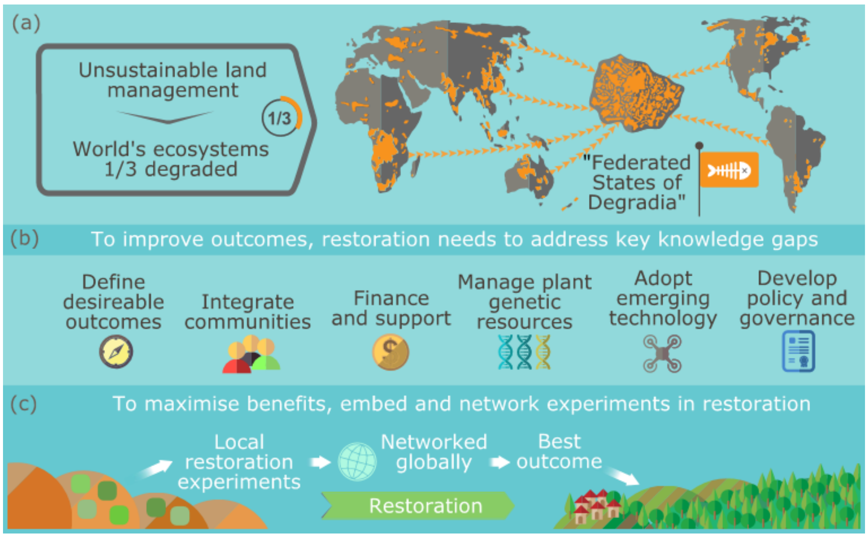

Figure 1

The Federated States of Degradia showing (a) the scale of the restoration challenge, (b) the key knowledge gaps in restoration (adapted from Perring *et al*. 2015, see WebTable 1 for more detail), and (c) to improve restoration outcomes the practical way forward is to embed experiments locally, and regionally, then ultimately globally network the process to encourage knowledge transfer.

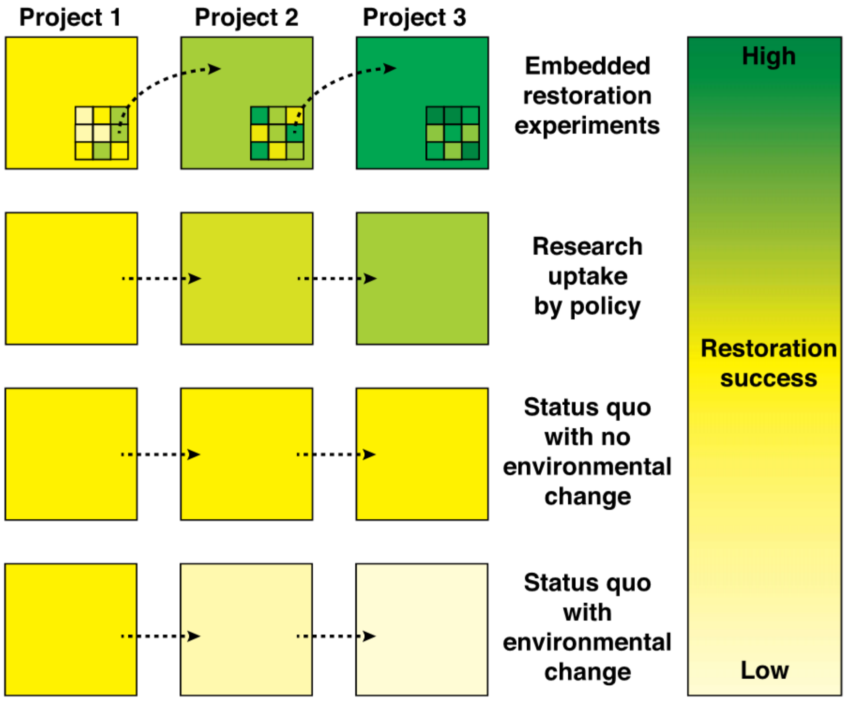

Figure 2

Embedding experiments during restoration would allow inefficient practices to be identified. Desirable traits for future climate scenarios (e.g. drought/frost/fire tolerance) could be routinely tested in common gardens locally. Targeted phenotypes could be robustly tested in reciprocal transplant trials using repeatable modular experimental units (see NutNet in Panel 2) that focus on the gaps in WebTable 1. Site effects would be promptly identified then minimized, and networks could identify trends. The principle of embedding experiments is illustrated above, where restoration success ranges from low (yellow) to high (green). Arrow directions represent the flow of knowledge gained from past restoration projects.

Panel 1. Success stories of embedded experiments

Adaptree: The Canadian forest sector provides a good model of how to improve the link between research, application and outcomes. In 2013, forestry added CAD$19.8 billion to the Canadian economy, however economic losses due to climate change impacts were forecasted to be as high as 35% (or a loss of CAD$6.93 billion) by 2100 (Adaptree 2012). In an attempt to provide the evidence-base for policy and to address suboptimal tree growth and maladaptation associated with climate change AdapTree was formed and ran from 2011-2015. Adaptree assembled a transdisciplinary team that integrated genomics and climate mapping technologies for the primary economic trees of Canada and resulted in a portfolio of climate scenario options for the forestry sector. More broadly speaking, North American land managers can now use this knowledge to justify strategies of assisted gene flow to combat climate change (Rosner 2015). The concerns identified by the AdapTree project over maladaptation due to climate change in forestry are mirrored in restoration sector (Breed *et al*. 2013).

Methods used by Adaptree (e.g. common garden experiments, Figure 3) could be applied as embedded experiments in restoration to address many of the six key knowledge gaps identified in Figure 1.

Figure 3. AdapTree team measures heights and prepares to plant out 2-year old Lodgepole pine seedlings in a validation field trial. Photo credit: P. Smets

Atlantic Forest Restoration Pact: At a regional scale, the Brazilian Atlantic forest restoration practices have matured due to the success and failures of experimental trials (e.g. exotic species in the 1980s, to plantings of remnant forest species in the 1990s, and onto the practices that seek to mimic and accelerate ecological succession in the early 2000s). Early work led to a unified regional restoration alliance in 2009, called The Atlantic Forest Restoration Pact (AFRP) with an ambition of restoring 1 million hectares by 2020 (Rodrigues *et al.* 2009) that has now grown to 15 million ha by 2050.The AFRP has extensively used embedded experiments and subprojects have been identified that have real social benefits. For example, communities have been involved in seed collection and propagation and new cottage industries have emerged (e.g. nurseries and seed enterprises). Plus, the education of communities has encouraged participation in the monitoring of projects. These outreach programs have further enhanced land stewardship and enabled the exchange of indigenous knowledge back into the AFRP. Local policymakers have also pioneered legal instruments for regulating restoration that have introduced mandatory requirements for projects that increasingly rely on evidence and oversight obtained from embedded experiments (Chaves *et al.* 2015).

The AFRP is successfully developing sustainable restoration approaches derived from the evidence obtained in embedded experiments.

Panel 2. Networking experiments pays dividends

TreeDivNet: An integration of an experimental mindset has been successfully applied in the Tree Diversity Network (http://www.treedivnet.ugent.be/). This alliance of projects aims to estimate the impact of tree diversity on ecosystem functioning and stability across different biomes. The experimental designs may differ marginally between projects but they broadly focus on progressing monospecific plantations into more sustainable practices that incorporate diverse genotypes, species and structures.

In its second decade of operation, the scale of TreeDivNet is impressive (e.g. coordinated plantings of more than 1,000,000 trees spanning four biomes). Projects have not generally been integrated into active restoration as we are proposing because of the forestry theme of the network, but the Ridgefield TreeDivNet Experiment (Figure 4) in Western Australia is a notable exception, and highlights the network's flexibility (Perring *et al*. 2012). Improving future TreeDivNet projects by expanding spatial, temporal and operational scales was proposed by Kris Verheyen and colleagues in a recent review (Verheyen *et al*. 2016), and if adopted these changes are likely to reinforce TreeDivNet's relevance to evidence based restoration practices.

By emphasizing the benefits of a networked experimental approach to decision making TreeDivNet promotes the coordinated knowledge transfer that we believe is essential in the restoration sector.

Figure 4. The Ridgefield TreeDivNet Experiment, Photo credit: R.J. Hobbs

NutNet: The Nutrient Network (NutNet; http://www.nutnet.umn.edu/) is a cooperative research network that tackles questions of biogeochemical cycling and species interactions in natural ecosystems at a global scale. The genesis of NutNet came from a workshop in 2005 where students realized the paucity of replicated experimental data for grassy biomes globally (Stokstad 2011). NutNet provides good evidence that with clear goals and well-designed experimental treatments, ecological networks do not have to be financially restrictive (costing ca. US$300/year/treatment for site setup and maintenance) (Borer *et al.* 2014).

Despite NutNet focusing on natural ecosystems without a restoration theme, after a decade of networked research, some key recommendations of this network have particular relevance to embedding and networking experimental design into restoration (Borer *et al.* 2014). These recommendations include the following considerations:

i. Communicating clear scientific goals to participants is essential.
ii. Plain language and simple protocols are indispensable.
iii. Standardizing both treatment and sampling strategies has power in its replication. Any deviation from this format will degrade the data set.
iv. Long-term, transparent strategies to overcome participation, cost, data ownership and authorship hurdles should be developed early.
v. Simple, inexpensive and modular designed experiments will assist in uptake, but keep it flexible enough to incorporate additional studies.
vi. A critical mass of contributors will ensure short generation times of data, when the benefits are articulated this will help in growth.
vii. Data integration and management needs to be planned.

A similar standardized protocol that is used in NutNet (Figure 5) could be applied in a restoration context to allow the analysis, extrapolation and forecasting required to tackle the challenges of scale arising in the 21st century.

Figure 5. The Doane Audubon Spring Creek Prairie NutNet site in Nebraska, USA. Photo credit: R. Laungani

CHAPTER 3: LOCAL MALADAPTATION

Local maladaptation in a foundation tree species: implications for restoration

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Name of Principal Author (Candidate)	Nicholas Gellie		
Contribution to the Paper	Formulated the ideas, conducted field trial, undertook the analysis, and composed figures, wrote manuscript and addressed reviewer's comments.		
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Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	24/01/2017

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

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Formulated the ideas, conducted field trials, undertook analysis and composed figure, wrote manuscript and addressed reviewers comments

I hereby certify that this statement of contribution is accurate

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Formulated the ideas, undertook analysis, prepared figures and tables commented and edited subsequent manuscript drafts and revision

I hereby certify that this statement of contribution is accurate and consent to the inclusion of this manuscript in Nick Gellie's PhD thesis

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Involved in developing ideas and contributed to revisions of the manuscript.

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Kennedy S. A.

Involved in developing ideas, supervised planting logistics and contributed to revisions of the manuscript.

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Formulated the ideas, commented and edited subsequent manuscript drafts

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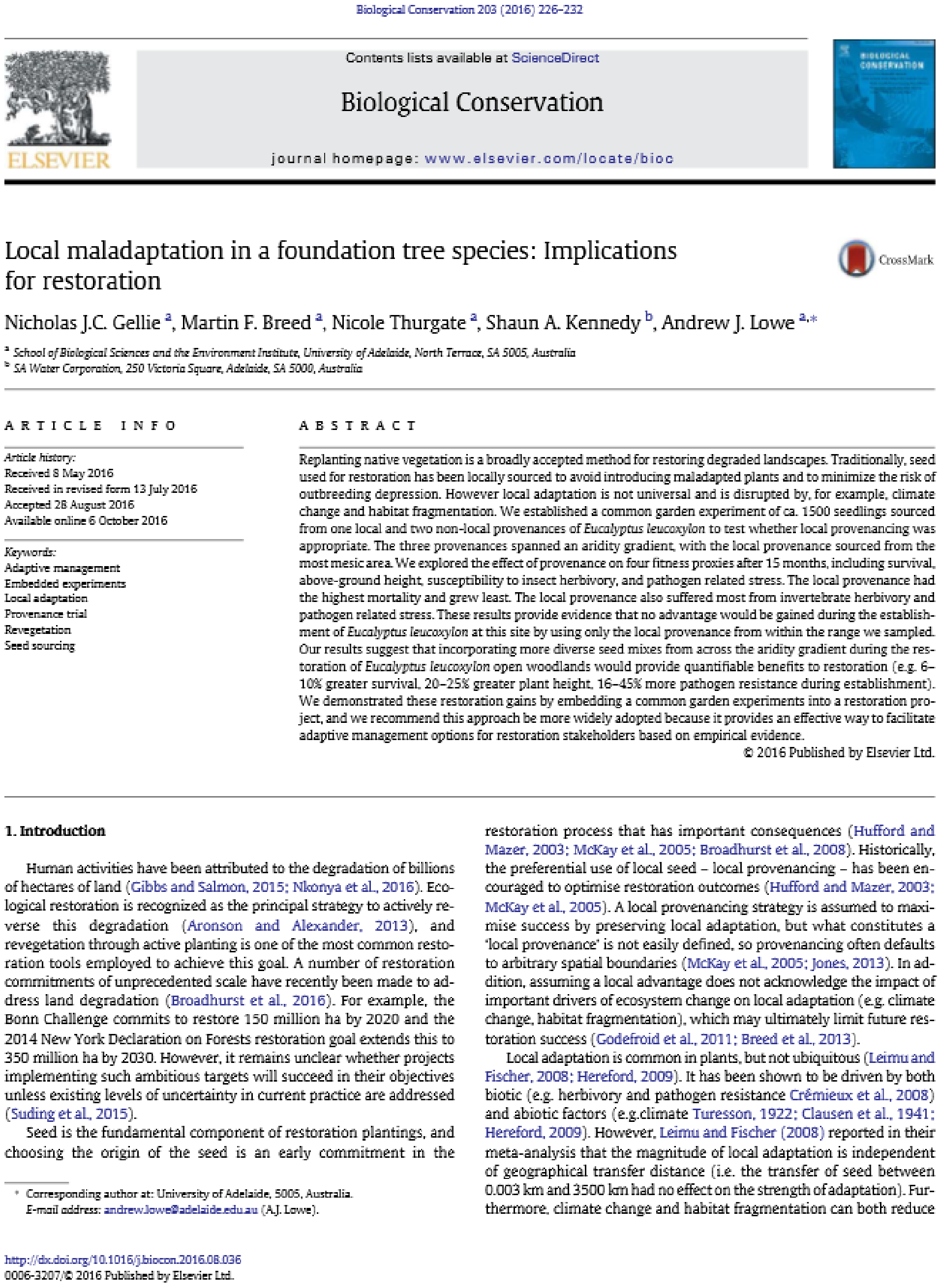
Abstract

Replanting native vegetation is a broadly accepted method for restoring degraded landscapes. Traditionally, seed used for restoration has been locally sourced to avoid introducing maladapted plants and to minimise the risk of outbreeding depression. However local adaptation is not universal and is disrupted by, for example, climate change and habitat fragmentation. We established a common garden experiment of *ca.* 1500 seedlings sourced from one local and two non-local provenances of *Eucalyptus leucoxylon* to test whether local provenancing was appropriate. The three provenances spanned an aridity gradient, with the local provenance sourced from the most mesic area. We explored the effect of provenance on four fitness proxies after 15 months, including survival, above-ground height, susceptibility to insect herbivory, and pathogen related stress. The local provenance had the highest mortality and grew least. The local provenance also suffered most from invertebrate herbivory and pathogen related stress. These results provide evidence that no advantage would be gained during the establishment of *Eucalyptus leucoxylon* at this site by using only the local provenance from within the range we sampled. Our results suggest that incorporating more diverse seed mixes from across the aridity gradient during the restoration of *Eucalyptus leucoxylon* open woodlands would provide quantifiable benefits to restoration (e.g. 6-10% greater survival, 20-25% greater plant height, 16-45% more pathogen resistance during establishment). We demonstrated these restoration gains by embedding a common garden experiments into a restoration project, and we recommend this approach be more widely adopted because it provides an effective way to facilitate adaptive management options for restoration stakeholders based on empirical evidence.



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Local maladaptation in a foundation tree species: Implications for restoration

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ABSTRACT

Replanting native vegetation is a broadly accepted method for restoring degraded landscapes. Traditionally, seed used for restoration has been locally sourced to avoid introducing maladapted plants and to minimize the risk of outbreeding depression. However local adaptation is not universal and is disrupted by, for example, climate change and habitat fragmentation. We established a common garden experiment of ca. 1500 seedlings sourced from one local and two non-local provenances of *Eucalyptus leucoxylon* to test whether local provenancing was appropriate. The three provenances spanned an aridity gradient, with the local provenance sourced from the most mesic area. We explored the effect of provenance on four fitness proxies after 15 months, including survival, above-ground height, susceptibility to insect herbivory, and pathogen related stress. The local provenance had the highest mortality and grew least. The local provenance also suffered most from invertebrate herbivory and pathogen related stress. These results provide evidence that no advantage would be gained during the establishment of *Eucalyptus leucoxylon* at this site by using only the local provenance from within the range we sampled. Our results suggest that incorporating more diverse seed mixes from across the aridity gradient during the restoration of *Eucalyptus leucoxylon* open woodlands would provide quantifiable benefits to restoration (e.g. 6–10% greater survival, 20–25% greater plant height, 16–45% more pathogen resistance during establishment). We demonstrated these restoration gains by embedding a common garden experiments into a restoration project, and we recommend this approach be more widely adopted because it provides an effective way to facilitate adaptive management options for restoration stakeholders based on empirical evidence.

1. Introduction

Human activities have been attributed to the degradation of billions of hectares of land (Gibbs and Salmon, 2015; Nkonya et al., 2016). Ecological restoration is recognized as the principal strategy to actively reverse this degradation (Aronson and Alexander, 2013), and revegetation through active planting is one of the most common restoration tools employed to achieve this goal. A number of restoration commitments of unprecedented scale have recently been made to address land degradation (Broadhurst et al., 2016). For example, the Bonn Challenge commits to restore 150 million ha by 2020 and the 2014 New York Declaration on Forests restoration goal extends this to 350 million ha by 2030. However, it remains unclear whether projects implementing such ambitious targets will succeed in their objectives unless existing levels of uncertainty in current practice are addressed (Suding et al., 2015).

Seed is the fundamental component of restoration plantings, and choosing the origin of the seed is an early commitment in the restoration process that has important consequences (Hufford and Mazer, 2003; McKay et al., 2005; Broadhurst et al., 2008). Historically, the preferential use of local seed – local provenancing – has been encouraged to optimise restoration outcomes (Hufford and Mazer, 2003; McKay et al., 2005). A local provenancing strategy is assumed to maximise success by preserving local adaptation, but what constitutes a 'local provenance' is not easily defined, so provenancing often defaults to arbitrary spatial boundaries (McKay et al., 2005; Jones, 2013). In addition, assuming a local advantage does not acknowledge the impact of important drivers of ecosystem change on local adaptation (e.g. climate change, habitat fragmentation), which may ultimately limit future restoration success (Godefroid et al., 2011; Breed et al., 2013).

Local adaptation is common in plants, but not ubiquitous (Leimu and Fischer, 2008; Hereford, 2009). It has been shown to be driven by both biotic (e.g. herbivory and pathogen resistance Crémieux et al., 2008) and abiotic factors (e.g.climate Turesson, 1922; Clausen et al., 1941; Hereford, 2009). However, Leimu and Fischer (2008) reported in their meta-analysis that the magnitude of local adaptation is independent of geographical transfer distance (i.e. the transfer of seed between 0.003 km and 3500 km had no effect on the strength of adaptation). Furthermore, climate change and habitat fragmentation can both reduce

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the likelihood and strength of local adaptation. Fragmentation tends to increase inbreeding and reduce adaptive capacity, and climate change is shifting adaptive landscapes (i.e. the relationship between a given site and the optimal phenotype at that site) (Jump and Peñuelas, 2005; Lowe et al., 2005; Breed et al., 2015). As such, it has been argued that strictly adhering to local provenancing could limit the evolutionary potential of restoration plantings (Sgrò et al., 2011; Breed et al., 2013; Havens et al., 2015).

The effects of intentionally mixing local and non-local provenances on plant fitness was recognized by Darwin (1876), and has been exploited in plant breeding for centuries (Schnable and Springer, 2013). Mixing genotypes also comes with risks as it can lead to the introduction of maladapted individuals and outbreeding depression (Lesica and Allendorf, 1999), where interpopulation crosses experience a decline in progeny fitness (Hufford and Mazer, 2003; Vander Mijnsbrugge et al., 2010). These concerns have merit, chiefly when provenances with different ploidy levels are used (Weeks et al., 2011), where transfer is being considered over very long distances, or dramatic environmental gradients exist (Byrne et al., 2011; Breed et al., 2013). However, the concern of outbreeding depression has generally been overemphasised in the conservation genetics literature since the likelihood of outbreeding depression is low for crosses of non-threatened, predominantly outcrossing species that are used in restoration (Frankham et al., 2011).

Traditional and novel seed collection recommendations were reviewed in Breed et al. (2013), who argued for provenancing approaches that mitigated the impacts of climate change and habitat fragmentation on provenance fitness. Two themes came out of this review. First, it was suggested that local provenances should be supplemented with provenances from further afield to augment adaptive potential of plantings (e.g. composite and admixture provenancing). Secondly, it was suggested that particular provenances should be selected to match future environmental conditions based on climate modelling (e.g. predictive provenancing). Further strategies have suggested to explicitly incorporate climate resilience, as in Prober et al. (2015), who encouraged a directional selection to seed collection in line with climate predictions (i.e. climate adjusted predictive provenancing), and regionally developed cultivars selected for specific traits have also been recommended (e.g. vigour, drought tolerance and disease resistance Baer et al., 2014). With mounting evidence from translocation studies showing that some populations lack distinct local adaptation (Hancock et al., 2012; Breed et al., 2016a; Lu et al., 2016), and the numerous quantitative reviews also questioning the ubiquity of local adaptation (Leimu and Fischer, 2008; Hereford, 2009), provenance studies of core restoration species are needed to help guide the selection of appropriate provenancing strategies (Breed et al., 2013; Prober et al., 2016).

In this study we investigated how provenance influenced first season survival, growth, herbivory and pathogen resistance for a foundation tree species commonly used in restoration of southern Australian habitat, *Eucalyptus leucoxylon ssp leucoxylon* (hereafter *E. leucoxylon*). We used three provenances orientated along an east–west aridity gradient in the southern Mt. Lofty Ranges in South Australia. The local provenance was western and most mesic, and the distant provenance was eastern and most xeric (Fig. 1). The three provenances were grown in a common garden experiment to explore the following questions: (1) what effect does *E. leucoxylon* provenance have on survival, growth, herbivory or pathogen resistance? If variation is observed, (2) is this variation in line with local adaptation? From the evidence we present, we derive regional management recommendations for optimising seed sourcing strategies for *E. leucoxylon*. The findings of this study has implications for other species in the region and for conservation and restoration more generally.

2. Materials and methods

2.1. Study species and site

Eucalyptus leucoxylon is a tree that grows 8 to 30 m (Nicolle, 2013), occurs in southern Australian open woodland communities on fertile soils with a loamy horizon over clay (Armstrong et al., 2003), particularly where annual rainfall is >400 mm (Boomsma and Lewis, 1980). It is largely pollinated by birds, and to a lesser extent by insects and small mammals, and is predominantly outcrossing (Ellis and Sedgley, 1993; Ottewell et al., 2009). *E. leucoxylon* is protandrous and the close proximity of sequentially hermaphroditic inflorescences allows selfing to occur from adjacent flowers on the same plant (Ellis and Sedgley, 1993; House, 1997).

We established a common garden experiment within a 238 ha restoration site owned and managed by the South Australian Water Corporation (SA Water), near the township of Clarendon (−35.0882°S, 138.6236°E). The site was cleared >100 years ago and was managed under a grazing lease agreement until 2010. Restoration began in 2011 by SA Water and is ongoing. The local climate is Mediterranean, with hot dry summers and moderately wet winters (mean maximum summer temperature = 21.9 °C; mean maximum winter

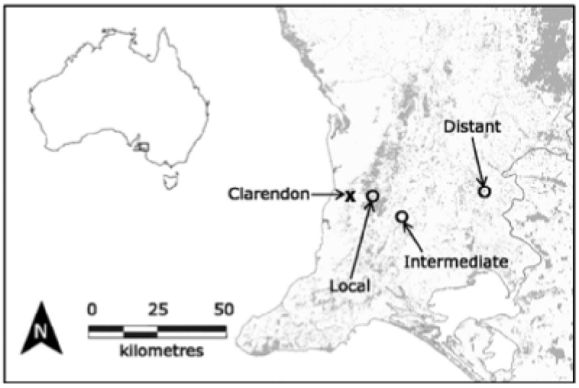


Fig. 1. Provenance localities (open circles), location of restoration site (x) and extent of remnant vegetation (grey shading).

temperature = 11.2 °C; mean annual rainfall = 790 mm. http://www.bom.gov.au/climate/data/, additional environmental data Table A1).

2.2. Seed collection and germination

We sourced seed from maternal trees of three provenances across a west-east aridity gradient to capture variation in precipitation and temperature that might contribute to establishment and performance differences across the provenances. The provenances were selected from intact native stands of >100 individuals. The stand densities of these provenances were 140 ± 21.3 SE plants ha^{-1}. Open-pollinated seed was collected from the canopies of at least 10 mature donor trees at each provenance. The provenance localities were Mt. Bold (35.1043°S, 138.6901°E; ca. 5 km from the restoration site; hereafter local provenance); Macclesfield (35.1612°S, 138.8517°E; ca. 20 km from the restoration site; hereafter intermediate provenance); and Monarto (35.1178°S, 139.1295°E; ca. 45 km from the restoration site; hereafter distant provenance) (Fig. 1).

The restoration site has a similar climate to the local provenance, with aridity, temperature and rainfall trending towards drier and hotter conditions in an easterly direction, towards the distant provenance. The restoration site has an aridity index of 1.01 (aridity index = mean annual precipitation / potential evapotranspiration), which is near the mean aridity index for the species (mean aridity index = 0.8 ± 0.01 SE). The selected provenances span a large proportion of the total range of aridity that *E. leucoxylon* occurs (e.g. 43% of the total aridity index present in the records for the species; local = 1.01; intermediate = 0.85; distant = 0.39, Atlas of Living Australia; http://spatial.ala.org.au/; Fig. A1).

Germination and rearing of seedlings was conducted in full-sun at a commercial nursery in South Australia (35.1264°S, 139.2359°E). A subset (n = 1434) of all the *E. leucoxylon* plants raised for this restoration project (n = 12,320) was randomly selected in the nursery and pots were marked for use in the common garden experiment (final sample sizes for analysis described below).

2.3. Common garden experiment

A fully randomised design was used where provenance was randomly assigned to a planting location within the restoration site (35.0882°S, 138.6235°E), and planting took place in June–July 2012. Plants were individually geo-referenced during planting using a Trimble Juno 3D GIS mobile field data collector (i.e. 1434 plants; $n_{local} = 477$; $n_{intermediate} = 513$; $n_{distant} = 444$). Each seedling was planted into ground which was mechanically prepared using a plough. A 200 × 200 × 400 mm UV stabilised corflute tree guard (Geofabrics) surrounded each seedling to protect against vertebrate herbivores (e.g. rabbits and kangaroos). We sprayed glyphosate herbicide in a 1 m radius of the planting site, with one follow-up spray 12 months post-planting. Each seedling was planted with a slow-release fertiliser tablet (Typhoon™ for Natives), and none were watered during or after planting. A mix of canopy species was planted over the entire restoration project at a density of ca. 150 stems ha^{-1}.

2.4. Fitness proxies

We scored four fitness proxies in November–December 2013 (ca. 15 months after planting; 19 months after germination) as follows. First, we scored plant survival. Plants were scored as either 'alive' if green foliage and/or a green stems were present or scored as 'dead' if no green foliage was present or no plant was found within the plant guard of a marked stake.

Plant fitness should in part be proportional to wood and stem production, which can be expressed as a function of height (Falster and Westoby, 2003). We scored aboveground height for each plant with a graduated telescopic surveyor's stave (Alumi Staff Pty. Ltd). Height was recorded as the vertical distance between the ground and the most distal photosynthetic tissue of each plant.

We scored each plant for the presence/absence of invertebrate herbivory (hereafter herbivory) and pathogen related stress (hereafter stress) to provide a proxy of biotic interactions of the plants (e.g. herbivory and stress resistance Linhart and Grant, 1996). The presence of herbivory was scored as present when ca. >5% of the entire foliage showed signs of herbivory. Stress was scored as present if leaf browning and fall were evident or leaf blight or rust was observed.

We excluded 35 ($n_{local} = 8$; $n_{intermediate} = 14$; $n_{distant} = 13$) of the 1434 plants that were planted but could not be relocated during our survey. A total of 1399 plants ($n_{local} = 469$; $n_{intermediate} = 499$; $n_{distant} = 431$) remained and were used for determining survival. Of these 1399 plants, 179 were dead ($n_{local} = 84$; $n_{intermediate} = 59$; $n_{distant} = 36$) and were excluded from provenance performance analyses. Hence, provenance effects on height, herbivory and stress was conducted on the remaining 1220 plants ($n_{local} = 385$; $n_{intermediate} = 440$; $n_{distant} = 395$).

2.5. Data analysis

We explored provenance effects on the four fitness proxies with generalised linear models in R v 3.2.3 (R Core Team, 2015). Provenance was treated as a fixed factor. The four fitness proxy response variables were treated as follows: survival, herbivory and stress were binary variables and a binomial link function was used; plant height was a continuous variable and data identity was used. Model residuals were visually assessed for normality and we used Box-Cox transformations of the data to meet normality of residuals assumptions where appropriate (Box and Cox, 1964).

Since plant height is likely to be associated with herbivory and stress among provenances, we explored the provenance effects on plant height controlling for variation of these two factors. We did this by including herbivory and stress as predictor variables together with provenance in the model exploring the variation in height, and including the herbivory × provenance and stress × provenance 2-way interactions.

To help demonstrate the influence of provenance on the fitness proxies, we calculated the relative home-site advantage for each fitness proxy by dividing the difference between local and non-local provenance fitness proxy values by the local fitness proxy value (e.g. for the relative home site height advantage of the intermediate provenance, we calculated [local height − intermediate height] / local height).

3. Results

We observed a striking difference in survival across provenances, with significantly higher survival of distant and intermediate

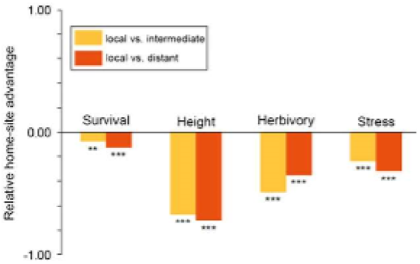
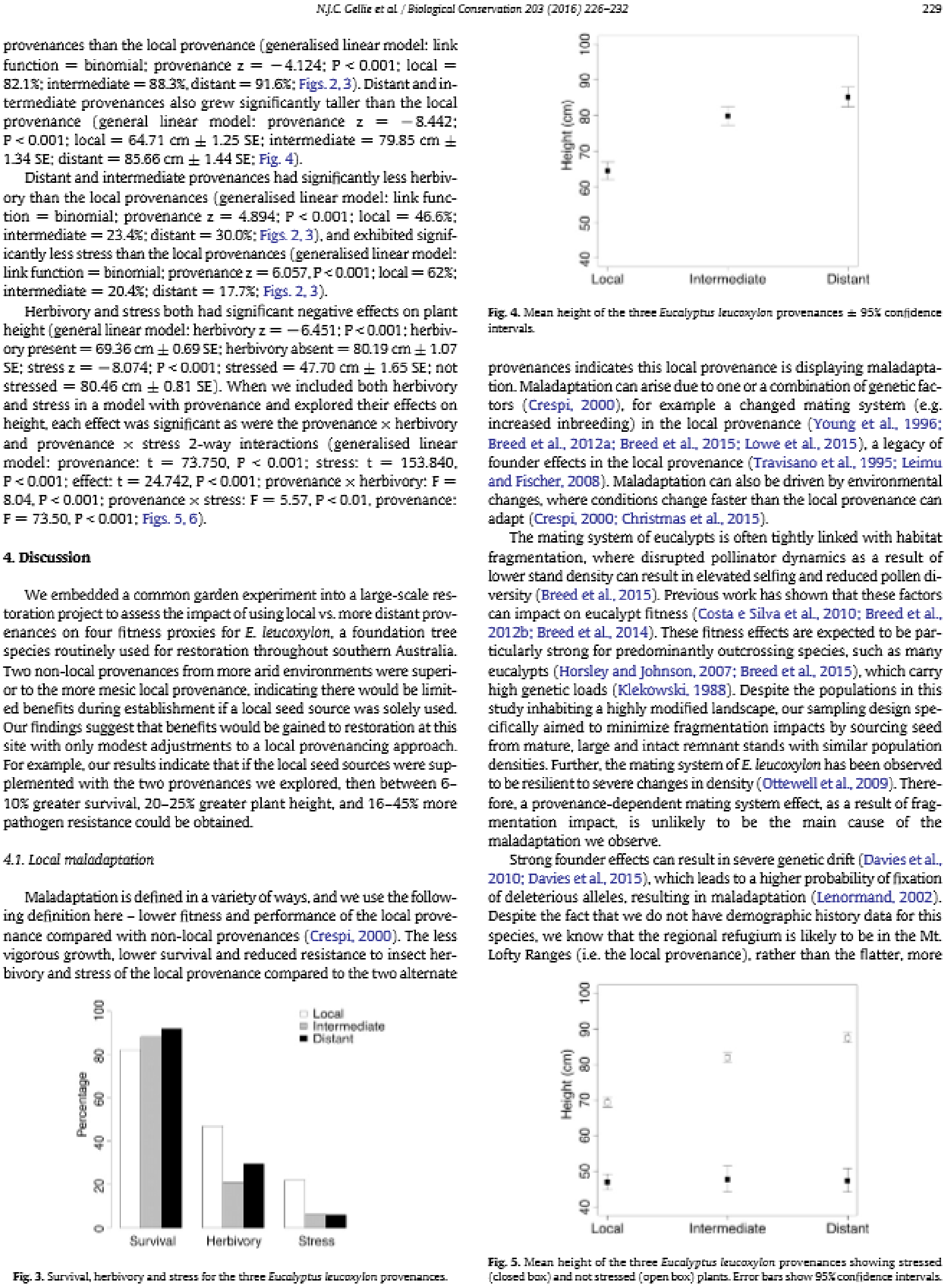


Fig. 2. Relative home-site advantage of the provenances for each of four fitness proxies. Results above the horizontal line would indicate a local advantage, and results below the line indicate local is not best. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.



provenances than the local provenance (generalised linear model: link function = binomial; provenance $z = -4.124$; $P < 0.001$; local = 82.1%; intermediate = 88.3%, distant = 91.6%; Figs. 2, 3). Distant and intermediate provenances also grew significantly taller than the local provenance (general linear model: provenance $z = -8.442$; $P < 0.001$; local = 64.71 cm ± 1.25 SE; intermediate = 79.85 cm ± 1.34 SE; distant = 85.66 cm ± 1.44 SE; Fig. 4).

Distant and intermediate provenances had significantly less herbivory than the local provenances (generalised linear model: link function = binomial; provenance $z = 4.894$; $P < 0.001$; local = 46.6%; intermediate = 23.4%; distant = 30.0%; Figs. 2, 3), and exhibited significantly less stress than the local provenances (generalised linear model: link function = binomial; provenance $z = 6.057$, $P < 0.001$; local = 62%; intermediate = 20.4%; distant = 17.7%; Figs. 2, 3).

Herbivory and stress both had significant negative effects on plant height (general linear model: herbivory $z = -6.451$; $P < 0.001$; herbivory present = 69.36 cm ± 0.69 SE; herbivory absent = 80.19 cm ± 1.07 SE; stress $z = -8.074$; $P < 0.001$; stressed = 47.70 cm ± 1.65 SE; not stressed = 80.46 cm ± 0.81 SE). When we included both herbivory and stress in a model with provenance and explored their effects on height, each effect was significant as were the provenance × herbivory and provenance × stress 2-way interactions (generalised linear model: provenance: $t = 73.750$, $P < 0.001$; stress: $t = 153.840$, $P < 0.001$; effect: $t = 24.742$, $P < 0.001$; provenance × herbivory: $F = 8.04$, $P < 0.001$; provenance × stress: $F = 5.57$, $P < 0.01$, provenance: $F = 73.50$, $P < 0.001$; Figs. 5, 6).

4. Discussion

We embedded a common garden experiment into a large-scale restoration project to assess the impact of using local vs. more distant provenances on four fitness proxies for *E. leucoxylon*, a foundation tree species routinely used for restoration throughout southern Australia. Two non-local provenances from more arid environments were superior to the more mesic local provenance, indicating there would be limited benefits during establishment if a local seed source was solely used. Our findings suggest that benefits would be gained to restoration at this site with only modest adjustments to a local provenancing approach. For example, our results indicate that if the local seed sources were supplemented with the two provenances we explored, then between 6–10% greater survival, 20–25% greater plant height, and 16–45% more pathogen resistance could be obtained.

4.1. Local maladaptation

Maladaptation is defined in a variety of ways, and we use the following definition here – lower fitness and performance of the local provenance compared with non-local provenances (Crespi, 2000). The less vigorous growth, lower survival and reduced resistance to insect herbivory and stress of the local provenance compared to the two alternate provenances indicates this local provenance is displaying maladaptation. Maladaptation can arise due to one or a combination of genetic factors (Crespi, 2000), for example a changed mating system (e.g. increased inbreeding) in the local provenance (Young et al., 1996; Breed et al., 2012a; Breed et al., 2015; Lowe et al., 2015), a legacy of founder effects in the local provenance (Travisano et al., 1995; Leimu and Fischer, 2008). Maladaptation can also be driven by environmental changes, where conditions change faster than the local provenance can adapt (Crespi, 2000; Christmas et al., 2015).

The mating system of eucalypts is often tightly linked with habitat fragmentation, where disrupted pollinator dynamics as a result of lower stand density can result in elevated selfing and reduced pollen diversity (Breed et al., 2015). Previous work has shown that these factors can impact on eucalypt fitness (Costa e Silva et al., 2010; Breed et al., 2012b; Breed et al., 2014). These fitness effects are expected to be particularly strong for predominantly outcrossing species, such as many eucalypts (Horsley and Johnson, 2007; Breed et al., 2015), which carry high genetic loads (Klekowski, 1988). Despite the populations in this study inhabiting a highly modified landscape, our sampling design specifically aimed to minimize fragmentation impacts by sourcing seed from mature, large and intact remnant stands with similar population densities. Further, the mating system of *E. leucoxylon* has been observed to be resilient to severe changes in density (Ottewell et al., 2009). Therefore, a provenance-dependent mating system effect, as a result of fragmentation impact, is unlikely to be the main cause of the maladaptation we observe.

Strong founder effects can result in severe genetic drift (Davies et al., 2010; Davies et al., 2015), which leads to a higher probability of fixation of deleterious alleles, resulting in maladaptation (Lenormand, 2002). Despite the fact that we do not have demographic history data for this species, we know that the regional refugium is likely to be in the Mt. Lofty Ranges (i.e. the local provenance), rather than the flatter, more

Fig. 3. Survival, herbivory and stress for the three *Eucalyptus leucoxylon* provenances.

Fig. 4. Mean height of the three *Eucalyptus leucoxylon* provenances ± 95% confidence intervals.

Fig. 5. Mean height of the three *Eucalyptus leucoxylon* provenances showing stressed (closed box) and not stressed (open box) plants. Error bars show 95% confidence intervals.

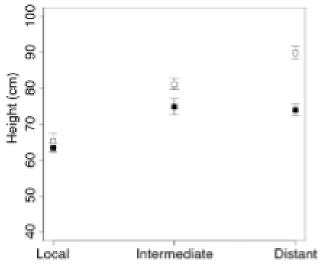


Fig. 6. Mean height of the three *Eucalyptus leucoxylon* provenances with herbivory (closed box) and without herbivory (open box). Error bars show 95% confidence intervals.

arid surrounding areas (i.e. the intermediate and distant provenances) (Byrne et al., 2008; Guerin et al., 2016). Thus, it is also unlikely that founder effects explain the maladaptation we observed in our study.

Environmental conditions might be changing faster than the local provenance can adapt, which may have driven the maladaptation we observed (Christmas et al., 2015). Aridity is likely to be a strong agent of selection in many eucalypt species (Steane et al., 2014; Booth et al., 2015; Dillon et al., 2015; Breed et al., 2016a), and it may be that we are detecting a signature of climate change impacts on the local provenance. Climate models suggest that southern Australia (including the study region) is undergoing significant increases in aridity (CSIRO and BoM, 2014). *E. leucoxylon* forms large populations with high inter-population gene flow (Ottewell et al., 2009; Nicolle, 2013), suggesting that effective population size and genetic diversity should be high (Petit and Hampe, 2006; Ottewell et al., 2010). Consequently, selection should have ample genetic variation to act upon, thus allowing selection to act efficiently (Lenormand, 2002) and not constraining adaptation in this system (Christmas et al., 2015). However, the long-lived nature of *E. leucoxylon* indicates that the selection that took place on the adult generation (the provenances used in our study) was under a pre-climate change environment, possibly resulting in an adaptation lag to the rapid climate change occurring today (Kremer et al., 2012). To further explore the extent of climate adaptation lag in *E. leucoxylon*, it is imperative to extend monitoring of this trial into the future, with a particular focus on differential recruitment between provenances. As aridity increases with climate change, a greater adaptation lag should manifest by maladaptation further increasing in years to come.

We also observed that the local provenance was more susceptible to herbivores and pathogens than the two non-local provenances in the common garden environment of this study. These results support the findings that pathogen severity in planted eucalypts is strongly affected by provenance (Stone et al., 1998), and strong negative correlations between leaf diseases and growth rate are the norm (e.g. in *Eucalyptus globulus*Carnegie et al., 1994). The differential herbivore impacts between provenances we observed was similarly observed in *Eucalyptus tereticornis* (Hancock and Hughes, 2014), where local provenances were more susceptible to phytophagous insects than non-local provenances. Follow-up studies are needed to fully explore whether the herbivore and pathogen responses identified in the local provenance were directly due to shifting herbivores and pathogens, or indirectly as a result of the poorer performance of the local provenance increasing its susceptibility to local herbivores and pathogens.

Overall, our study provides evidence of local maladaptation in *E. leucoxylon* from the southern Mt. Lofty Ranges in South Australia. To increase our understanding of the generality and spatial extent of maladaptation in this species, we need to perform more extensive trials, expanding on the number of trial sites and the number of provenances tested. Such trials could include peripheral provenances (e.g. testing performance of leading and trailing edge), which are likely to respond differently to selection and have different levels of adaptive potential (Kremer et al., 2014), or provenances that occupy past climate refugia as these could hold cryptic sources of genetic diversity (Temunovic et al., 2013). Furthermore, as highlighted recently in Prober et al. (2016), managing the potential interactions of local maladaptation with cryptic population structures, and non-climate related adaptations (e.g. community ecology issues such as pollinators), need to be managed at a site level. It would also be useful to test whether the intermediate and distant provenances were locally adapted, and a reciprocal transplant trial would resolve this query (Kawecki and Ebert, 2004).

4.2. Implications for management

We provide clear evidence that no advantage would be gained during the establishment of *E. leucoxylon* at this site by solely using the local provenance rather than including two additional provenances from more arid environments. The local and most mesic provenance performed considerably worse than two alternative and more arid provenances for each of four fitness proxies. Thus, we recommend that for *E. leucoxylon*, at least in this area, non-local and more arid provenances should be incorporated into additional restoration trials. We show that alternative and superior provenances could span up to 45 km into more arid locations. In this case, for example, even a conservative addition of more distant provenances into the seed mix, described in Broadhurst et al. (2008) as composite provenancing, would substantially reduce negative effects of exclusively using local provenancing. Thus, the results of this study provide a management option that is not reliant on local provenance (Breed et al., 2013), and is consistent with the directionality of provenance choice recommended by Prober et al. (2015). It is important that our trial is monitored into the future to study undetected responses of the non-local provenances, such as monitoring flowering time and the genotypes of recruits. It would also be important to establish additional trials that explore the responses of additional provenances from across a broader range of environments.

The ability of large restoration projects to achieve their goals will rely on the scalability of current practices to meet global demand (Merritt and Dixon, 2011), the certainty of seed supply (Broadhurst et al., 2016), and the capacity of restoration plantings to be dynamic and adjust to global change (Perring et al., 2015; Breed et al., 2016b). We were fortunate to be able to incorporate our experiment directly into a restoration project, and information garnered from this trial can be directly incorporated into the adaptive management framework of the stakeholders. Embedding experiments into restoration projects, such as we have done here, promises to improve the efficacy of restoration practices, and lead to innovation with real end-user impact (Suding et al., 2015). Undertaking these activities in partnership with the end-users will empower stakeholders and help develop strategies to scale-up restoration efforts to face the challenges set down by current global targets.

Data accessibility

Common garden data available from Aekos Digital repository for survival (Gellie et al., 2016a) DOI: 10.4227/05/57ECADFA02465, and growth (Gellie et al., 2016b) DOI: 10.4227/05/57ECB10786A57.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.earlhumdev.2016.08.012.

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Supplementary Information

Supplementary Table A1. Precipitation and aridity data at common garden

Common Garden[1]	**Climate data**
Annual mean aridity index[2]	1.01
Annual precipitation (mm)[3]	
Mean (20 year)	736.8
Mean (since 1868)	790.0
2010	829.1
2011	731.0
2012	748.0
2013	719.8
2014	583.4

CHAPTER 4: FITNESS AND FUNCTIONAL TRAIT DIFFERENTIATION

Fitness and functional trait differentiation of *Eucalyptus gracilis* across an aridity gradient

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Principal Author

Name of Principal Author (Candidate)	Nicholas Gellie		
Contribution to the Paper	Contributed to ideas, assessed field trials, undertook analysis composed figures and wrote manuscript and subsequent revisions.		
Overall percentage (%)	70		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	24/01/2017

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Contribution to the Paper	Formulated the ideas, commented on and edited subsequent manuscript drafts		
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***Gellie N. J**., Breed M. F., Baruch Z., Hill K. E., and A. J. Lowe (submitted). Fitness and functional trait differentiation of Eucalyptus gracilis across an aridity gradient. Evolutionary Applications*

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I hereby certify that this statement of contribution is accurate

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Formulated the ideas, undertook analysis, prepared figures and tables commented and edited subsequent manuscript drafts.

I hereby certify that this statement of contribution is accurate and consent to the inclusion of this manuscript in Nick Gellie's PhD thesis

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Involved in developing ideas and contributed to revisions of the manuscript.

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Abstract

Preserving local adaptation is often stated as a principle intention of ecological restoration, but is seldom tested. Exploring intraspecific variation in fitness and functional traits through reciprocal transplant experiments can identify local adaptation and the adaptive potential of source populations used for revegetation under climate change. In this study, we established two common gardens in 2010 at either end of an aridity gradient (300 km apart; 238 vs. 386 mm long-term mean annual rainfall) for three *Eucalyptus gracilis* provenances – a species commonly used in restoration plantings across southern Australia. We included provenances local to both sites, plus a third provenance geographically and climatically intermediate to the two local provenances. In 2015, we measured fitness (e.g. survival, height, reproductive activity) and functional traits known to relate to aridity adaptation (e.g. wood density, specific leaf area) of *ca*. 100 plants per provenance per trial. Planting site was a strong predictor of survival, which was lower for all provenances at the arid site. Plant height and reproductive activity displayed adaptive differentiation, where the local provenance performed best at both sites. All functional traits had a significant provenance effect, indicating a genetic basis to aridity adaptation. Each trait also showed greater intra-provenance variation at the more arid site, which is consistent with the observation that unfavourable conditions increase genetic variation in functional traits. We conclude that *E. gracilis* occurs across a range of climatic conditions by combining specific adaptations to aridity and adjusting to environmental change via phenotypic plasticity. With the increasing aridity that is predicted in southern Australia under climate change, our results support arid-to-mesic provenance sourcing strategies for restoration of this species to help build the adaptive potential of newly planted populations.

INTRODUCTION

Plant seed is versatile and has become the preferred propagule of revegetation because it transports, stores and sows efficiently (Galatowitsch 2012) however the restoration sector needs enormous quantities of quality seed to meet demand for current global initiatives (Perring et al. 2015; Broadhurst et al. 2016). Climate change and habitat fragmentation are important determinants of seed quality, and a number of novel seed sourcing methods have been proposed to mitigate these impacts (Broadhurst et al. 2008; Breed et al. 2013; Prober et al. 2015), but need evidence-based approaches to achieve desired targets (Suding 2011; Chazdon et al. 2015, Miller et al 2016).

Applying principles of evolutionary biology to restoration can improve restoration outcomes (Mijangos et al. 2015) by, for example, helping to overcome genetic quality issues of seed sourced from fragmented populations or seed that is unable to adapt quickly enough to climate change (Sgrò et al. 2011; Carroll et al. 2014). The genetic consequences of habitat fragmentation are well studied, and fragmented tree populations generally experience increased inbreeding and decreased pollen diversity (Eckert et al. 2010; Breed et al. 2015b; Lowe et al. 2015). Thus avoiding highly fragmented populations will reduce negative fitness consequences and maintain the adaptive potential of seed used for restoration (Lowe et al. 2005, 2015; Breed et al. 2012).

Seed sourcing alternatives have been proposed to mitigate the impacts of climate change but require more sophistication than simply avoiding isolated trees. Alternative strategies include translocating seed from non-local sources (e.g. predictive provenancing; Crowe and Parker 2008) or mixing multiple sources that include some ‘local’ seed (e.g. climate-adjusted provenancing ; Prober et al. 2015, composite provenancing; Broadhurst et al. 2008, and admixture provenancing; Breed et al. 2013). However these strategies are not universally accepted as they suffer from a lack of empirical studies (Gibson et al. 2016), and the perception of risk of malaptation from translocation (Bucharova 2016).

The theoretical pros and cons of such alternative seed sourcing strategies are well documented (Broadhurst et al. 2008; Crowe and Parker 2008; Sgrò et al. 2011; Breed et al. 2013; Prober et al. 2015, Bucharova et al. 2016). However there is no universally optimal strategy and uptake of these strategies will ultimately be determined by a mix of conservation concerns, flow on effects, restoration objectives, operational feasibility and evidence. Notwithstanding the complex nature of restoration decision making, the perception of genetic risk in these alternative strategies (e.g. increased risk of outbreeding depression and the associated disruption of local adaptation) remains a major barrier to implementation. Though, with risks of outbreeding depression often overstated, particularly for outcrossing and common species (Frankham et al. 2011) used for restoration, it is reasoned that if local adaptation is already being eroded by climate change, these genetic risks are worth taking (Hoffmann and Sgro 2011; Aitken and Bemmels 2015; Gellie et al. 2016). Despite this ongoing debate, both empirical and theoretical evidence generally supports the need for changes to seed sourcing practices to help reduce the impacts of climate change (Aitken et al. 2008; Broadhurst et al. 2008; O'Neill et al. 2008; Breed et al. 2013; Breed et al. 2016b), and a clear way forward to help provenance decision-making on this complex issue is to test provenance performance *in situ* during restoration (Gellie et al. 2017).

Reciprocal transplant experiments that incorporate a range of provenances and are planted along environmental gradients provide a powerful experimental tool for restoration ecologists (Mátyás 1996; McLean et al. 2014; Christmas et al. 2015; Caddy-Retalic et al. 2017). However, deciding on the traits to measure in provenance trials of long-lived trees that reflect fitness or adaptation is a nontrivial exercise. Traditional fitness components (reproductive success and lifespan) are impractical (Petit and Hampe 2006), and most tree species used in restoration are non-model species that lack the substantial trait or genomic resources available for model and commercial species. Survival, reproduction and growth metrics are commonly measured in provenance trials (Funk et al. 2008; Sandel et al. 2011) because survival and reproductive traits clearly relate to fitness, and growth traits are expected to correlate with resource acquisition efficiency.

Functional traits (e.g. wood density, specific leaf area, and stomatal size) affect a plant's ability to acquire, use, and conserve resources (Reich et al. 2003). These traits relate to plant fitness in the environment but are less commonly measured in provenance trials, despite widespread trees commonly show variation in these traits across climate gradients (McLean et al. 2014). Therefore, describing the variation in these functional traits within and among provenances can provide an effective way to distinguish the resource acquisition strategies of plants in response to their origin (i.e. genetic effects) and at planting sites (i.e. environmental effects; Sandel et al. 2011; Funk et al. 2016). Hence, identifying trends in functional traits for key restoration species has considerable merit as this information can assist land managers to select appropriate seed for restoration.

In this study we established two common garden experiments in the winter of 2010 of three *Eucalyptus gracilis* (F. Muell.) provenances that differed markedly in aridity (e.g. the aridity index = mean annual precipitation/potential evapotranspiration and ranged from 0.15 (arid site) to 0.37 (mesic site) Figure S1). Common gardens were established local to the two most environmentally dissimilar provenances (i.e. arid vs. mesic). A third provenance geographically and climatically intermediate to the two locals was also planted at the gardens. We used these provenance trials to address the following questions: (1) is there fitness and functional trait differentiation across provenances? If so, (2) are fitness and trait differences consistent with aridity adaptation? Since adaptive differentiation is expected in most plant species and our provenances cross a considerable environmental gradient, we expect our provenances to display local adaptation for both fitness and functional traits (Leimu and Fischer 2008; Hereford 2009).

METHODS

Study species

Eucalyptus gracilis is a multi-stemmed, sclerophyllous tree common throughout sand and sand-over-limestone soils (Nicolle 1997). *Eucalyptus gracilis* is common in Mediterranean type environments of southern Australia that are characterised by hot dry summers and cooler wet winters. *Eucalyptus gracilis* generally grows from 2 to 6 m high, it has small white hermaphroditic flowers and is pollinated primarily by small insects and, to a lesser degree, by birds and small marsupials (Slee et al. 2006; Morrant et al. 2010). *Eucalyptus gracilis* probably has a late-acting self-incompatibility mechanism, resulting in mixed mating to preferential outcrossing (tm generally >0.80) (Horsley and Johnson 2007). This assumption is supported by published *E. gracilis* mating system data (tm = 0.75-0.95) (Breed et al. 2014; Breed et al. 2015b). Serotinous fruit (i.e. seed released in response to an environmental trigger that often includes fire) are held over numerous years, with drying triggering seed release. Seed are small (<2 mm diameter) and gravity dispersed. Based on published data from the ecologically similar species *Eucalyptus incrassata* and our own field observations of *E. gracilis*, ants harvest the majority of newly fallen seed, except during particularly heavy seed release events (e.g. fire mediated serotiny; Wellington 1985a, 1985b).

Provenance collections

To capture aridity differences that might contribute to fitness and functional trait variation, we chose three provenances of *E. gracilis* in the mallee vegetation of the Murray-Darling Basin (Fig. 1 and S2). The least arid southwest provenance was at Monarto (35.12° S, 139.16° E), the intermediate provenance was at Yookamurra Sanctuary (34.52° S, 139.47° E; hereafter Yookamurra), and the most arid provenance was at Scotia Sanctuary (33.22° S, 141.15° E; hereafter Scotia). Scotia is *ca.* 300 km northeast of Monarto, and *ca.* 220 km northeast of Yookamurra. Climate data indicate that Scotia is more arid than both Yookamurra and Monarto (aridity index: Scotia = 0.15, Yookamurra = 0.25, Monarto = 0.37; aridity index = mean annual precipitation/potential evapotranspiration; mean annual rainfall: Scotia = 238 mm, Yookamurra = 278 mm, Monarto 386 mm) (Williams et al. 2010; 2012). Our provenances represent the central range of the climatic envelope of *E. gracilis* (http://spatial.ala.org.au/; Supplementary Fig. S1).

We collected open-pollinated seed from >100 mature fruit across the canopies of 20 trees at each provenance in January 2010. Nearest neighbours were excluded from sampling to minimise the risk of re-sampling maternal trees. Additionally a separation of >20m between donor tree sources was made to reduce the inclusion of clonal clusters or close relatives in the seed mix. Fruit from each mother tree were processed separately. Fruit were dried for *ca*. 2-3 weeks in individual plastic containers under greenhouse conditions. Dried fruit were then shaken in these plastic containers to encourage seed release. These seed were then sown in February 2010 (details below). The mean height of mother trees was 5.88 m ± SD 2.37 at Scotia, 6.96 m ± SD 1.58 at Yookamurra, and 6.03 m ± SD 1.23 at Monarto.

Figure 1.

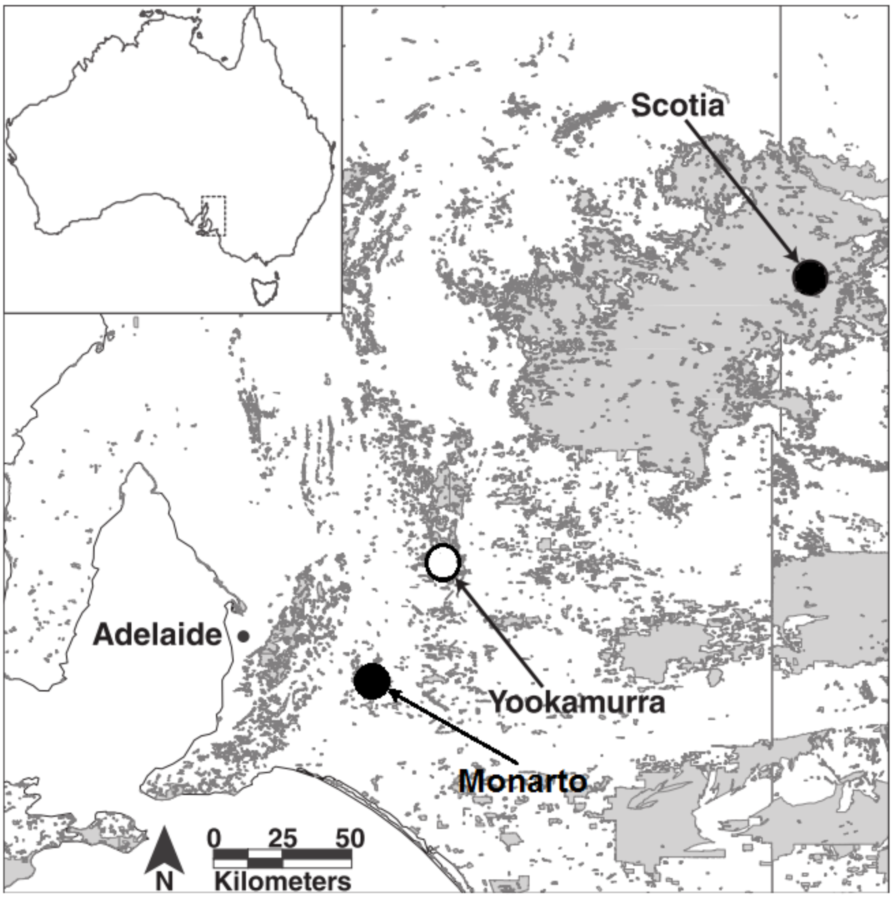

Figure 1. Map showing the locations of the *Eucalyptus gracilis* provenances and transplant experiments, Monarto and Scotia (closed circles) and intermediate provenance Yookamurra (open circle). Shading indicates the extant of remnant vegetation. The inset map shows the study location in Australia.

Reciprocal transplant trial

To assess fitness and functional trait differences between the three provenances, we established common garden experiments in 2010 within 10 km of the mother trees at Scotia and at Monarto (Fig. 1). The experimental plantings in the common gardens were part of restoration projects undertaken on both of these properties.

Ten replicates (i.e. pots) of *ca*. 20 seed from each mother tree were sown onto saturated, commercial potting mix. Germination was conducted under glasshouse conditions in Adelaide, South Australia (34.92° S, 138.61° E). Four-week-old seedlings were moved to a full-sun nursery at the Mt Lofty Botanic Gardens, South Australia (34.99° S, 138.72° E). Family cohorts of seedlings (i.e. all progeny from one mother tree) were grown in crates that were shifted and rotated weekly to minimise confounding effects of location in glasshouse/nursery. To minimise selection on seedling fitness, the most central seedling within each pot was chosen, and the rest were removed prior to planting.

Plantings took place at Scotia (33.12° S, 141.78° E) and Monarto (35.83° S, 139.9° E) in June 2010. We used a randomised complete block design (Addelman 1969), with five seedlings per family per site. Seedlings from each mother tree were present in a random location once in every second of 10 rows per site. Two seedlings died in the nursery prior to planting (for sample sizes see Table 1). Planting sites were prepared by rotary hoeing to remove residual surface vegetation, parallel rip-lines were drawn through at 3 m intervals, and seedlings were spaced at 3 m intervals within these rip lines. Planting took place in May of 2010 and no manual watering or fertilisation took place at the time of planting or thereafter. A 200 x 200 x 500 mm tree guard (Global Land Repairs, Fyshwick) was installed which surrounded each seedling to protect it against vertebrate herbivores (e.g. rabbits, kangaroos).

Table 1. Number of *Eucalyptus gracilis* seedlings planted in 2010 as part of the reciprocal transplant experiment, number of surviving plants at the time of sampling (2015), and surviving plant reproductively activity (%), height, wood density, specific leaf area and stomatal size mean ± SE.

	2010 Planted (count)	**2015 survival (count)**	**Reproductive (%)**	**Height (cm)**	**Wood density (g/cm^3)**	**Specific leaf area (cm^2/g)**	**Stomatal size (μm^2)**
Planted at Monarto (mesic)							
Monarto provenance	100	89	36.0	96.40 ± 7.64	0.73 ± 0.02	34.26 ± 0.80	745 ± 51
Yookamurra provenance	100	87	19.6	82.61 ± 8.17	0.72 ± 0.04	34.92 ± 0.92	784 ± 67
Scotia provenance	99	81	26.3	74.44 ± 7.96	0.74 ± 0.02	33.79 ± 0.84	815 ± 69
Planted at Scotia (arid)							
Monarto provenance	99	69	24.5	194.12 ± 12.80	0.76 ± 0.02	35.27 ± 1.20	754 ± 64
Yookamurra provenance	100	78	22.2	208.59 ± 11.51	0.77 ± 0.02	35.25 ± 1.02	797 ± 97
Scotia provenance	100	69	61.5	213.77 ± 11.21	0.80 ± 0.02	33.35 ± 0.92	925 ± 56

Fitness and functional trait data

In July 2015 (5 years, 1 month after planting) we measured the aboveground stem height of all plants (distance from ground to highest point of plant; hereafter height), plant survival (yes, no), whether plants were reproductively active or not (yes, no; yes = signs of buds, flowers, fruit; no = no signs of any reproductive activity), and collected plant samples to measure leaf and wood functional traits.

Branchlets with *ca*. 10 leaves and woody stem with diameter >5mm were used as a standard sampling unit for all plants. Branchlets were excised from the northern aspect of all plants with secateurs. These branchlets and stems were stored in moist, cool (*ca*. 4° C) hermetic plastic bags until processed. The youngest, fully developed and expanded leaf on each branchlet was sampled to estimate leaf area, by scanning and measuring with ImageJ (Schneider et al. 2012). Leaves were then oven dried at 65ºC for 48 hours and weighed. Specific leaf area is the ratio of leaf area and mass.

Wood density is the volumetric mass found by determining the ratio between dry weight and volume. Wood density was obtained by cutting 2–3 cm of uniform segments from the proximal end of one branchlet for all plants. Length and diameter were measured with a digital calliper to obtain volume. Afterwards, the segments were dried at 85 ºC for 48-72 hours and weighed.

Stomatal size was estimated on 13 (±2 SE) of the *ca.* 100 plants per provenance-site combination. We cut 1 x 1 cm^2 leaf sections from halfway along the lamina to prepare the cuticles, placed them in sterile test tubes and submerged in a 2:1 solution of 35% hydrogen peroxide: 80% ethanol (v/v), then warmed them until the leaf sections became translucent and the cuticle began to separate from the leaf tissue. Crystal violet 0.05% w/v was used to stain the cuticles, which were then fixed to slides with warmed phenol glycerine jelly. An Olympus UC50 camera mounted on an AX70 Olympus microscope (Olympus™ Australia) was used to image both the abaxial and adaxial surfaces of each leaf using AnalySIS (Soft Imaging System, Münster, Germany). ImageJ (Schneider et al. 2012) was used to measure guard cell-pair length and width of five stomata for both the abaxial and adaxial leaf surfaces with ten stomata measured per leaf. Guard cell-pair length and width were multiplied together to obtain stomatal size (μm^2). Leaves from 50 individuals at the Scotia common garden (i.e. Scotia n = 18; Yookamurra n = 18; Monarto n = 14) and, 26 individuals at the Monarto common garden (i.e. Scotia n = 6; Yookamurra n = 10; Monarto n = 10) were used for analysis. Sample numbers varied due to availability of suitable material.

Data analysis

We used general and generalised linear mixed effects models in the package nlme v. 3.1-120 (Pinheiro et al. 2015) in R v. 3.2.3 (R Core Team 2015) to assess the effects of plant provenance and trial site on *E. gracilis* fitness and functional traits. Plant provenance and trial site were treated as fixed effects, and family (i.e. mother tree) was treated as a random effect nested within provenance. A binomial distribution with a logit link function was used for survival and reproductive data and a Gaussian distribution with no link function was fitted for height, wood density, specific leaf area and stomata area. Planting row was not included in models because model residuals showed no spatial autocorrelation, and not including row in the model reduced model parameterisation. Fitted model residuals were visually assessed for normality and were normally distributed in each case (except for binomial models). We had too few individuals within families to confidently estimate trait heritability.

RESULTS

Climate variation

During the first two years of the reciprocal transplant experiment (2010 and 2011) the observed annual rainfall was the highest on record at both locations (Table S1), more than twice the historical mean annual rainfall at Scotia and 1.4 times the historical mean annual at Monarto. The rainfall in 2012, 2013, 2014, and 2015 was more similar to long term mean annual rainfall trends.

Fitness variation

Trial site had a significant effect on plant survival, with higher survival at Monarto (85.6%) than at Scotia (71.9%). We observed no significant provenance or provenance*site effects on plant survival (Table 1, Fig. 2A).

Trial site and provenance*site had significant effects on plant height (Fig. 2B). Scotia plants grown at Scotia grew taller than either Yookamurra or Monarto plants at Scotia (i.e. the height of plants grown at Scotia: from Monarto 194.1 cm ± 6.5 SE; from Yookamurra 208.3 cm ± 5.6 SE; from Scotia 213.4 cm ± 5.9 SE). Monarto plants grew taller than either Yookamurra or Scotia plants when grown at Monarto (i.e. the height of plants grown at Monarto: from Monarto 96.4 cm ± 3.9 SE; from Yookamurra 82.9 cm ± 4.1 SE; from Scotia 72.8 cm ± 4.2 SE). Overall, plants, regardless of origin, were significantly taller at Scotia (205.4 cm ± 3.5 SE) than those grown at Monarto (84.7 cm ± 2.4 SE) (Table 1, Fig. 2B).

Provenance and provenance*site had significant effects on reproductive activity, with site being marginally not significant (Fig. 2C). Scotia plants grown at Scotia had considerably more reproductively active plants (61.5%) than those from Monarto (25.5%) or Yookamurra (22.2%). At Monarto, local plants were again the most reproductively active (36.0%), with Scotia (26.3%) and Yookamurra (19.6%) plants showing lower rates of reproductive activity. Plants from Yookamurra were the least reproductively active, and plants from Scotia were the most reproductively active (Scotia = 42.1%; Yookamurra = 21.3%; Monarto = 30.6%) regardless of where they were planted. We observed higher levels of reproductively active plants at Scotia (34.9%) than at Monarto (27.0%), but the overall effect of planting site was marginally not significant (P = 0.055).

Figure 2.

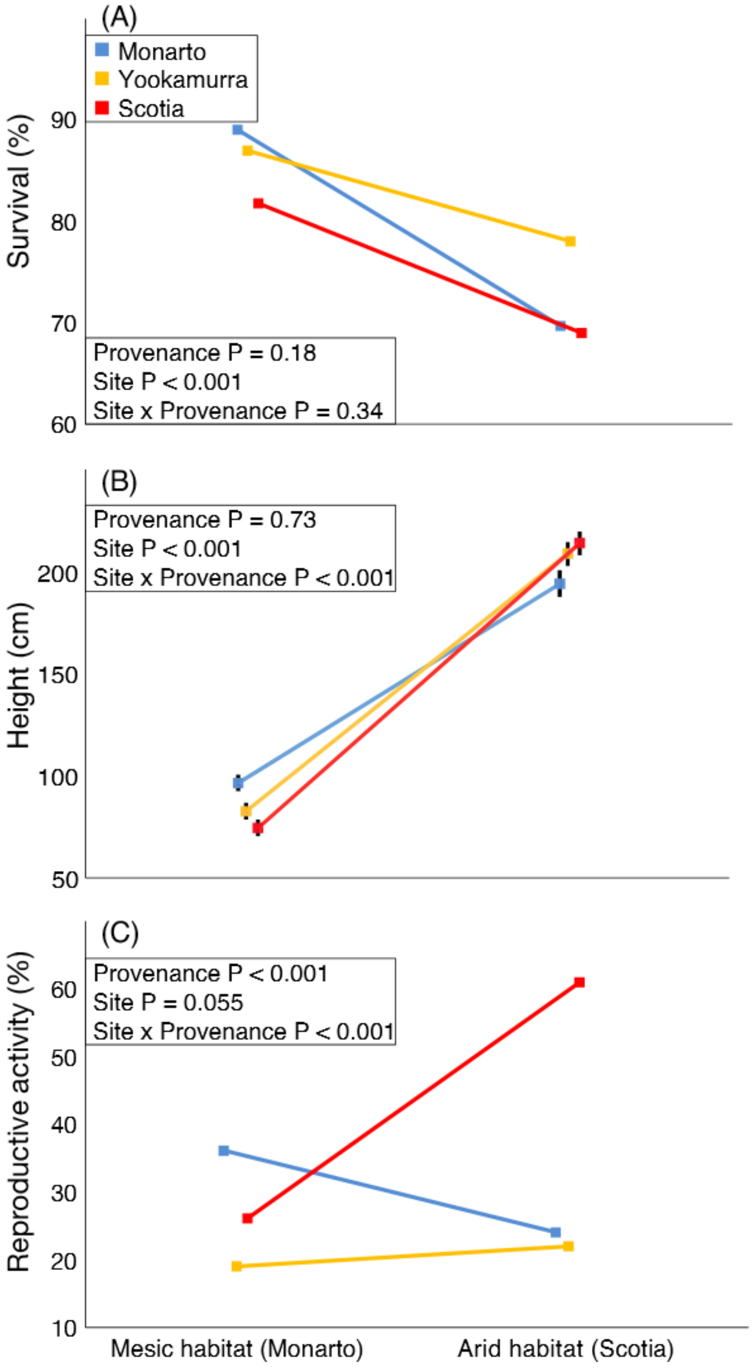

Figure 2. Fitness traits, (A) survival, (B) height, and (C) reproductive activity of the three *Eucalyptus gracilis* provenances in the two common garden trials. Error bars show SE.

Functional trait variation

Trial site, provenance and provenance*site all had significant effects on wood density (Fig. 3A). We observed clear differences in wood density among all provenances grown at Scotia, (i.e. the wood density of plants grown at Scotia: from Monarto = 0.764 gcm^{-3} ± 0.007 SE; from Yookamurra = 0.772 g/cm^3 ± 0.006 SE; from Scotia = 0.802 g/cm^3 ± 0.011 SE). At Monarto, we identified weaker differences between provenance wood densities, but Scotia plants again had the highest wood density (i.e. the wood density of plants grown at Monarto: from Monarto = 0.733 g/cm^3 ± 0.005 SE; from Yookamurra = 0.721 g/cm^3 ± 0.007 SE; from Scotia = 0.743 g/cm^3 ± 0.008 SE). Overall, wood density was significantly higher at the more arid Scotia site (0.779 g/cm3 ± 0.005 SE) than at Monarto (0.732 g/cm^3 ± 0.004 SE), and was also significantly higher for Scotia sourced plants (0.768 g/cm^3 ± 0.028 SE) than either Yookamurra (0.739 g/cm^3 ± 0.023 SE) or Monarto (0.743 g/cm^3 ± 0.022 SE) sourced plants (Table 1, Fig. 3B).

Provenance had a significant effect on specific leaf area (Fig. 3B). Specific leaf area was largest for Yookamurra plants (35.18 cm^2/g ± 0.36 SE), with Scotia having the lowest specific leaf area (Monarto = 34.68 cm^2/g ± 0.35 SE; Scotia = 33.53 cm^2/g ± 0.32 SE). There was no significant effect of planting site or provenance*site on specific leaf area.

Provenance had a significant effect on stomatal size (Fig. 3C), where Scotia plants had the largest stomata (Monarto = 750.6 µm ± 22 SE; Yookamurra = 793 µm ± 34 SE; Scotia = 898 µm ± 25 SE) (Table 1, Fig. 3C). There was no significant effect of planting site or provenance*site on stomatal size.

Figure 3.

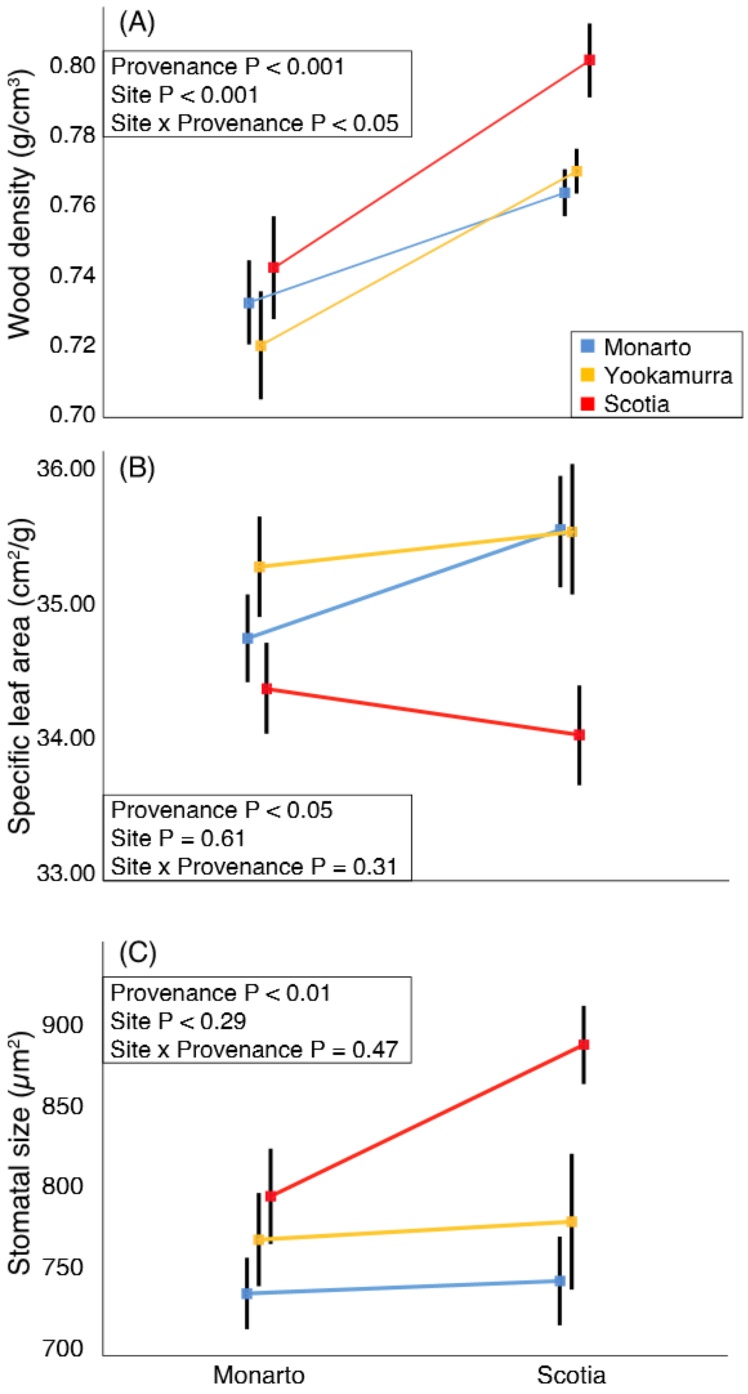

Figure 3 Functional traits, (A) wood density, (B) specific leaf area, and (C) stomatal size of the three *Eucalyptus gracilis* provenances in the two common garden trials. Error bars show SE.

DISCUSSION

By embedding common garden experiments of *Eucalyptus gracilis* into two large-scale restoration projects *ca.* 300 km apart in semi-arid Australian mallee, we observed a combination of adaptive differentiation and plastic responses to environmental conditions. The environment at the planting site was a strong predictor of survival, which was lower for all provenances when grown at the more arid site. Plant fitness – as expressed by height and reproductive activity – showed patterns consistent with adaptive differentiation. Patterns of functional trait variation – as expressed by leaf and wood architecture – were largely consistent with aridity adaptation, and also corresponded with theory that unfavourable conditions will increase genetic variation in functional traits (Hoffmann and Merilä 1999). Together, our findings suggest that the *E. gracilis* provenances studied have substantial adaptation to aridity and they also have some capacity to physiologically acclimate to changing conditions. With the trajectory of increasing aridity predicted in southern Australia under climate change (CSIRO and BoM 2014), there would be some benefit in seed sourcing strategies for this species in restoration that seeks to combine seed sources in an arid-to-mesic direction. Orientating seed selection in a prescribed direction is likely to provide a low cost, low risk amendment to current practice that will help build the adaptive potential of planted populations to climate change.

Variation in fitness traits

We observed a significant genotype-by-environment interaction for height and reproductive activity, indicating local adaptation and a home-site advantage for these traits. These results suggest a current local advantage is being maintained within provenances. The provenance-site combination with the largest plants in our experiment was Scotia plants grown at Scotia (i.e. the most arid site), which indicates adaptation to aridity. This taller, arid provenance should have a competitive advantage over smaller non-local provenances. Our observations are largely consistent with previous work on eucalypts that has shown aridity is a strong agent of selection in eucalypts (Steane et al. 2014; Booth et al. 2015; Breed et al. 2016a).

Aridification and the formation of the Australian sandy deserts since the mid-Pleistocene has meant the ability to exploit water availability is a major factor in the evolutionary history of eucalypts (Parsons 1969). It is currently thought that eucalypts in southern Australia responded to the aridification during the mid-Pleistocene by *in situ* adaptation and persistence, rather than large-scale migration (Byrne 2008). Indeed, our study species *E. gracilis* forms large populations in Mediterranean-type mallee environments of southern Australia with high intra-population gene flow (Breed et al. 2015a), suggesting that the effective population size and genetic diversity of *E. gracilis* is likely to be large (Petit and Hampe 2006; Kremer et al. 2012). Therefore, selection should have adequate genetic variation to act efficiently (Lenormand 2002), and adaptation to aridity is unlikely to be constrained by low genetic diversity in this system (Christmas et al. 2015).

However, *E. gracilis* provenances grown at the arid site outgrew plants at the more mesic site, which appears counterintuitive if we consider the arid site to be water limited. This inconsistency can in part be explained by a high degree of phenotypic plasticity in the species and the exceptionally high water availability at the arid site in 2010 and 2011 (Table S1). This inter-annual variation in rainfall is likely to have provided a low water stress environment for the arid site plants during these establishment years, which in turn is likely to have led to greater than expected growth. Furthermore, during the latter stages of the trial we observed a higher weed load at the mesic compared to arid site (e.g. introduced grasses *Avena* sp., *Briza* sp., *Vulpia* sp.; personal observations). This weed load is likely to have increased interspecific competition for resources (e.g. water and nutrients) at the mesic site. Resource competition is known to dramatically reduce biomass of other eucalypt species during establishment (Ball et al. 2002). Despite these observations, the relative height of provenances within sites was consistent with aridity being a strong stressor and important agent for selection.

The long-lived nature of mallee eucalypts, including *E. gracilis* (e.g. 100s of years; Clarke et al. 2010), means that selection most likely took place on the adult generation in an environment before anthropogenic climate change. This pattern of selection could perpetuate an adaptation lag to contemporary conditions, under which rapid climate change is occurring (Kremer et al. 2012). To further explore the extent of climate adaptation lag in *E. gracilis* it will be important to maintain monitoring of these experimental trials into the future, focusing on fecundity and the differential recruitment or introgression between provenances. If an adaptation lag occurs at the rear edge of the distribution of this species (i.e. more arid provenances), it should result in these more arid provenances having higher fitness in a future drying climate, as suggested by Hampe and Petit (2005). We would therefore expect that the arid provenance will out-perform the mesic provenances at the mesic site in years to come, and it is our intension to continue this research.

In our study, local plants had higher reproductive activity than non-local plants. These reproductive differences were most pronounced at the more arid site where the local, and larger plants were approximately 2.5 times more reproductively active than the smaller plants from the two more mesic provenances. Similar results have been observed in *E. globulus* ssp. *globulus,* where reproductive activity was dependent on plant size (Jordan et al. 2000). The correlation of reproductive output with plant size is not limited to eucalypts (Samson and Werk 1986), and can have important consequences for plant population dynamics. The relationship between reproduction and plant size should be a consideration for land managers undertaking restoration with *E. gracilis*, but further work on pollinator visitation, and ultimately recruitment, is required to understand the significance of plant size in this system.

Functional trait variation

Functional traits are those traits that should have a significant effect on fitness, giving plants the ability to acquire, use, and store resources in their given environment (Reich et al. 2003). For example, plants tended to employ a suite of strategies (e.g. slow tissue turnover, low transpiration rate, or strong plant defence traits) to exploit low-resource environments, and these strategies are physiologically linked to growth-related traits (e.g. leaf morphology for photosynthesis and defence, wood density for nutrient transport, resource allocation and strength). Therefore, if functional traits are found to have a significant genetic component then these traits are likely to influence provenance fitness if provenances are transferred to different resource environments (e.g. more arid sites).

Our results showed that the three functional traits observed (specific leaf area, wood density, stomatal size) each had a significant genetic effect. As such, the more mesic provenances are likely to be functionally constrained under resource limited conditions (e.g. lower water availability during times of drought). Furthermore, the significant genetic effect we observed for our functional traits indicate that our *E. gracilis* provenances have a genetic base to their adaptation to aridity, and we believe that these findings can serve as a suitable predictor of environmental suitability for provenances. We observed an amplification of phenotype differentiation between provenances in the more resources limited site at Scotia, which is consistent with the theory that unfavourable conditions increase genetic variation in functional traits (Hoffmann and Merilä 1999).

Site, provenance and site*provenance each had significant effects on wood density, where denser wood for all provenances was observed at Scotia, indicating trait plasticity, but also the Scotia provenance had the highest wood density across both common gardens, indicating a heritable component to this functional trait. Higher wood density is usually associated with narrower vessels that can safeguard plants against the loss of conductivity that may occur due to embolism (Lens et al. 2013). Denser wood should allow mallee species such as *E. gracilis* to survive and grow under conditions of very low soil water availability (Pfautsch et al. 2016). In our case, the aridity index (mean annual precipitation/potential evapotranspiration) was considerably lower at the arid site (0.15) compared to the mesic site (0.37). Observing wood density to be heritable is consistent with a recent study of 28 eucalypt species that found hydraulic architecture to be adapted to water availability (Pfautsch et al. 2016).

Specific leaf area represents a trade-off between resource allocation for construction and photosynthetic ability of the leaf structure. Specific leaf area tends to decrease with increasing aridity (Cornelissen et al. 2003; Wright et al. 2004), however this relationship is not ubiquitous (Schulze et al. 2006; Warren et al. 2006). We observed trends in specific leaf area that were consistent with the former expectations. Our results were also consistent with the intraspecific variation in specific leaf area observed in *E. tricarpa* (McLean et al. 2014) and a common garden trial of 29 eucalypt species by Warren et al. (2006). However, the absence of a significant provenance*site effect most likely indicates that selection has not acted strongly enough on specific leaf area to differentiate values between provenances. Despite the small observed range of specific leaf area (33.35 cm^2/g for Scotia provenance planted at Scotia to 35.27 cm^2/g for Monarto provenance planted at Scotia), the significant provenance effect does indicate a genetic component. In a similar fashion to wood density, we observed greater variation in specific leaf area among provenances when planted at the more arid site.

Gas exchange in leaves is a function of the stomatal size and density that along with light intensity, carbon dioxide concentration and temperature control the photosynthetic rate of plants. To conserve water, stomatal size is generally observed to decrease with increasing aridity (Franks et al. 2009; Carlson et al. 2015). Contrary to this trend, stomata of the arid provenance were the largest in our study which would seem to be a disadvantage for water use efficiency in this arid environment. There is some evidence of vernal geophytes (e.g. herbaceous open woodland plants) developing larger stomata as an avoidance strategy to preclude growth in the hotter drier months (Hodgson 2010), but we have no evidence to support this strategy being adopted by this species. Interestingly, greater intra-provenance variation in this trait also manifest at the more arid site while the intraspecific stomatal sizes tended to converge at the mesic site. It would therefore be useful to investigate the leaf architecture of this species further (e.g. stomatal density, guard cells and boundary layer) and directly measure gas exchange to help reconcile the stomatal size anomaly we have reported on. Overall, leaf architecture observed in the Scotia provenance indicate a relatively conservative strategy that suits arid conditions (Hetherington and Woodward 2003) which are predicted for our study region in the coming decades (CSIRO and BoM 2014).

CONCLUSIONS

Considerable differentiation was observed in fitness and functional traits between provenances of *E. gracilis* with common phenotypic trends emerging. These complex genetic vs. environment and multi-trait patterns convey a more complete picture of provenance performance than studies based on just one or two fitness or functional traits. The evolutionary insight provided by the broader suite of traits we explored in situ through transplant experiments has great utility for land managers undertaking restoration, especially when applied across the species range. With the climate change projections indicating a trajectory of increasing aridity for southern Australia, our findings suggest that, at least for our study species, natural stands have a strong genetic basis to aridity adaptation. Therefore, mixing *E. gracilis* seed sources during restoration in an arid-to-mesic direction on a spatial scale beyond what would normally be considered 'local' may be the best climate change mitigation strategy for this species as it should increase their adaptive capacity.

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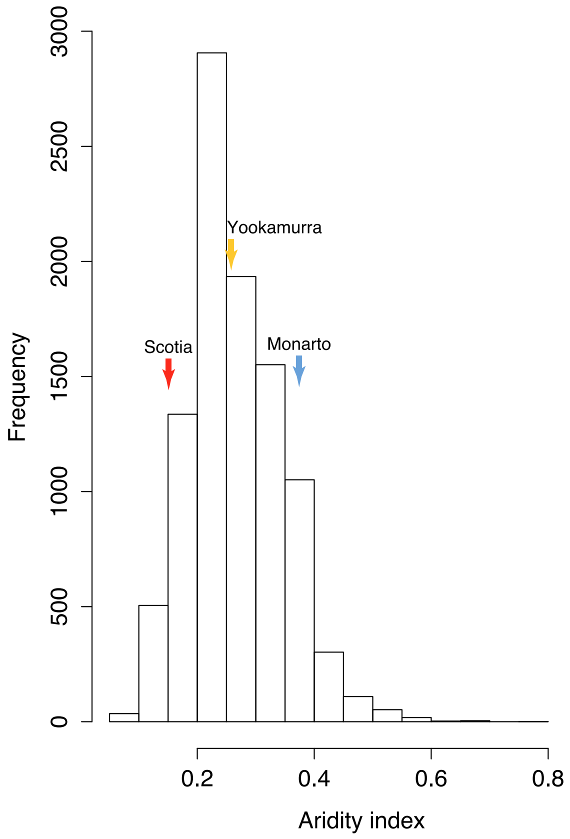
Data archiving statement

Data for this study will be available at the AEKOS digital repository if the paper is accepted for publication.

Supplementary Information

Figure S1. Annual mean aridity index for *Eucalyptus gracilis* presence data post-1980 from the Atlas of Living Australia (http://www.ala.org.au/). Arrows indicate the aridity index of the three provenances.



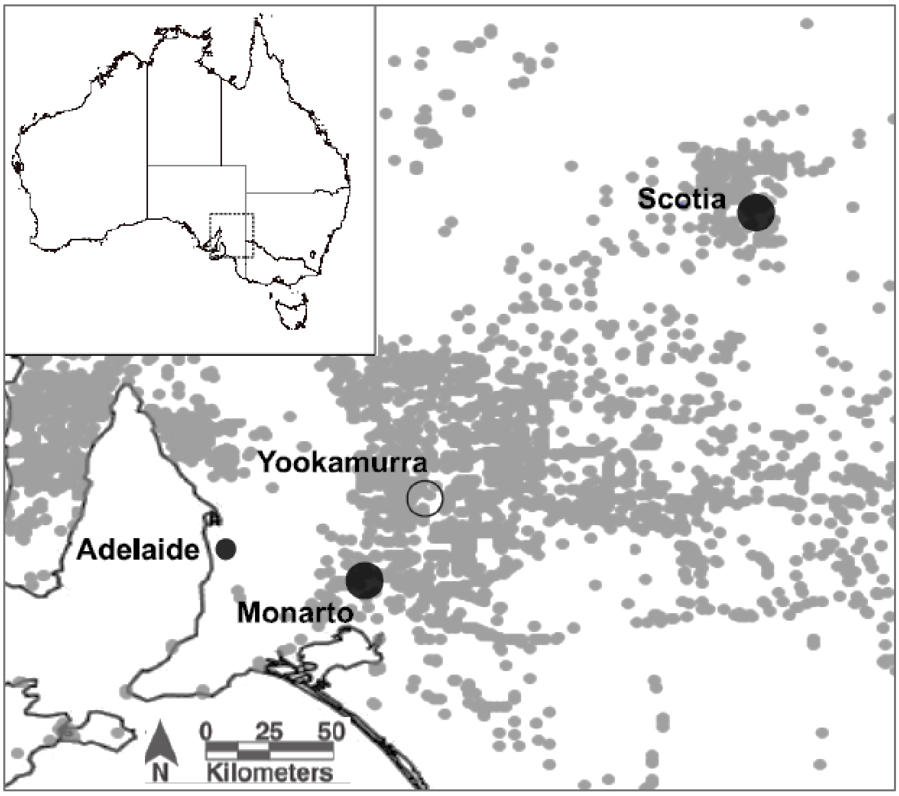


Figure S2. Map showing the locations of the *Eucalyptus gracilis* provenances and transplant experiments, Monarto and Scotia (closed circles) and intermediate provenance Yookamurra (open circle). Grey points indicate the incidence of records for *Eucalyptus gracilis* in the study location http://spatial.ala.org.au.

Table S1. Annual mean aridity index and precipitation data at Monarto and Scotia, plus the observed rainfall for 2010, 2011, 2012, 2013, 2014 and 2015.

	Monarto	**Scotia**
Annual mean aridity index	0.37*	0.14*
Annual precipitation (mm*)*		
Mean	386^	238^
2010	525.4	480.7
2011	445.4	454.6
2012	465.9	164.5
2013	410.0	172.8
2014	359.0	214.2
2015	397.9	235.1

Footnote
*Data sourced from Williams et al. (2010)
^Data sourced from http://www.bom.gov.au

CHAPTER 5: THE IMPACTS OF SEED CHOICE

Impacts of provenance on establishment of four Myrtaceae species

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Publication Details	**Gellie N. J.**, Breed M. F., and A. J. Lowe. Impacts of provenance on establishment of four Myrtaceae species. Restoration Ecology

Principal Author

Name of Principal Author (Candidate)	Nicholas Gellie		
Contribution to the Paper	Formulated the ideas conducted field trials and wrote manuscript		
Overall percentage (%)	80		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	24/01/2017

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Andrew Lowe (corresponding author and principal supervisor)		
Contribution to the Paper	Formulated the ideas commented and edited subsequent manuscript drafts		
Signature		Date	24/01/2017

CHAPTER 5: THE IMPACTS OF SEED CHOICE

***Gellie N. J.**, Breed M. F., and A. J. Lowe (submitted). Impacts of provenance on establishment of four Myrtaceae species. Restoration Ecology*

Gellie N. J. C. (candidate)

Formulated the ideas, conducted field trials, undertook analysis, wrote manuscript

I hereby certify that this statement of contribution is accurate

Signed Date: 23rd January 2017

Breed M. F. (co-supervisor)

Formulated the ideas, undertook analysis, prepared figures and tables commented and edited subsequent manuscript drafts.

I hereby certify that this statement of contribution is accurate and consent to the inclusion of this manuscript in Nick Gellie's PhD thesis

Signed Date: 23rd January 2017

Lowe A. (primary supervisor)

Formulated the ideas, commented and edited subsequent manuscript drafts

I hereby certify that this statement of contribution is accurate and consent to the inclusion of this manuscript in Nick Gellie's PhD thesis

Signed Date: 23rd January 2017

Abstract

Few ecosystems are as diverse yet remain as vulnerable as those found in Mediterranean type landscapes. The heavily altered and degraded Australian Mediterranean biome is testimony to this fragility. Replanting locally sourced native vegetation is an accepted method for actively restoring these degraded landscapes and assumes plants to be universally locally adapted. However, this common practice fails to acknowledge the disruptions to local adaptation that can be caused by a changing climate and habitat fragmentation. We established two common garden experiments (4 species × 4 provenances) of *ca*. 1900 seedlings each to test the suitability of local provenances on the Yorke Peninsula, a highly degraded part of the Mediterranean biome in Australia. The provenances spanned a 250 km aridity gradient running the length of the peninsula (aridity index = 0.28 for Port Pirie at the northern, arid end; aridity index = 0.49 at Point Yorke at the southern, mesic end). The common gardens coincided with current restoration projects at the two southern provenance localities that (i.e. Point Pearce and Point Yorke). We explored the effect of provenance on four fitness proxies; survival, above-ground height, susceptibility to insect herbivory, and pathogen related stress of the surviving plants in these gardens after 10 months of establishment. We found that the local provenance of each species did not have the highest survival and the most arid provenance showed significantly more growth than all other provenances in 5 of the 8 species × environment combinations. Site and species were stronger predictors of the incidence of invertebrate herbivory and pathogen related stress than the source of the plants. We conclude that although additional work is required to fully explore the potential risks of translocation in this system, arid to mesic transfer may provide a low risk management strategy to increase climate resilience on Yorke Peninsula.

CHAPTER 5: THE IMPACTS OF SEED CHOICE

INTRODUCTION

The Mediterranean biome is characterized by mild wet winters and warm dry summers. This biome occupies <5% of the world's terrestrial land surface and is spread across five continents. It is disproportionally rich in biodiversity and harbours almost 20% of the world's vascular plant species (Cowling et al. 1996). In Australia, the Mediterranean biome is disjunct but concentrated on the south-central and south-western continental margins (Rundel et al 2016). Floristically, the Australian Mediterranean biome is heavily represented by eucalypt dominated woodland and grassland communities.

Historically the arability and productive nature of this biome has led to widespread land conversion (e.g. since European settlement it is estimated that approximately 47 million ha of eucalypt woodlands have been cleared in Australia (Booth et al. 2015)). Ecological restoration has been proposed as the principal strategy to reverse these trends (Aronson and Alexander 2013) through the reinstatement and/or rehabilitation of self-sustaining communities (Hobbs and Norton 1996). However, the current day scale of restoration (e.g. The Bonn Challenge aspires to restore 350 million ha by 2030) has no historical precedent, so optimal outcomes will require honing the efficiency of strategies. Restoration is evolving to meet these new challenges by pursuing practices that are backed by scientific rigour (Miller et al 2016) and as a result restoration has become a truly multi-disciplinary pursuit (Choi et al. 2008).

Supplying adequate amounts of appropriate seed is a major challenge for restoration projects (Galatowitsch 2012). Genetic quality of seed can be compromised by the effects of climate change (Sgrò et al. 2011; Carroll et al. 2014) and habitat fragmentation (Breed et al. 2015). The usual method to source and supply seed in restoration takes a local provenancing approach (i.e. collecting seed from remnant plant populations in as close proximity to the restoration site as possible in order to exploit local adaptation; Callaham 1963). Despite local-adaptation investigation being the foundation of forestry trials for the past 250 years (Langlet, 1971) and retrospective assessment of these trials giving great insight to the effects of climate change (Mátyás, 1994, 1996; Leites et al, 2012), local adaptation is seldom empirically tested in a restoration context (Gibson et al. 2016).

Local-provenancing is a prescriptive strategy that by definition is static and has no internal mechanism to account for the dynamic nature of climate-change or fragmentation on restoration habitats. Hence the uniform application of local-provenancing in dynamic systems has attracted close academic scrutiny in recent years because it lacks the nuances necessary to react to rapid abiotic changes (Broadhurst et al. 2008; Sgrò et al. 2011; Breed et al. 2013). In response, seed sourcing practices are evolving to meet the challenges of climate change and fragmentation. Many alternative strategies have been proposed, for example, augmenting seed sources with a mix of seed to increase genetic diversity (e.g. composite or admixture provenancing; Broadhurst et al. 2008; Breed et al. 2013) or matching provenances to future climates (e.g. climate adjusted or predictive provenancing; Sgrò et al. 2011; Prober et al. 2015). These conceptual advances have prompted some researchers to empirically test these alternative strategies (e.g. Breed et al. 2016b; Gellie et al. 2016) but further testing is required.

Exploring the validity of alternative provenancing methods is analogous to exploring local adaptation which has a long history in plant ecology (Clausen, Keck & Hisey 1941), evolutionary biology (Turreson 1922) and forestry (Mátyás 1996). Classically, investigating local adaptation has been done with reciprocal transplant experiments (Kawecki & Ebert 2004). Understanding the direction and magnitude of adaptation within a species, and the role the environment plays on it, allows us to determine whether non-local plants can survive and potentially thrive under particular environmental conditions. With alternative provenancing strategies being seriously considered in restoration practice (McDonald, Jonson and Dixon 2016), there remains an urgent need to test their efficacy.

In this study, we measured fitness responses of four provenances of four foundation tree species (*E. oleosa, E. porosa, E. socialis* and *M. lanceolata*) in a reciprocal transplant experiment on Yorke Peninsula, South Australia. We selected these species as they are commonly used for restoration in the Mediterranean biome in southern Australian, and each lacks comprehensive provenance data. The four provenances we used span the strong climate gradient on Yorke Peninsula. We embedded our garden experiments within two active restoration projects in the more mesic southern half of Yorke Peninsula. This experiment was used to explore the following questions: (1) Does provenance affect survival, growth, herbivory and the impact of pathogens in our study species? If so, (2) is variation in these traits in line with local adaptation? We extend our interpretation of these two questions with regional management recommendations for optimising restoration strategies of our four focal species, however this study will have broader implications for temperate plant species more generally.

METHODS

Study species

We used three sclerophyllous mallee eucalypt tree species in our study - *Eucalyptus oleosa, E. porosa*, and *E. socialis* (all in the Myrtaceae family). Each species is widespread in southern Australia, well represented in mallee communities and span the Mediterranean biome (see Supporting Information Fig. S1-S3). They all have hermaphroditic flowers that are pollinated primarily by insects and, to a lesser degree, birds and small marsupials (Nicolle 2013). Each species is also likely to have a mixed mating system that has been either directly observed (*E. socialis*: Breed et al. 2012, 2016b) or based on observations from closely related eucalypts (Horsley and Johnson 2007). All grow to approximately 10 m or slightly larger in the case of *Eucalyptus porosa* in higher rainfall areas (Nicolle 2013). Each produces small seed (<2 mm diameter) that are gravity dispersed.

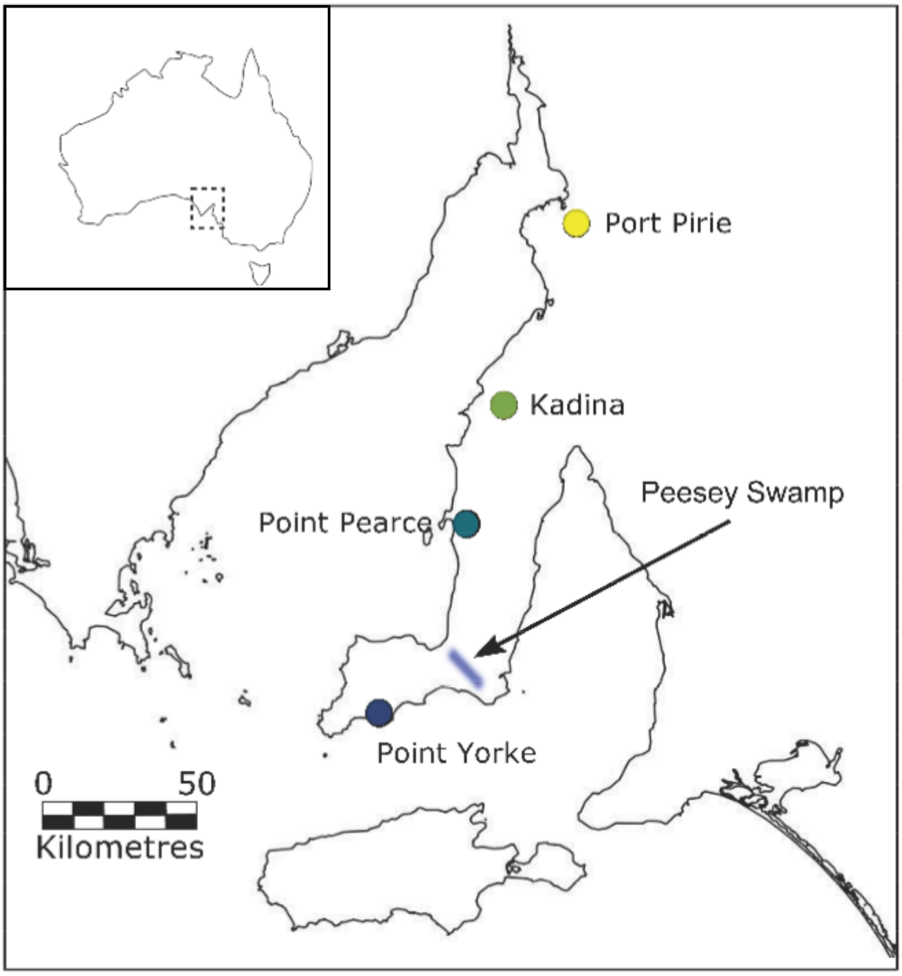
Our fourth study species is *Melaleuca lanceolata* and is also a member of the Myrtaceae family. *M. lanceolata* is widespread across southern Australia and found throughout the Mediterranean biome (see Supporting Information Fig. S4). It is an evergreen shrub growing to approximately 5 m in lower rainfall areas. It produces lanceolate glaucous leaves (Brophy, Craven and Doran 2013). This species exhibits a mixed mating system with long lasting hermaphroditic inflorescences that are most likely pollinated by insects and birds (Brophy, Craven and Doran 2013) and to a lesser extent small marsupials (Pestell & Petit 2008). Like the eucalypts, the seed of *M. lanceolata* are small and gravity dispersed (<1 mm diameter *pers. obs.*).

Populations and seed collection

Similar to other regions in the Mediterranean biome (e.g. south-western Western Australia and the Cape region of South Africa) the mallee woodlands and associated plant communities of Yorke Peninsula, South Australia have experienced substantial modification. The peninsula has been extensively cleared for agriculture and grazing, with most remaining biodiversity hugs the coastline, road networks, less arable ridgelines and the southern extremity. The peninsula runs north-south, is *ca.* 60 km wide and 240 km long, with over 560 km of coastline. The local climate is Mediterranean, with hot dry summers and moderately wet winters, with the north being more arid than the south (Port Pirie: mean annual precipitation = 362 mm, aridity index = 0.28; Point Yorke: mean annual precipitation = 485 mm, aridity index = 0.49).

To capture aridity differences that might contribute to fitness variation, we sourced seed from four provenances along the peninsula for each of the four study species in in the spring of 2014 (see Supporting Information Fig. S1-S4). The provenance localities were Port Pirie (-33.1279°, 138.1449°), Kadina (-34.0731°, 137.7473°), Point Pearce (-34.3839°, 137.5001°), and Point Yorke (-35.2122°, 137.1468°) (Fig. 1). The provenances were each intact native stands of >100 individuals. Open-pollinated seed was collected from the canopies of at least 10 donor trees in each case. Fruit for each provenance-species combination was pooled prior to sowing. Germination and rearing of seedlings was conducted in full sun at a commercial nursery in South Australia (Brooklyn Park, -34.9328°, 138.5340°) for approximately 6 months prior to planting.

Figure 1. Map showing the extent of Yorke Peninsula and the locations of provenance selection. Reciprocal transplant experiments were located at Point Yorke and Point Pearce and the Peesey swamp ecotone is highlighted.



Common garden trials

We used a fully randomised block design at common gardens located at Point Pearce (-34.4073°, 137.4936°) and Point Yorke (-35.2277°, 137.1781°). In each common garden, we planted a total of *ca.* 120 plants per provenance-species combination, where 40 plants per provenance were randomly assigned to a planting location in one of three blocks at each common garden (Table 1 has planting numbers). Each seedling was hand-planted into ground that was mechanically prepared with a V-plough to remove weeds and produce a rip line. A 200 × 200 × 400 mm (Geofabrics) UV stabilised corflute tree guard was put around each seedling to protect it against vertebrate herbivores that are present at both sites (e.g. rabbits, kangaroos). None of the seedlings were watered or fertilized at the time of planting or thereafter. Our experimental plots were embedded into larger restoration projects where canopy species were direct seeded at a stocking density of *ca*. 150 stems ha^{-1}.

Fitness proxies

We scored four recognised fitness proxies that included survival, growth, insect herbivory, and pathogen impact in May 2015, *ca*. 10 months after planting and 16 months after germination. We scored survival as either ‘alive’ if green foliage and/or a green stems were present, or ‘dead’ if no green foliage was present or no plant was found within the plant guard at a marked stake.

Plant fitness is also known to be proportional to wood and stem production, which can be expressed as a function of height (Falster & Westoby, 2003). We therefore scored aboveground height for each plant with a graduated telescopic surveyor's stave (Alumi Staff Pty. Ltd). Height was recorded as the vertical distance (cm) between the ground and the most distal photosynthetic tissue of each plant.

We scored each plant for the presence/absence of invertebrate herbivory (hereafter herbivory) and pathogen related stress (hereafter stress) to provide a proxy of biotic interactions of the plants, which is also an important component of plant fitness (e.g. herbivory and stress resistance; Linhart & Grant, 1996). The presence of herbivory was scored as present when *ca.* 5% of the entire foliage showed signs of herbivory. Stress was scored as present if leaf browning and fall were evident or leaf blight or rust was observed.

Of the 3840 plants reared for the two common gardens (= 120 plants × 4 provenances × 4 species × 2 common gardens), 99 plants did not survive from the nursery, leaving 3741 seedlings. At Point Yorke, a total of 1850 plants (*E. oleosa* = 419, *E. lanceolata* = 477, *E. porosa* = 480, *E. socialis* = 474) were used to assess survival, of which 1734 survived (*E. oleosa* = 388*, E. lanceolata* = 458, *E. porosa* = 453, *E. socialis* = 435). These survivors were used to assess growth, herbivory and pathogen impact. At Point Pearce, 1891 plants (*E. oleosa* = 457, *E. lanceolata* = 478, *E. porosa* = 478, *E. socialis* = 478) were used to assess survival, of which 1779 survived (*E. oleosa* = 403, *E. lanceolata* = 458, *E. porosa* = 465, *E. socialis* = 453). These survivors were used to assess growth, herbivory and pathogen impact (see Table 1 for further details of provenance sizes at the common gardens).

Data analysis

We used general and generalised linear mixed effects models in the package nlme v. 3.1-120 (Pinheiro et al. 2015) in R v 3.2.3 (R Core Team, 2015) to explore the effects of plant provenance and trial site on plant fitness. Plant provenance and trial site were treated as fixed effects. A binomial distribution with a logit link function was used for survival, herbivory and stress data, and a Gaussian distribution with no link function was fitted for the continuous variable height where the data identity was used. Fitted model residuals were visually assessed for normality and were normally distributed in each case (except for binomial models).

Since plant height has previously been seen to be associated with herbivory and stress among provenances (Hancock et al. 2012; Gellie et al. 2016) we explored the effects of provenance on the height of plants controlling for variation of these two biotic factors. We did this by including herbivory and stress as covariate predictors together with provenance in a model exploring variation in height, and including the herbivory × provenance and stress × provenance 2-way interactions.

RESULTS

Eucalyptus oleosa

The local provenance at Point Yorke had significantly higher survival than the Point Pearce provenance ($P < 0.05$), but survived similarly to the two most northern provenances (Table 1). The Point Pearce provenance at Point Yorke had the lowest survival of any species-provenance-garden combination (82.7%). No overall difference in survival was observed among provenances at Point Pearce, however Point Yorke – the southernmost provenance – had the second lowest survival of any species-provenance-garden combination (83.0%).

At Point Yorke, provenance had a significant effect on plant height ($P < 0.001$) with the local provenance growing higher than all other provenances (Figure 2). At Point Pearce, provenance again had a significant effect on height ($P < 0.01$), where plants from Port Pirie – the northern most provenance – grew the largest.

At Point Yorke, provenance had no significant effect on herbivory rate ($P = 0.34$). At Point Pearce, provenance did have a significant effect on herbivory rate, where the local plants (6.4%) had significantly more herbivory than Point Yorke (0%; $P < 0.05$), Kadina (1.9%; $P < 0.05$), and Port Pirie (1.8%; $P < 0.05$). When controlling for this effect in the model the same growth trends remained.

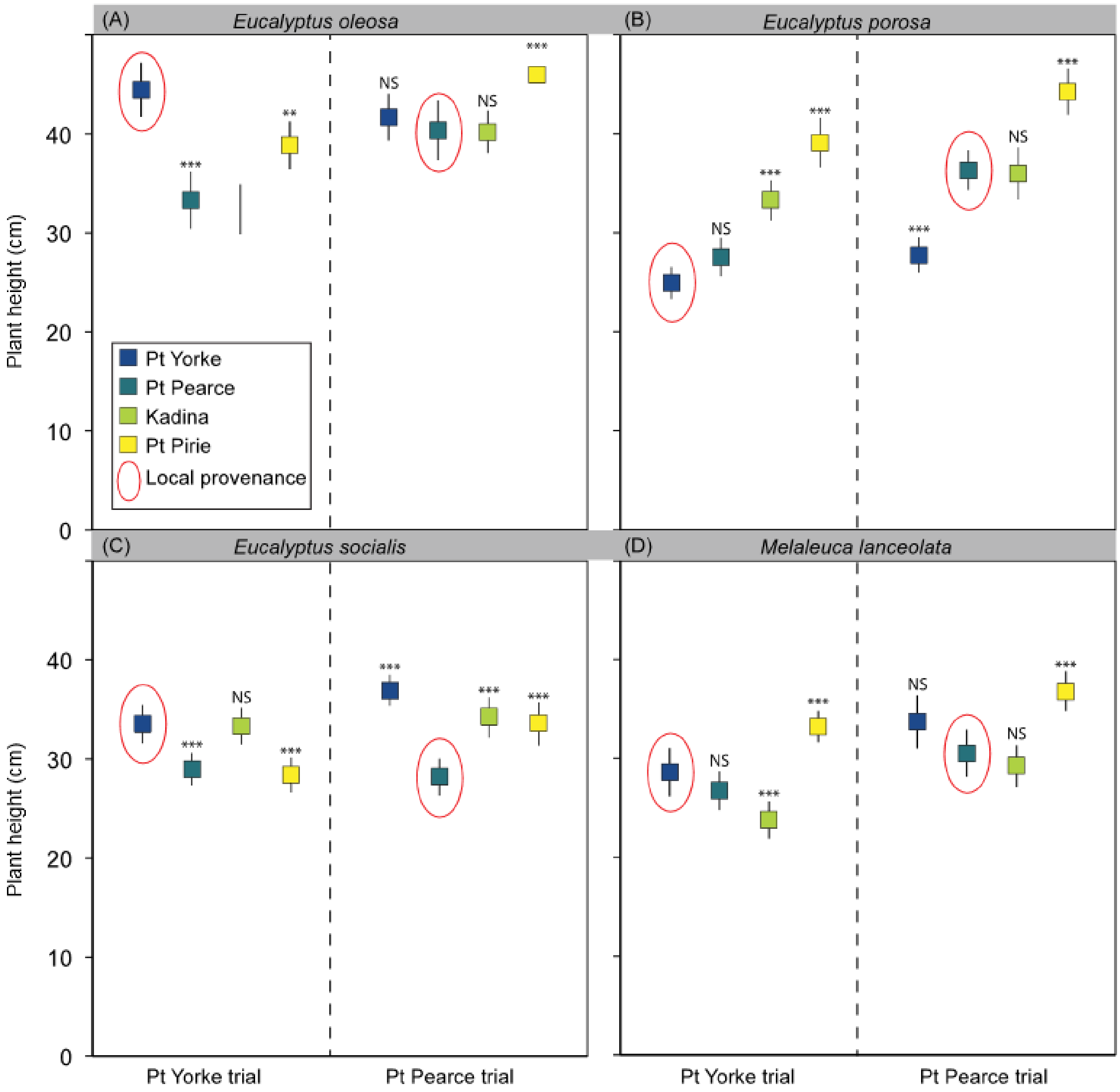
At Point Yorke, provenance had a significant effect on stress where the local provenance had significantly less stress than Point Pearce, Kadina and Port Pirie (all had $P< 0.001$). There was a strong negative effect of stress on height ($P< 0.001$). When controlling for this effect in the model, Point Yorke provenance still grew substantially better than Point Pearce and Kadina, but the Port Pirie provenance did significantly better than any other provenance. No significant stress*provenance interaction was detected at Point Yorke. At Point Pearce, provenance had no effect on stress. There was however a strong negative effect of stress on height ($P < 0.001$), when controlling for this effect in the model the trends remained similar. The Port Pirie provenance still did significantly better than all other provenances, and Point Pearce, the local provenance, had similar performance to Kadina and Point Yorke. No significant stress*provenance interaction was detected at Point Pearce.

Table 1 *E. oleosa* provenance performance at Point Yorke and Point Pearce common gardens, bold script indicates local provenance

Species	Site	Provenance	Survival %	Height ±SE (cm)	Herbivory %	Stress %
E. oleosa	Point Yorke	**Point Yorke**	**95.5**	**44.5±1.39**	**1.0**	**6.5**
		Point Pearce	82.7*	33.3±1.47 ***	0 ^{ns}	23.5 **
		Kadina	93.6 ^{ns}	32.4±1.30 ***	3.4 ^{ns}	22.7 **
		Port Pirie	97.5 ^{ns}	38.9±1.22 **	1.7 ^{ns}	33.6 **
	Point Pearce	Point Yorke	83.0 ^{ns}	41.7±1.20 ^{ns}	0 *	19.4 ^{ns}
		Point Pearce	**85.5**	**40.4±1.54**	**6.4**	**25.5**
		Kadina	90.5 ^{ns}	40.2±1.10 ^{ns}	1.9 *	18.7 ^{ns}
		Port Pirie	92.4 ^{ns}	46.0±1.14 **	1.8 *	22.9 ^{ns}

ns =not significant, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

Figure 2. Height of the focal species from the four provenances in the two common gardens. Error bars show SE. Red circles indicate the local provenance. Statistical effect of provenance for growth is shown, where NS = not significant, **= P< 0.01, and *** = P < 0.001.



Eucalyptus porosa

At Point Yorke and Point Pearce, there were no significant differences in survival between the four provenances (Table 2).

At Point Yorke, provenance had a significant effect on plant height ($P < 0.001$, see Figure 2). The local provenance (25.0 cm ± 0.84 SE) were significantly shorter than Kadina (33.3 cm ± 1.05 SE; $P< 0.001$) and Port Pirie (39.2 cm ± 1.27 SE; $P < 0.001$) and only marginally less than Point Pearce (27.6cm ± 1.00 SE; $P = 0.089$). At Point Pearce, provenance significantly affected the height of plants ($P < 0.001$). The local provenance was significantly shorter (36.4 cm ± 1.04 SE) than Point Pirie (44.3cm ± 1.20 SE), but equally as tall as Kadina, and taller than Point Yorke. The plants that originated from Port Pirie outperformed all other provenances at Point Yorke by *approx.* 40% and at Point Pearce by *approx.* 33%.

Provenance had no significant effect on herbivory rate at either Point Yorke ($P = 0.34$) or Point Pearce ($P = 0.33$).

At Point Yorke, provenance did not have a significant effect on stress. However, there was a significant negative effect of stress on height ($P < 0.001$), and when controlling for stress in the model the local plants at Point Yorke remained significantly smaller than all other provenances. No significant stress*provenance interaction was detected at point Yorke. At Point Pearce, stress was not significantly different between provenances. There was a significant negative effect of stress on height ($P < 0.001$), but controlling for this effect led to similar trends, where Point Pearce did better than Point Yorke but worse than Port Pirie and similar to Kadina. No significant stress*provenance interaction was detected at Point Pearce.

Table 2 *E. porosa* provenance performance at Point Yorke and Point Pearce common gardens. Bold script indicates local provenance

Species	Site	Provenance	Survival %	Height ±SE (cm)	Herbivory %	Stress %
E. porosa	Point Yorke	**Point Yorke**	**94.2**	**25.0±0.84**	**1.0**	**16.8**
		Point Pearce	92.5 ^{ns}	27.6±1.00 ^{ns}	3.6 ^{ns}	8.1 ^{ns}
		Kadina	95.0 ^{ns}	33.3±1.05 ***	4.4 ^{ns}	10.5 ^{ns}
		Port Pirie	95.8 ^{ns}	39.2±1.27 ***	8.7 ^{ns}	8.7 ^{ns}
	Point Pearce	Point Yorke	94.2 ^{ns}	27.8±0.92 ***	7.9 ^{ns}	33.6 ^{ns}
		Point Pearce	**98.3**	**36.4±1.04**	**15.3**	**28.8**
		Kadina	98.3 ^{ns}	36.1±1.36 ^{ns}	12.7 ^{ns}	31.4 ^{ns}
		Port Pirie	98.3 ^{ns}	44.3±1.20 ***	12.7 ^{ns}	20.3 ^{ns}

ns =not significant, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

Eucalyptus socialis

At Point Yorke, provenance had a significant effect on survival (P < 0.047), where the local provenance (94.0%) had significantly less mortality than Point Pearce (87.2%), but similar levels of survival to the other two provenances (Table 3). At Point Pearce, provenance had no significant effect on survival.

At Point Yorke, provenance had a significant effect on height (P < 0.001) (see Figure 2). The local plants (33.5 cm ± 1.0 SE) grew significantly larger than both Point Pearce (28.9 cm ± 0.84 SE; P < 0.001) and Port Pirie (28.3 cm ± 0.90 SE; P < 0.001) but equally as high as Kadina (33.2 cm ± 0.94 SE; P = 0.88). At Point Pearce, provenance had a significant effect on growth where the local provenance (28.1 cm ± 0.96 SE), grew significantly less than all other provenances (Point Yorke 36.9 cm ± 0.81 SE, P < 0.001; Kadina 34.2 cm ± 1.05 SE, P < 0.001; Port Pirie, 33.5 cm ± 1.12 SE, P < 0.001)

Provenance had no significant effect on herbivory rate at Point Yorke (P = 0.09) or Point Pearce (P = 0.09).

At Point Yorke, provenance had a significant effect on stress, where the local provenance had significantly less stress than Point Pearce (P > 0.05), Kadina (P < 0.001) and Port Pire (P < 0.001). There was a significant negative effect of stress on height (P < 0.01), but when controlling for this effect, the same trend in plant height remained (i.e. Point Yorke still did better than Point Pearce and Point Pirie, and similarly to Port Pirie). No significant stress*provenance interaction was detected at Point Yorke. At Point Pearce, stress did not differ significantly between provenances. However stress had a strong negative impact on height (P < 0.001), and when controlling for this effect, the same trends in plant height remained. The stress recorded for local plants at Point Pearce (34.3%) and the Port Pirie provenance (35.9%) at this garden were the highest recorded for any species-provenance-garden combination.

Table 3. *E. socialis* provenance performance at Point Yorke and Point Pearce common gardens. Bold script indicates local provenance.

Species	Site	Provenance	Survival %	Height ±SE (cm)	Herbivory %	Stress %
E. socialis	Point Yorke	**Point Yorke**	**94.0**	**33.5±1.00**	**2.7**	**6.2**
		Point Pearce	87.2 *	28.9±0.84 ***	4.9 ^{ns}	13.7 *
		Kadina	94.1 ^{ns}	33.2±0.94 ^{ns}	7.2 ^{ns}	18.0 **
		Port Pirie	90.8 ^{ns}	28.3±0.90 ***	1 ^{ns}	22.0 **
	Point Pearce	Point Yorke	96.7 ^{ns}	36.9±0.81 ***	0.9 ^{ns}	24.1 ^{ns}
		Point Pearce	**90.8**	**28.1±0.96**	**4.6**	**34.3**
		Kadina	95.8 ^{ns}	34.2±1.05 ***	5.2 ^{ns}	31.3 ^{ns}
		Port Pirie	92.0 ^{ns}	33.5±1.12 ***	4.4 ^{ns}	35.9 ^{ns}

ns =not significant, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

Melaleuca lanceolata

There were no significant differences in survival between the four provenances at both Point Yorke and Point Pearce (Table 4).

At Point Yorke, provenance had a significant effect on plant height (Figure 2). The local provenance was the second largest of the plants (28.6 cm ± 1.26 SE), grew similarly to Point Pearce (26.7 cm ± 0.85 SE; P = 0.20), and significantly better than Kadina (23.8 cm ± 0.96 SE, P < 0.001) and significantly worse than Port Pirie (33.3 cm ± 0.82 SE, P < 0.01). At Point Pearce, provenance also had a significant effect on height. The local provenance grew to (30.5 cm ± 1.23 SE), significantly less than Port Pirie (36.8 cm ± 1.01 SE, P < 0.001), but similar to Kadina (29.2 cm ± 1.01 SE, P = 0.44) and marginally less than Point Yorke (33.7 cm ± 1.38 SE, P = 0.056).

At Point Yorke, no herbivory was recorded for this species at this garden. At Point Pearce, provenance had no significant effect on herbivory (P = 0.09).

At Point Yorke, stress was not significantly differ between provenances, but had a large effect on height (P < 0.001). After controlling for this effect, the same trends in plant height remained (i.e. Point Yorke still doing significantly worse than Port Pirie, but significantly better than Kadina). No significant stress*provenance interaction was detected at Point Yorke. At Point Pearce, stress was not significant between provenances but had a large negative effect height (P < 0.001). After controlling for this effect, a similar trend in plant height remained (i.e. Point Pearce doing worse than Port Pirie, but similar to the other two provenances). No significant stress*provenance interaction was detected at Point Pearce.

Table 4 *M. lanceolata* provenance performance at Point Yorke and Point Pearce common gardens. Bold script indicates local provenance.

Species	Site	Provenance	Survival %	Height ±SE (cm)	Herbivory %	Stress %
M. lanceolata	**Point Yorke**	**Point Yorke**	**98.3**	**28.6±1,26**	**0**	**5.1**
		Point Pearce	93.3 ^{ns}	26.7±0.85 ^{ns}	0	4.5 ^{ns}
		Kadina	93.3 ^{ns}	23.8±0.96 **	0	4.5 ^{ns}
		Port Pirie	99.2 ^{ns}	33.3±0.82 **	0	8.6 ^{ns}
	Point Pearce	Point Yorke	96.6 ^{ns}	33.7±1.38***	3.5 ^{ns}	33.0 ^{ns}
		Point Pearce	**95.0**	**30.5±1.23**	**0**	**30.7** ^{ns}
		Kadina	91.6 ^{ns}	29.2±1.01 ^{ns}	1.0 ^{ns}	29.4 ^{ns}
		Port Pirie	100.0 ^{ns}	36.8±1.01 ***	1.7 ^{ns}	30.8 ^{ns}

ns =not significant, *P < 0.05, **P < 0.01, ***P < 0.001

DISCUSSION

By embedding common garden experiments of four restoration species into two large-scale restoration projects *ca.* 100 km apart in southern Australia, we observed a spectrum of responses to environmental conditions. For each species, we included four provenances that were collected across 250 km of aridity gradient, which is well beyond what is usually considered 'local'. Provenance had a strong effect on most fitness traits. However, across the experimental treatments we only observed the local provenance to perform significantly better than all non-locals on 3 of 96 unique combinations (2 gardens x 3 non-local-local provenance comparisons x 4 traits x 4 species = 96 combinations; of which 27 showed significant differences). Local plants did significantly best for height and stress in *E. oleosa* at Point Yorke, and stress for *E. oleosa* at Point Yorke. The most arid provenance (Port Pirie) performed best in 5 of the 96 species-provenance-garden-trait combinations. Our findings indicate that local adaptation is far from an expectation, at least for our study system during the period we recorded data. Therefore, with the trajectory of increasing aridity in southern Australia, combining seed in an arid-to-mesic direction would provide a low cost, low risk mitigation strategy to help build the adaptive potential of restored populations of these species to climate change.

Plant fitness during establishment

The local plants performed best for only one species, at one garden and only for two of the four traits we measured (*E. oleosa* at Point Yorke for height and stress). The most northern provenance – Port Pirie – significantly outperformed all other provenances in 5 contrasts of plant height (*E. oleosa* at Point Pearce, *E. porosa* at both gardens, and *M. lanceolata* at both gardens). The arid provenance had greater or equal survival than local provenances (e.g. 7 of 8 provenance x survival combinations) but none of these were significant. Therefore we observe little evidence to support strict local provenancing in our study system.

Our results should be interpreted with caution as we have only observed the early establishment of the study species chosen. Different stages of life history will need to be observed in follow up studies, as they will contribute to understanding the overall plant fitness and the persistence of these species on the peninsula. The effect of biotic factors were generally not significantly different between provenances (except for *E. oleosa* herbivory at Point Pearce, *E. oleosa* and *E. socialis* stress at Point Yorke). When controlling for herbivory in *E. oleosa* at Point Pearce, no significant effect on height remained, unlike other eucalypt systems where herbivory has been shown to have a strong effect on provenance performance (Hancock et al. 2012; Gellie et al. 2016).

Stress tended not to be significantly different between provenances, however often had a strong negative effect on plant height. This strong effect on height was also seen to be the case in a common garden study across a similar aridity gradient of *Eucalyptus leucoxylon* in southern Australia (Gellie et al. 2016). However, unlike the *E. leucoxylon* trial (Gellie et al. 2016) the initial plant height trends we observed generally remained after statistically controlling for stress, suggesting that stress had a similar effect on height across provenances. The contrasts where this was not the case both occurred at the more mesic Point Yorke garden, where the local *E. oleosa* and *E. socialis* plants showed a strong home site advantage in stress resistance over the more arid provenances. Coincidentally, local plants also did best or equal best at these species-garden combinations, indicating that augmenting seed selection with more distance provenances is generally not warranted. If mixing was to occur, we would recommend that only small proportion of alternate provenances be added and then only to increase the genetic diversity of plantings (i.e. a composite provenancing strategy as described in Broadhurst et al. 2008). As indicated by Bucharova et al. (2016), the consequences of biotic interactions on provenance selection are complex and important, and if significant trends are observed, the trophic interactions should always be considered before translocating seed.

Previous studies on eucalypts have shown aridity to be a strong agent of selection (Steane et al. 2014; Booth et al. 2015), and it is likely to also be the case for our study species. *E. socialis* has been the best studied of our four study species (Parsons & Rowan 1968; Breed et al. 2016b). An *E. socialis* provenance trial was recently published, and used provenances closer to the central aridity range of the species (aridity index 0.14 to 0.25; Breed et al. 2016b), similar to the most arid provenance we used (Port

Pirie aridity index = 0.28). Although our study spans a greater range in aridity, the trends in early *E. socialis* growth (years 0-2) in Breed et al. (2016) are consistent with our results, and the authors also observed a weak link between growth and provenance. In Breed et al. (2016), adaptive differentiation only emerged after several years (>4 years), and then only manifesting at the most arid site. *E. socialis* appears to be rarer on southern Yorke Peninsula than the other species in our study (ALA species records for southern Yorke below Peesey Swamp: *E. oleosa* >60, *M. lanceolata* >100, *E. porosa* >40*, E. socialis ca*. 30*,* http://www.ala.org.au/). Reduced fitness through changes in individual mating patterns driven by reductions in pollen diversity has been observed in this species (Breed et al., 2012), where pollen diversity better explained variation in growth than inbreeding alone. It has also been shown that fragmentation has a strong impact on inbreeding (Breed et al. 2015). As such, the severe habitat fragmentation and low density stands in this part of its range (*personal observation*) could potentially help explain these results. Further work on the population genetic structure and mating patterns of this species would assist in reconciling the departure from the trend for this and other species on Yorke Peninsula.

Follow-up studies should explore both phenotypic and genotypic target(s) of selection in greater detail, and in additional life stages, however measuring fitness in long-lived woody plants is logistically difficult (Petit & Hampe 2006). Therefore it is important to utilise these common gardens in the future to explore traits that should reflect lifespan plant fitness, such as functional ecological traits (e.g. specific leaf area, wood density), reproductive traits (e.g. phenology, fruit/seed production), along with genetic signatures of selection to more fully understand adaptation to aridity in our study system.

Management implications

In the Mediterranean Biome, where the dual threats of a climate change and habitat fragmentation are apparent (Klausmeyer & Shaw 2009), there is a strong push for alternative management strategies. This biome has a high degree of endemism (Cowling et al. 1994), and in this case species are obstructed from migrating at their leading edge (e.g. the Southern Ocean in Australia), which is likely to accelerate the need for implementing strategies to conserve and restore suitable habitat. The Yorke Peninsula is a particularly interesting case where pockets of high plant diversity remain, but they largely occur in small, isolated patches due to the legacy of land clearing. Since 2012, the Australian Government's Biodiversity Fund, and more recently Landcare, has promoted active restoration initiatives to help alleviate this disjunction of remnant vegetation on Yorke Peninsula.

Our findings suggest that restoration could expand seed sourcing beyond what is normally considered local for restoration in Yorke Peninsula. Though it must also be understood that manipulating the genetic makeup of restoration seed across strong environmental gradients does not come without risks (Byrne et al. 2011), as this may lead to maladaptation and outbreeding depression (Breed et al. 2013). However, our data do not indicate trends of strong adaptive differentiation in our study system and for predominantly outcrossing long lived species (Horsley & Johnson 2007; Breed et al. 2015), like our focal species, the risk of outbreeding depression is low (Frankham et al. 2011; Weeks et al. 2011).

With an oceanic barrier to the south and drying conditions predicted for these high yield agricultural lands, incorporating a genetically diverse seed mix would be precautionary. Performance and evolutionary potential would certainly be improved by following low risk strategies of mixing seed (e.g. composite and admixture provenancing; Broadhurst et al. 2008; Breed et al. 2013). Our precautionary conclusion of shifting seed in an arid-to-mesic direction remains consistent with previous trends found in other long lived Myrtaceae species of the southern Australian Mediterranean biome (Breed et al. 2016b; Gellie et al. 2016).

Data accessibility

Upon acceptance of this manuscript, the supporting data will be made available from Aekos Digital repository.

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Supporting information

Table S1. Annual mean aridity index and precipitation data at provenance locations, plus the observed rainfall for 2014 and 2015.

	Port Pirie	Kadina	Point Pearce	Point Yorke
Annual mean aridity index				
	0.28	0.31	0.32	0.49
Annual precipitation (mm)				
Mean	362	376	372	485
2014	330	336[1]	362	362[3]
2015	331	317[1]	n/a[2]	n/a[3]

Footnote

[1] closest weather station Moonta (137.59°E, 34.07°S)

[2] closest weather station with records Sandilands (137.77°E, 34.52°S) is at a similar latitude but has no records for 2015.

[3] closest weather station 022016 Stenhouse Bay (137.77°E, 34.52°S) and no records for 2015

Figure S1. Annual mean aridity index for *Eucalyptus oleosa* presence data post-1980 from the Atlas of Living Australia (http://www.ala.org.au/). Provenance name indicate the aridity index of the four provenances.

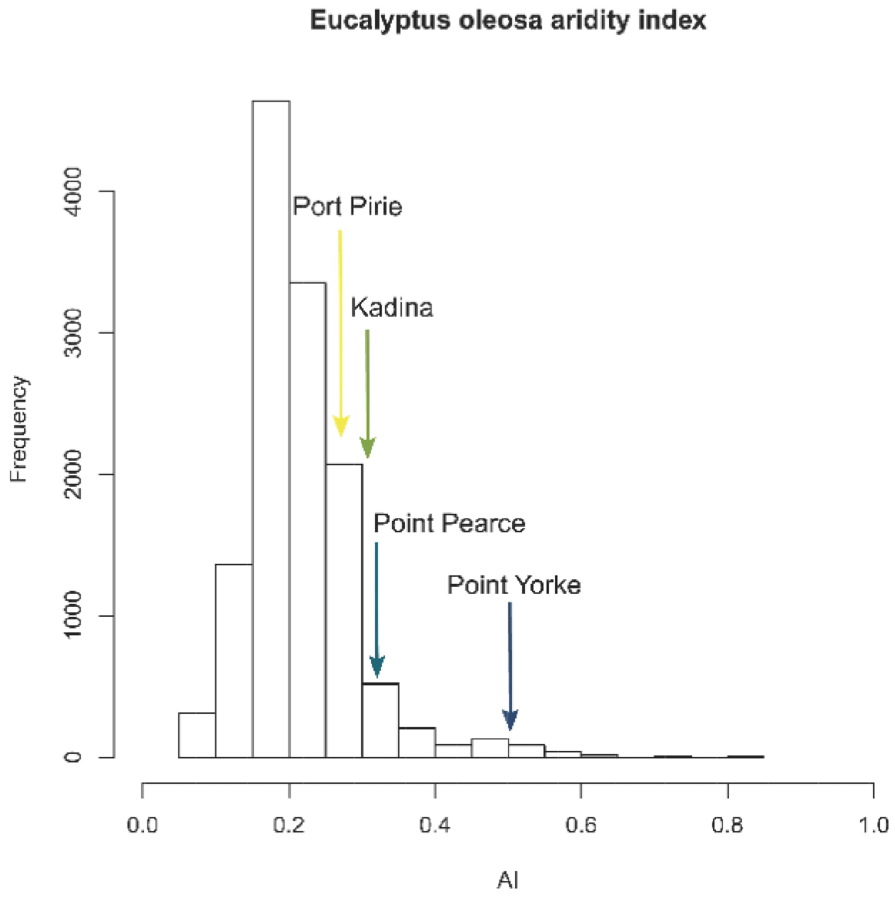


Figure S2. Annual mean aridity index for *Eucalyptus porosa* presence data post-1980 from the Atlas of Living Australia (http://www.ala.org.au/). Provenance name indicate the aridity index of the four provenances.

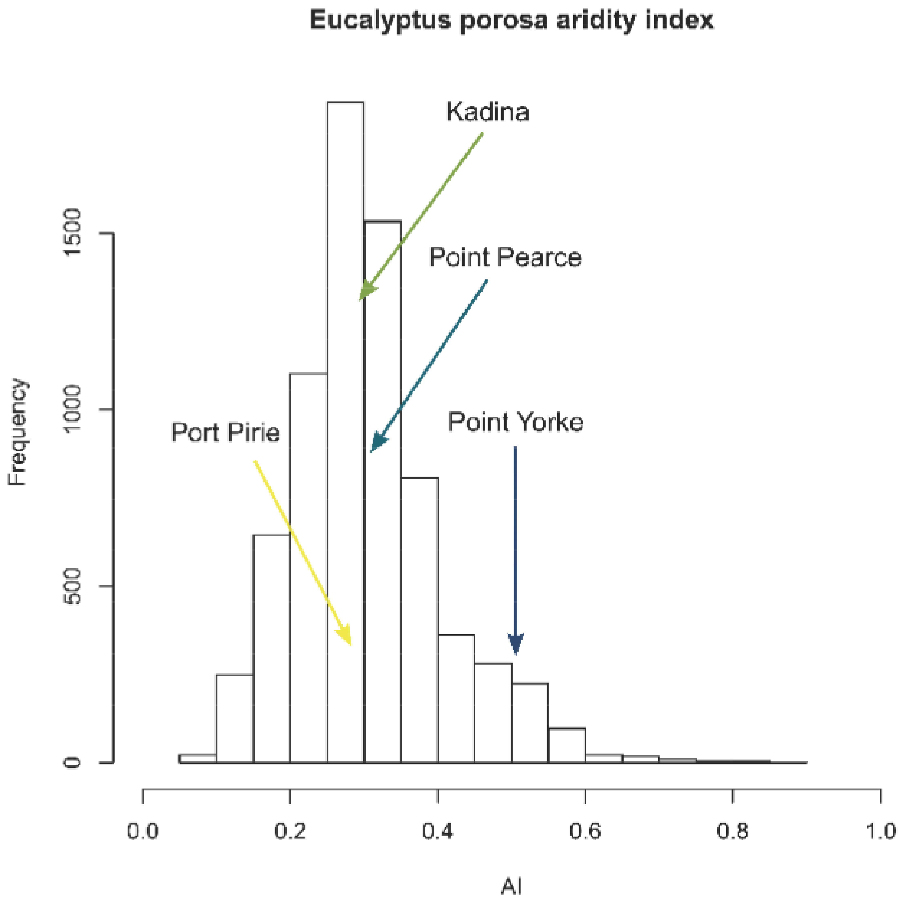


Figure S3. Annual mean aridity index for *Eucalyptus socialis* presence data post-1980 from the Atlas of Living Australia (http://www.ala.org.au/). Provenance name indicate the aridity index of the four provenances.

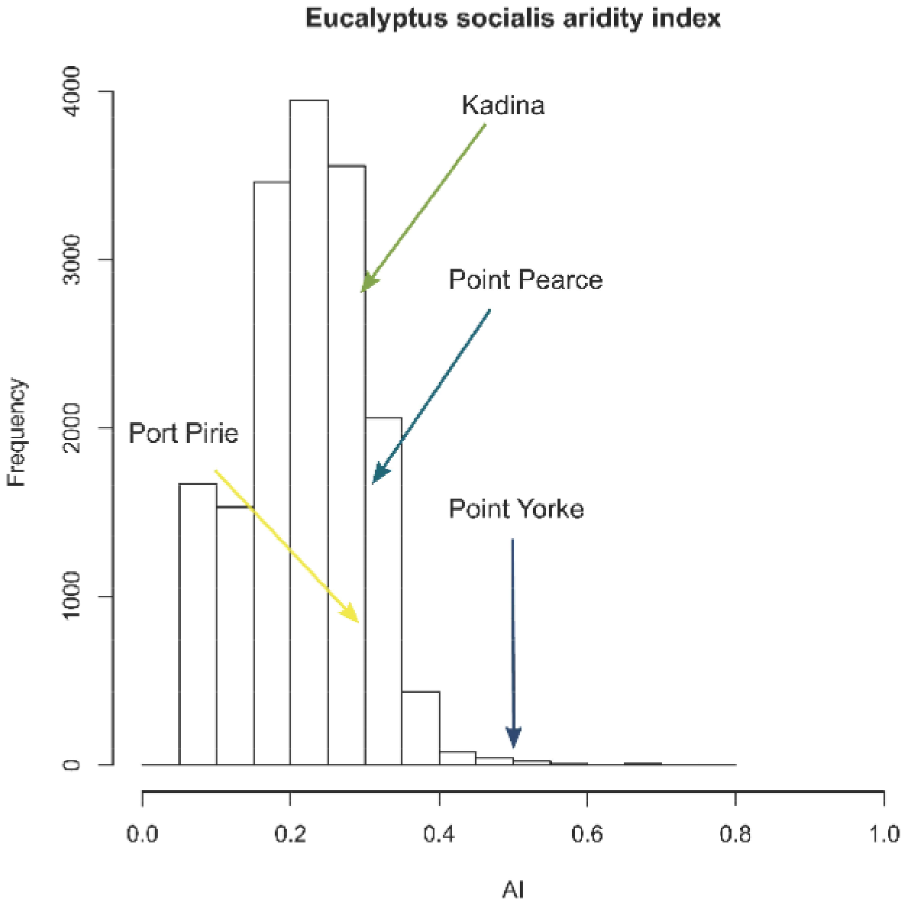
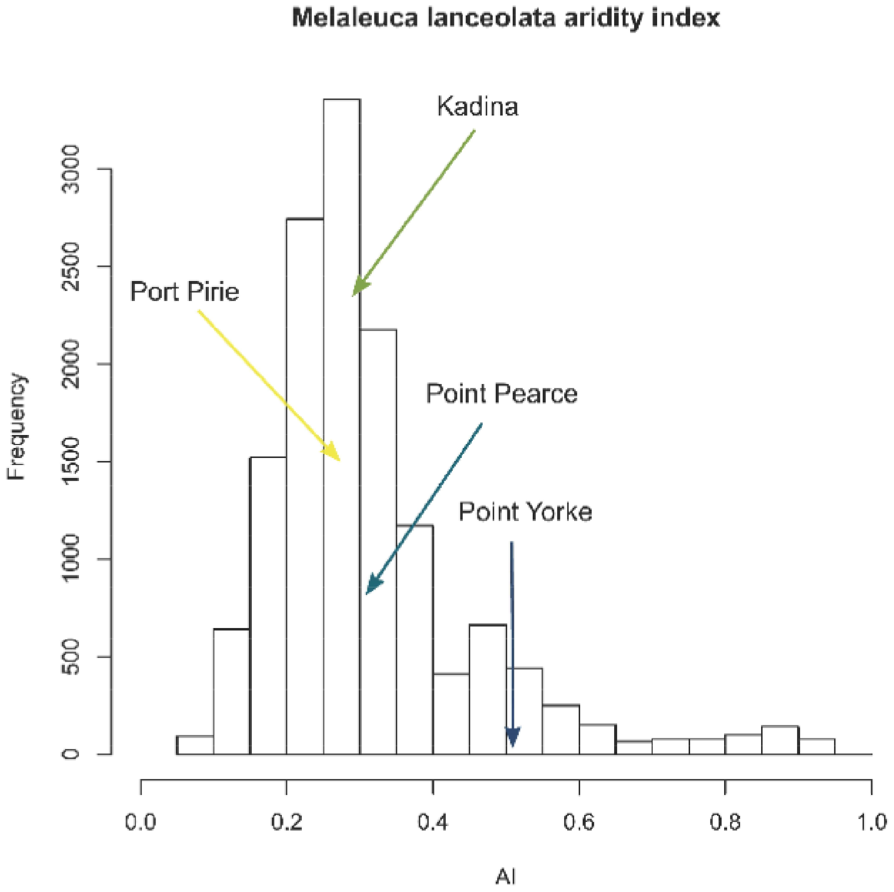


Figure S4. Annual mean aridity index for *Melaleuca lanceolata* presence data post-1980 from the Atlas of Living Australia (http://www.ala.org.au/). Provenance name indicate the aridity index of the four provenances.



CHAPTER 6: MICROBIOME REWILDING

Revegetation rewilds the soil bacterial microbiome of an old field

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Name of Principal Author (Candidate)	Nicholas Gellie		
Contribution to the Paper	Formulated the ideas conducted field trials wrote manuscript and addressed reviewers comments		
Overall percentage (%)	70		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	24/01/2017

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Andrew lowe(corresponding author and principal supervisor)		
Contribution to the Paper	Formulated the ides, commented and edited subsequent manuscript drafts		
Signature		Date	24/01/2017

Statement of Authorship

Gellie N. J*., Mills J. G., Breed M. F., and A. J. Lowe (accepted in press 2017). Revegetation rewilds the soil bacterial microbiome of an old field. Molecular Ecology*

Gellie N. J. C. (candidate)

Formulated the ideas, conducted field trials, wrote manuscript addressed reviewers comments

I hereby certify that this statement of contribution is accurate

Signed ______________ Date: 23rd January 2017

Mills J. G.

Conducted field trials, undertook analysis, prepared figures and tables commented and edited subsequent manuscript drafts.

I hereby certify that this statement of contribution is accurate and consent to the inclusion of this manuscript in Nick Gellie's PhD thesis

Signed Date: 23rd January 2017

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Formulated the ideas, undertook analysis, prepared figures and tables commented and edited subsequent manuscript drafts.

I hereby certify that this statement of contribution is accurate and consent to the inclusion of this manuscript in Nick Gellie's PhD thesis

Signed _ __ Date: 23rd January 2017

Lowe A. (primary supervisor)

Formulated the ideas, commented and edited subsequent manuscript drafts

I hereby certify that this statement of contribution is accurate and consent to the inclusion of this manuscript in Nick Gellie's PhD thesis

Signed Date: 23rd January 2017

Abstract

Ecological restoration is a globally important and well-financed management intervention used to combat biodiversity declines and land degradation. Most restoration aims to increase biodiversity towards a reference state, but there are concerns that intended outcomes are not reached due to unsuccessful interventions and land use legacy issues. Monitoring biodiversity recovery is essential to measure success, however most projects remain insufficiently monitored. Current field-based methods are hard to standardise and are limited in their ability to assess important components of ecosystems, such as bacteria. High-throughput amplicon sequencing of environmental DNA (metabarcoding of eDNA) has been proposed as a cost-effective, scalable and uniform ecological monitoring solution, but its application in restoration remains largely untested. Here we show that metabarcoding of soil eDNA is effective at demonstrating the return of the native bacterial community in an old field following native plant revegetation. Bacterial composition shifted significantly after 8 years of revegetation, where younger sites were more similar to cleared sites and older sites were more similar to remnant stands. Revegetation of the native plant community strongly impacted on the belowground bacterial community, despite the revegetated sites having a long and dramatically altered land use history (i.e. >100 years grazing). We demonstrate that metabarcoding of eDNA provides an effective way of monitoring changes in bacterial communities that would otherwise go unchecked with conventional monitoring of restoration projects. With further development, awareness of microbial diversity in restoration has significant scope for improving the efficacy of restoration interventions more broadly.

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Revegetation rewilds the soil bacterial microbiome of an old field

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Abstract

Ecological restoration is a globally important and well-financed management intervention used to combat biodiversity declines and land degradation. Most restoration aims to increase biodiversity towards a reference state, but there are concerns that intended outcomes are not reached due to unsuccessful interventions and land-use legacy issues. Monitoring biodiversity recovery is essential to measure success; however, most projects remain insufficiently monitored. Current field-based methods are hard to standardize and are limited in their ability to assess important components of ecosystems, such as bacteria. High-throughput amplicon sequencing of environmental DNA (metabarcoding of eDNA) has been proposed as a cost-effective, scalable and uniform ecological monitoring solution, but its application in restoration remains largely untested. Here we show that metabarcoding of soil eDNA is effective at demonstrating the return of the native bacterial community in an old field following native plant revegetation. Bacterial composition shifted significantly after 8 years of revegetation, where younger sites were more similar to cleared sites and older sites were more similar to remnant stands. Revegetation of the native plant community strongly impacted on the belowground bacterial community, despite the revegetated sites having a long and dramatically altered land-use history (i.e. >100 years grazing). We demonstrate that metabarcoding of eDNA provides an effective way of monitoring changes in bacterial communities that would otherwise go unchecked with conventional monitoring of restoration projects. With further development, awareness of microbial diversity in restoration has significant scope for improving the efficacy of restoration interventions more broadly.

Keywords: anthropocene, ecosystem restoration, eDNA, land degradation, microbiome next-generation sequencing

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Introduction

Ecosystem restoration is increasingly relied upon to combat the global declines in biodiversity, ecosystem services and land quality (IPBES 2014; Suding *et al.* 2015). The recent formation of the Intergovernmental Platform on Biodiversity and Ecosystem Services (IPBES) draws attention to the key role restoration has in combatting these global issues. Aspirational goals have been set and agreed to, such as restoring 350 million ha by 2030 at the Sept 2014 UN Climate Summit in New York, requiring substantial investment (Menz *et al.* 2013). These impressive ambitions will rely on effective restoration practices being employed and the addition of integrated policy support (Suding *et al.* 2015).

A recent meta-analysis of 221 restoration projects found highly variable and, in some cases, suboptimal outcomes (Crouzeilles *et al.* 2016), corroborating previous studies (Benayas *et al.* 2009; Moreno-Mateos *et al.* 2012; Wortley *et al.* 2013) and policy reviews (Baker & Eckerberg 2016), which together provide clear evidence of deficiencies in current restoration practices. These studies identify the lack of consistent and objective monitoring as a common factor jeopardizing the delivery of restoration goals. If restoration monitoring is not undertaken effectively, then adaptive management options for follow-up maintenance will be hindered by

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a lack of evidence and restoration investments risk being unduly wasted (Ruiz-Jaen & Aide 2005). Finding effective restoration monitoring tools that assess site viability and intervention efficacy, and provide follow-up directions has generally eluded the restoration community, and resolving this impasse is a priority of restoration (Ruiz-Jaen & Aide 2005).

Effective monitoring provides the evidence needed to make adaptive management interventions if, for example, restoration goals are being missed (Collen & Nicholson 2014). With consistent monitoring, the restoration end-users and practitioners can demonstrate their achievements relative to investor or policy goals. Traditionally, monitoring involves field-based visual surveys of ecological communities (e.g. taxonomic inventories) (Butchart *et al.* 2010), which rely on expert observers. These approaches are time-consuming, expensive and often not standardized across projects (Thomsen & Willerslev 2015) or between observers (Vittoz & Guisan 2007). Consequently, many restoration projects go unmonitored, or where monitoring is conducted, it remains limited in scope and utility (Ruiz-Jaen & Aide 2005). These limitations can hamper the ability of follow-up management to achieve desired restoration goals (Burton 2014; Collen & Nicholson 2014).

Traditional field-based monitoring methods focus on terrestrial macro-organisms, largely discounting microbial communities, which are the foundations of many ecosystem functions and services (e.g. nutrient cycling; Fierer *et al.* 2007; Zak *et al.* 2003). High-throughput amplicon sequencing of DNA present in environmental samples (metabarcoding of eDNA; e.g. soil) can provide a complimentary approach to field-based ecological monitoring (Thomsen & Willerslev 2015; Barnes & Turner 2016), and could revolutionize the restoration assessment process (Ji *et al.* 2013; Williams *et al.* 2014; Barnes & Turner 2016). An eDNA approach can identify and quantify the sources of the genetic material, resulting in a cost-effective, high-throughput and standard approach that can quantify biodiversity. Little prior knowledge is needed to identify a representative suite of species within a focal taxon. Metabarcoding has already proven to be an effective and efficient method to survey important groups such as soil bacteria and fungi where morphological identification is notoriously problematic (Taberlet *et al.* 2012), and can equally be applied to macrocommunities (e.g. insect, plant) (Bissett *et al.* 2016).

Land degradation causes a decline in microbial activity (Araújo *et al.* 2014), and agricultural practices have strong impacts on microbial community composition (Potthoff *et al.* 2006); however, there is only limited information on how restoration affects these communities. Soil bacteria exhibit biogeographical trends (Fierer & Jackson 2006), where bacterial species turnover often occurs in an ecologically predictable way, in line with soil biochemical changes (Fierer *et al.* 2007). By identifying the dynamics of this turnover throughout the life of restoration projects (i.e. before restoration starts, during restoration and after restoration is declared complete), there exists potential to develop uniform assessment and monitoring tools.

Metabarcoding of eDNA has few empirical examples of the effects of ecological restoration on microbial communities (Araújo *et al.* 2014). However, metabarcoding has recently been applied to a number of allied fields to describe ecological community turnover. For example, Clemmensen *et al.* (2015) used this approach to describe fungal community succession across 5000 years of boreal forest, and Rime *et al.* (2015) used this approach to describe fungal and bacterial succession across stages of soil development along a sequence of 110 years of glacial retreat. In an applied ecology context, Clarke *et al.* (2015) used this approach to show that re-introducing native vertebrates could change soil fungal communities in the Australian arid zone. Such an approach holds great promise to explore the effectiveness of ecological restoration to return soil microbial diversity and function, and to determine whether restoration interventions can overcome past land-use legacy issues.

Our study used high-throughput sequencing of bacterial small-subunit ribosomal DNA (16S rRNA) genes to describe the turnover in bacterial community across a 10-year revegetation chronosequence. This chronosequence spanned cleared sites, sites revegetated for restoration in 2009, 2008, 2007 and 2005, plus three different reference sites of native remnant vegetation. We specifically addressed the following questions: (i) Do soil bacterial communities differ across stages of ecological restoration? (ii) How strong is the influence of past vs. contemporary land-use patterns on bacterial community composition? (iii) Are bacterial communities in older restoration sites similar to reference sites, and are bacterial communities in younger restoration sites similar to currently cleared sites? (iv) Which of these bacterial taxa are characteristic of the different ages of ecological restoration?

Methods

Study system

Our study system was an active restoration site at Mt Bold, a water catchment reserve of the Mt Lofty Ranges in South Australia (35.07°S, 138.42°E; Fig. 1). This catchment was dominated by an open eucalypt woodland that has historically been subjected to tree clearance and grazing that began early in the 20th century. In this

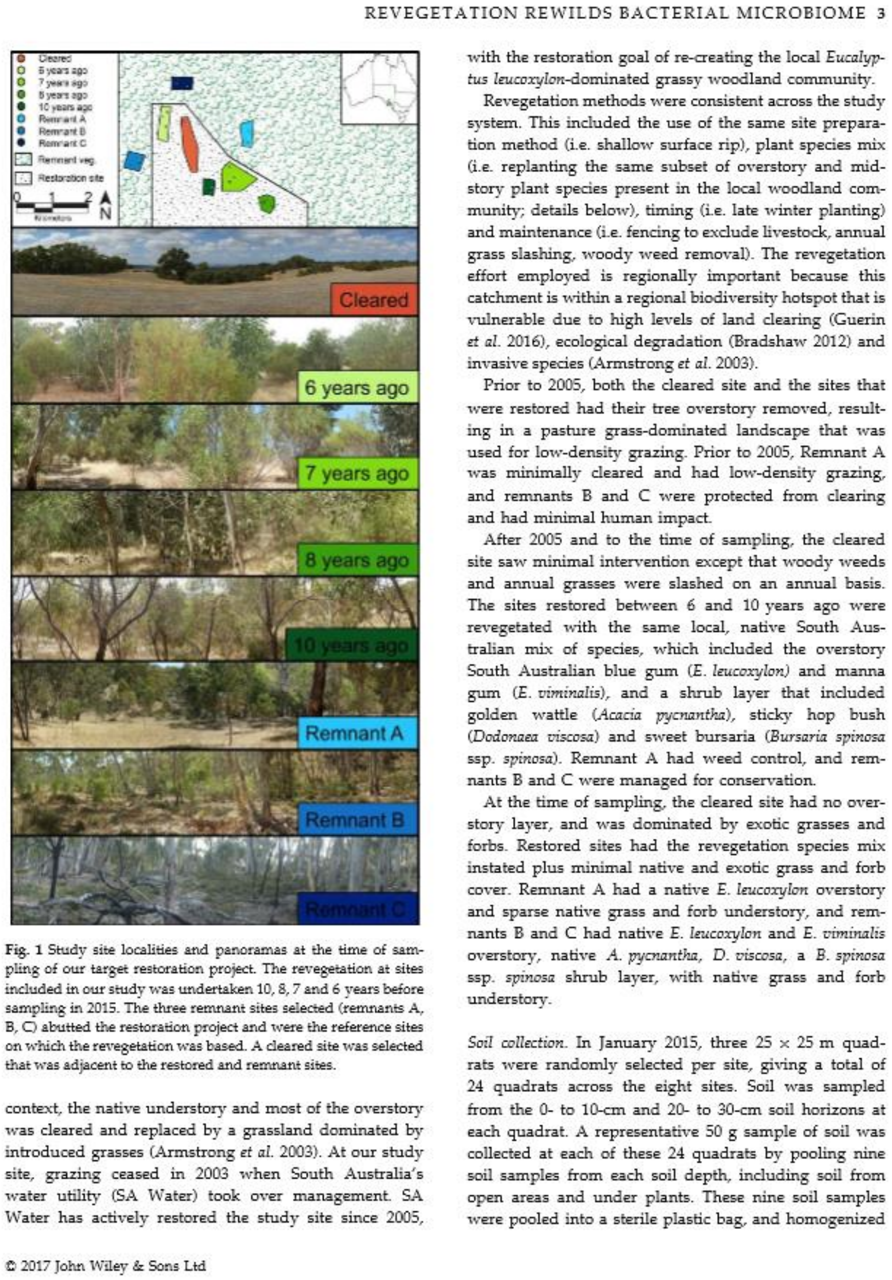


Fig. 1 Study site localities and panoramas at the time of sampling of our target restoration project. The revegetation at sites included in our study was undertaken 10, 8, 7 and 6 years before sampling in 2015. The three remnant sites selected (remnants A, B, C) abutted the restoration project and were the reference sites on which the revegetation was based. A cleared site was selected that was adjacent to the restored and remnant sites.

context, the native understory and most of the overstory was cleared and replaced by a grassland dominated by introduced grasses (Armstrong *et al.* 2003). At our study site, grazing ceased in 2003 when South Australia's water utility (SA Water) took over management. SA Water has actively restored the study site since 2005, with the restoration goal of re-creating the local *Eucalyptus leucoxylon*-dominated grassy woodland community.

Revegetation methods were consistent across the study system. This included the use of the same site preparation method (i.e. shallow surface rip), plant species mix (i.e. replanting the same subset of overstory and midstory plant species present in the local woodland community; details below), timing (i.e. late winter planting) and maintenance (i.e. fencing to exclude livestock, annual grass slashing, woody weed removal). The revegetation effort employed is regionally important because this catchment is within a regional biodiversity hotspot that is vulnerable due to high levels of land clearing (Guerin *et al.* 2016), ecological degradation (Bradshaw 2012) and invasive species (Armstrong *et al.* 2003).

Prior to 2005, both the cleared site and the sites that were restored had their tree overstory removed, resulting in a pasture grass-dominated landscape that was used for low-density grazing. Prior to 2005, Remnant A was minimally cleared and had low-density grazing, and remnants B and C were protected from clearing and had minimal human impact.

After 2005 and to the time of sampling, the cleared site saw minimal intervention except that woody weeds and annual grasses were slashed on an annual basis. The sites restored between 6 and 10 years ago were revegetated with the same local, native South Australian mix of species, which included the overstory South Australian blue gum (*E. leucoxylon*) and manna gum (*E. viminalis*), and a shrub layer that included golden wattle (*Acacia pycnantha*), sticky hop bush (*Dodonaea viscosa*) and sweet bursaria (*Bursaria spinosa* ssp. *spinosa*). Remnant A had weed control, and remnants B and C were managed for conservation.

At the time of sampling, the cleared site had no overstory layer, and was dominated by exotic grasses and forbs. Restored sites had the revegetation species mix instated plus minimal native and exotic grass and forb cover. Remnant A had a native *E. leucoxylon* overstory and sparse native grass and forb understory, and remnants B and C had native *E. leucoxylon* and *E. viminalis* overstory, native *A. pycnantha*, *D. viscosa*, a *B. spinosa* ssp. *spinosa* shrub layer, with native grass and forb understory.

Soil collection. In January 2015, three 25 × 25 m quadrats were randomly selected per site, giving a total of 24 quadrats across the eight sites. Soil was sampled from the 0- to 10-cm and 20- to 30-cm soil horizons at each quadrat. A representative 50 g sample of soil was collected at each of these 24 quadrats by pooling nine soil samples from each soil depth, including soil from open areas and under plants. These nine soil samples were pooled into a sterile plastic bag, and homogenized using a sterilized trowel. All soil samples ($n = 48$) were frozen on site in sterile 50-mL falcon tubes until DNA extraction – hereafter referred to as technical replicates. An additional 300 g soil was sampled from these pooled samples and used for soil physical and chemical analysis. We quantified soil moisture, ammonium, nitrate, available phosphorus, sulphur, organic carbon and soil pH (H_20 and $CaCl_2$) (for more detailed methods, see Bissett *et al.* 2016).

DNA extraction and genomic analyses

DNA was extracted and then pooled from 3 × 0.25 g soil samples per technical replicate at the Australian Genome Research Facility (AGRF, Adelaide, Australia) using MoBio powersoil DNA extraction kits according to the Earth Microbiome Project (http://www.earthmicrobiome.org/emp-standard-protocols/dna-extraction-protocol/). We PCR-amplified the bacterial 16S ribosomal DNA for each technical replicate with negative controls (laboratory-grade water) used on each plate using the forward 27F and reverse 519R primers (Lane 1991), including the 12-bp Golay barcodes as described by Caporaso *et al.* (2012), and with 1U Immolase DNA polymerase (Bioline) per reaction with the following PCR protocol: 10 min of activation at 95 °C, 35 cycles of 30 s at 94 °C, 10 s at 55 °C and 45 s at 72 °C, and 10 min of final extension at 72 °C (reagents, volumes and final concentrations are found at https://downloads-qcif.bioplatforms.com/bpa/base/methods/16S/16S_method.pdf). PCR products (ca. 530 bp) were visualized by electrophoresis in 2% agarose gel 0.5× TAE buffer. PCR plates showing bands for the negative controls were reamplified until they appeared blank (for more methods on controls, see Bissett *et al.* 2016). Products were purified with Agencourt AMPure XP bead clean-up. Amplicon concentrations were quantified using the Quant-iT™ $^{\text{PicoGreen®}}$ dsDNA Assay Kit, normalized by diluting to 10 nM with variable volumes of 10 mM Tris (pH 8.5), and sized on an Agilent Bioanalyzer. Equal volumes of these normalized PCR products were then pooled and diluted to 4 nM, and then sequenced on an Illumina MiSEQ platform.

Read quality was thoroughly assessed and trimmed prior to operational taxonomic unit (OTU) picking. Read merging and screening based on quality scores was performed with FLASH using default settings (Magoč & Salzberg 2011), and merged reads <400 bp and those containing Ns or homopolymer runs >8 bp were removed using mothur v1.34.1 (Schloss *et al.* 2009). We used an OTU picking workflow similar to the QIIME pipeline (Rideout *et al.* 2014), with minor changes detailed in Bissett *et al.* (2016). We used the following workflow on the reads: (i) demultiplex reads; (ii) make an otu fasta mapping file split into chimeric and nonchimeric reads by sorting reads by abundance, remove reads with <6 copies, cluster reads into OTUs of ≥97% similarity using UPARSE (Edgar 2013) and identify chimeric reads as detailed in Bissett *et al.* (2016); (iii) map original reads from (i) back to the otu fasta mapping file from (ii) and exclude chimeric-mapped OTUs and retaining nonchimeric OTUs. Non-mapped reads were rerun through steps (1–3) but with a lower threshold for copy number from >6 to 2. All nonchimeric reads were then combined into a final mapped file, which was converted to an OTU table.

Operational taxonomic units were identified using Greengenes (DeSantis *et al.* 2006), using the Wang classifier (Wang *et al.* 2007) in MOTHUR at 60% confidence in the assignment of each taxon based on sequence similarity. We discarded OTUs not identified as belonging to bacteria, unidentified at the phylum level, or having <100 reads across all technical replicates. Further details of our molecular methods, including the details of the use of mock communities to guide the analyses, can be found in Bissett *et al.* (2016), and commands used are in http://www.bioplatforms.com/wp-content/uploads/OTU_pipelines.pdf.

Statistical analyses

Operational taxonomic unit abundance was rarefied to the technical replicate with the lowest number of reads with the rarefy function in Vegan v 2.3-2 (Oksanen *et al.* 2015) implemented in r v 3.2.3 (R Core Team 2015). Species richness, effective species number (Jost 2006) and Shannon's diversity and evenness were used to describe site diversity. Variation in rarefied OTU abundances and phyla was visualized using principal coordinates analysis (PCoA) using Bray–Curtis (rarefied abundance) and Jaccard (presence–absence) distance matrices. Results of PCoAs were compared to principle components analyses of centred log-ratio-transformed OTU and phyla abundances (Gloor & Reid 2016). Differences in rarefied abundances of the dominant phyla and soil characteristics across the revegetation chronosequence were analysed using a permuted analysis of variance with the aovp function in Lmperm v 1.1-2 (Wheeler 2010) implemented in r with 5000 permutations.

Results

We analysed a total of 3 002 411 quality-filtered bacterial 16S rRNA gene sequences in the 48 technical replicates across the eight revegetation chronosequence sites (Table S1, Supporting information), consisting of 3316 OTUs. Nine bacterial phyla dominated our data set, including Acidobacteria, Actinobacteria, Bacteroidetes,

Table 1 Effect of restoration site and soil depth on rarefied relative abundance and diversity of bacteria phyla (for details on Shannon's diversity and effective species number ANOVAS, see Table S4, Supporting information)

	Rarefied abundance				
Phylum	Site ($F_{7,32}$)	Direction of effect	Depth ($F_{1,32}$)	Direction of effect	Site x Depth ($F_{7,32}$)
Acidobacteria	25.89^{***}	Increasing	56.30^{***}	Increasing	0.94^{ns}
Actinobacteria	5.32^{***}	Decreasing	18.45^{***}	Decreasing	0.53^{ns}
Bacteroidetes	7.07^{***}	Variable	46.51^{***}	Decreasing	0.39^{ns}
Chloroflexi	2.38^{*}	Variable	0.75^{ns}		0.65^{ns}
Firmicutes	37.05^{***}	Decreasing	0.33^{ns}		1.11^{ns}
Gemmatimonadetes	7.44^{***}	Decreasing	8.14^{**}	Decreasing	0.44^{ns}
Planctomycetes	8.06^{***}	Increasing	14.27^{***}	Decreasing	0.46^{ns}
Proteobacteria	2.09^{ns}		2.71^{ns}		0.51^{ns}
Verrucomicrobia	11.25^{***}	Increasing	31.64^{***}	Increasing	1.65^{ns}
Rare	1.51^{ns}		5.17^{*}	Increasing	0.96^{ns}

Permuted analysis of variance $^{\mathrm{ns}}$not significant, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

concentrations significantly increased, across the revegetation chronosequence (Table 2). Soil phosphorous, organic carbon, ammonium and sulphur significantly decreased with depth (Table 2). Other soil parameters (e.g. pH and ammonium) did not change in a directional fashion (Table 2), and there was no clear general trend when all soil traits were combined apart from remnant sites clustering together (Fig. S5, Supporting information).

Discussion

Using high-throughput amplicon sequencing of environmental DNA that we sampled from soils across a revegetation chronosequence, we demonstrated clear changes in the bacterial community at an active restoration site in southern Australia. The identification of these changes adds to our understanding of the effect of restoration on soil bacterial communities and contributes to the growing evidence that these communities can alter dramatically with ecological processes (Fierer *et al.* 2007). Most previous studies that have documented bacterial community changes have focussed on natural processes, such as plant community succession and stages of soil development (Jesus *et al.* 2009; Rime *et al.* 2015). Few studies have explored bacterial responses to active ecological restoration (Potthoff *et al.* 2006; Banning *et al.* 2011; Cavagnaro *et al.* 2016). To the best of our knowledge, only one other study has used metabarcoding of eDNA as a method for assessing the impact of restoration on soil microbiomes (Araújo *et al.* 2014). Araújo *et al.* (2014) explored the effect of high- vs. low-diversity non-native planting treatments on degraded land bacterial communities. However, unlike our study, Araújo and colleagues did not explore changes in bacteria across a restoration chronosequence, different responses at depth or responses to alternate restoration interventions with native plant species.

The bacterial phyla that displayed the greatest change in abundance across our revegetation chronosequence were Acidobacteria and Firmicutes. These trends support previous work that has shown Acidobacteria to be more abundant in forest and grassland soils rather than agricultural soils, and less abundant in nutrient-rich soils such as pastures (Fierer *et al.* 2007). Firmicutes abundance has previously been shown to be higher in pasture habitats than in forests (Jesus *et al.* 2009). These two phyla are associated with important functional roles in ecosystems (e.g. Acidobacteria have been shown to breakdown complex, recalcitrant sugars; Ward *et al.* 2009; and Firmicutes such as Clostridium are well-known human commensals and pathogens). Additional work is required to confirm the functionality of OTUs identified in our study (e.g. soil enzyme assays, Potthoff *et al.* 2006; proteomic investigation, Bastida *et al.* 2009).

We observed an increase in soil organic carbon across the revegetation sequence, which is consistent with previous work on restoration of a Californian grassland (Potthoff *et al.* 2006), and the positive effect of revegetation on soil organic carbon sequestration more generally (Wang *et al.* 2011). The direction of this change was towards the concentrations found at the remnant sites, which appears consistent with a previous study of Jarrah forest restoration in Western Australia (George *et al.* 2010). George *et al.* (2010) observed organic matter accumulated in the topsoil with increasing time since restoration, which was a trajectory towards a native soil carbon profile. We found nitrate and phosphorous decreased with age of restoration,

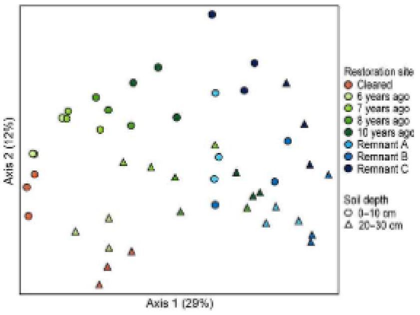


Fig. 2 Principal coordinates analysis of restoration sites based on Bray–Curtis distance matrix of bacterial 16S rRNA OTU rarefied abundance.

Chloroflexi, Firmicutes, Gemmatimonadetes, Planctomycetes, Proteobacteria and Verrucomicrobia (each with >1.5% abundance). These dominant phyla totalled 97.1% of the total read data set. Rare phyla were grouped and included 2.9% of the total reads (each phylum <1.5% total abundance). Technical replicate OTU abundance was rarefied to account for within technical replicate differences in abundance (a 0- to 10-cm Remnant C technical replicate had the lowest number of reads = 33 797).

Bacterial alpha-diversity was similar across sites (Table S1, Supporting information). However, we observed a striking directional change in community composition across the revegetation chronosequence (Figs 2 and 3). Recently revegetated sites had bacterial communities similar to the cleared area, and older revegetated sites were more similar to remnants. These bacterial community trends were largely similar between soil depths. The observed changes in community structure were constant for both rarefied abundance (Figs 2 and 3) and presence–absence richness (Fig. S1, Supporting information) distance matrices, as well as centred log-ratio-transformed abundance (Figs S2, S3 and S4, Supporting information).

Acidobacteria and Firmicutes were the phyla that exhibited the greatest change in abundance across the revegetation chronosequence (Fig. 3; Table 1). Acidobacteria showed a significant increase in abundance and Firmicutes showed a significant decrease in abundance with time since revegetation (Table 1). Acidobacteria also showed significantly increased abundance with depth (Table 1). Acidobacteria displayed a significant negative correlation with increasing nitrate ($r^2_{0-10} = 0.21$; $r^2_{20-30} = 0.42$) and phosphorous concentrations ($r^2_{0-10} = 0.37$; $r^2_{20-30} = 0.38$), and positive correlations with organic carbon ($r^2_{0-10} = 0.19$; $r^2_{20-30} = 0.17$) and sulphur ($r^2_{0-10} = 0.16$; $r^2_{20-30} = 0.23$) (Tables S2 and S3, Supporting information). Firmicutes displayed weak positive correlations with increasing concentrations of nitrate ($r^2_{0-10} = 0.15$; $r^2_{20-30} = 0.41$) and phosphorous ($r^2_{0-10} = 0.13$; $r^2_{20-30} = 0.48$), and negative correlations with organic carbon ($r^2_{0-10} = 0.24$; $r^2_{20-30} = 0.27$) and sulphur ($r^2_{0-10} = 0.21$; $r^2_{20-30} = 0.24$).

Soil nitrate and phosphorous concentrations significantly decreased, and organic carbon and sulphur

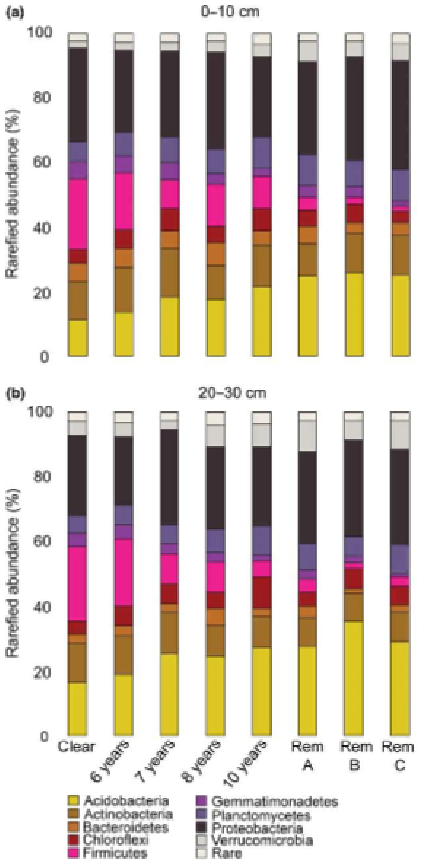


Fig. 3 Rarefied abundances of the nine dominant bacterial phyla and rare bacteria at (a) 0–10 cm and (b) 20–30 cm depths across the restoration chronosequence.

Table 1 Effect of restoration site and soil depth on rarefied relative abundance and diversity of bacteria phyla (for details on Shannon's diversity and effective species number ANOVAS, see Table S4, Supporting information)

	Rarefied abundance				
Phylum	Site ($F_{7,32}$)	Direction of effect	Depth ($F_{1,32}$)	Direction of effect	Site x Depth ($F_{7,32}$)
Acidobacteria	25.89***	Increasing	56.30***	Increasing	0.94^{ns}
Actinobacteria	5.32***	Decreasing	18.45***	Decreasing	0.53^{ns}
Bacteroidetes	7.07***	Variable	46.51***	Decreasing	0.39^{ns}
Chloroflexi	2.38*	Variable	0.75^{ns}		0.65^{ns}
Firmicutes	37.05***	Decreasing	0.33^{ns}		1.11^{ns}
Gemmatimonadetes	7.44***	Decreasing	8.14**	Decreasing	0.44^{ns}
Planctomycetes	8.06***	Increasing	14.27***	Decreasing	0.46^{ns}
Proteobacteria	2.09^{ns}		2.71^{ns}		0.51^{ns}
Verrucomicrobia	11.25***	Increasing	31.64***	Increasing	1.65^{ns}
Rare	1.51^{ns}		5.17*	Increasing	0.96^{ns}

Permuted analysis of variance ^{ns}not significant, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

concentrations significantly increased, across the revegetation chronosequence (Table 2). Soil phosphorous, organic carbon, ammonium and sulphur significantly decreased with depth (Table 2). Other soil parameters (e.g. pH and ammonium) did not change in a directional fashion (Table 2), and there was no clear general trend when all soil traits were combined apart from remnant sites clustering together (Fig. S5, Supporting information).

Discussion

Using high-throughput amplicon sequencing of environmental DNA that we sampled from soils across a revegetation chronosequence, we demonstrated clear changes in the bacterial community at an active restoration site in southern Australia. The identification of these changes adds to our understanding of the effect of restoration on soil bacterial communities and contributes to the growing evidence that these communities can alter dramatically with ecological processes (Fierer *et al.* 2007). Most previous studies that have documented bacterial community changes have focussed on natural processes, such as plant community succession and stages of soil development (Jesus *et al.* 2009; Rime *et al.* 2015). Few studies have explored bacterial responses to active ecological restoration (Potthoff *et al.* 2006; Banning *et al.* 2011; Cavagnaro *et al.* 2016). To the best of our knowledge, only one other study has used metabarcoding of eDNA as a method for assessing the impact of restoration on soil microbiomes (Araújo *et al.* 2014). Araújo *et al.* (2014) explored the effect of high- vs. low-diversity non-native planting treatments on degraded land bacterial communities. However, unlike our study, Araújo and colleagues did not explore changes in bacteria across a restoration chronosequence, different responses at depth or responses to alternate restoration interventions with native plant species.

The bacterial phyla that displayed the greatest change in abundance across our revegetation chronosequence were Acidobacteria and Firmicutes. These trends support previous work that has shown Acidobacteria to be more abundant in forest and grassland soils rather than agricultural soils, and less abundant in nutrient-rich soils such as pastures (Fierer *et al.* 2007). Firmicutes abundance has previously been shown to be higher in pasture habitats than in forests (Jesus *et al.* 2009). These two phyla are associated with important functional roles in ecosystems (e.g. Acidobacteria have been shown to breakdown complex, recalcitrant sugars; Ward *et al.* 2009; and Firmicutes such as Clostridium are well-known human commensals and pathogens). Additional work is required to confirm the functionality of OTUs identified in our study (e.g. soil enzyme assays, Potthoff *et al.* 2006; proteomic investigation, Bastida *et al.* 2009).

We observed an increase in soil organic carbon across the revegetation sequence, which is consistent with previous work on restoration of a Californian grassland (Potthoff *et al.* 2006), and the positive effect of revegetation on soil organic carbon sequestration more generally (Wang *et al.* 2011). The direction of this change was towards the concentrations found at the remnant sites, which appears consistent with a previous study of Jarrah forest restoration in Western Australia (George *et al.* 2010). George *et al.* (2010) observed organic matter accumulated in the topsoil with increasing time since restoration, which was a trajectory towards a native soil carbon profile. We found nitrate and phosphorous decreased with age of restoration,

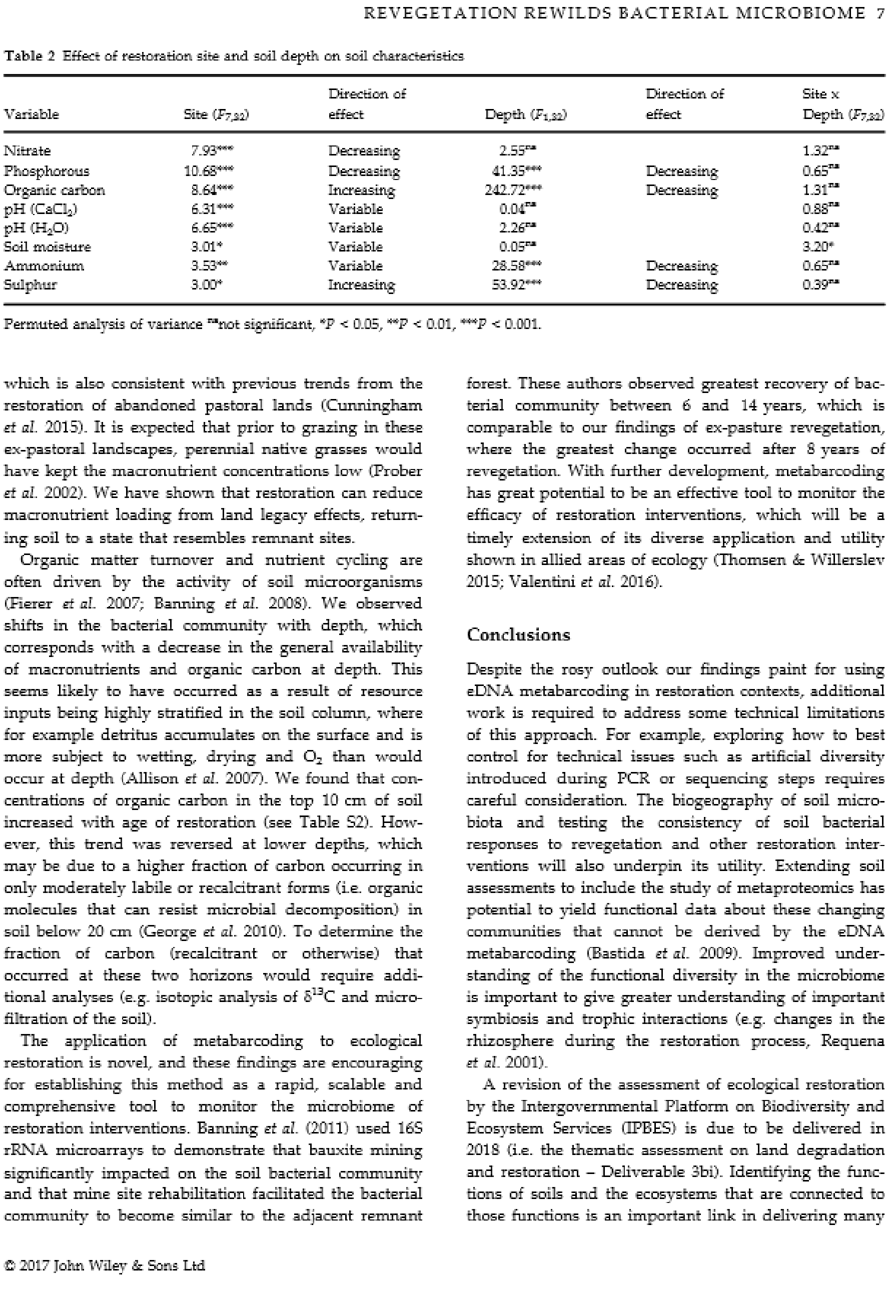


Table 2 Effect of restoration site and soil depth on soil characteristics

Variable	Site ($F_{7,32}$)	Direction of effect	Depth ($F_{1,32}$)	Direction of effect	Site x Depth ($F_{7,32}$)
Nitrate	7.93***	Decreasing	2.55^{ns}		1.32^{ns}
Phosphorous	10.68***	Decreasing	41.35***	Decreasing	0.65^{ns}
Organic carbon	8.64***	Increasing	242.72***	Decreasing	1.31^{ns}
pH ($CaCl_2$)	6.31***	Variable	0.04^{ns}		0.88^{ns}
pH (H_2O)	6.65***	Variable	2.26^{ns}		0.42^{ns}
Soil moisture	3.01*	Variable	0.05^{ns}		3.20*
Ammonium	3.53**	Variable	28.58***	Decreasing	0.65^{ns}
Sulphur	3.00*	Increasing	53.92***	Decreasing	0.39^{ns}

Permuted analysis of variance ^{ns}not significant, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

which is also consistent with previous trends from the restoration of abandoned pastoral lands (Cunningham *et al.* 2015). It is expected that prior to grazing in these ex-pastoral landscapes, perennial native grasses would have kept the macronutrient concentrations low (Prober *et al.* 2002). We have shown that restoration can reduce macronutrient loading from land legacy effects, returning soil to a state that resembles remnant sites.

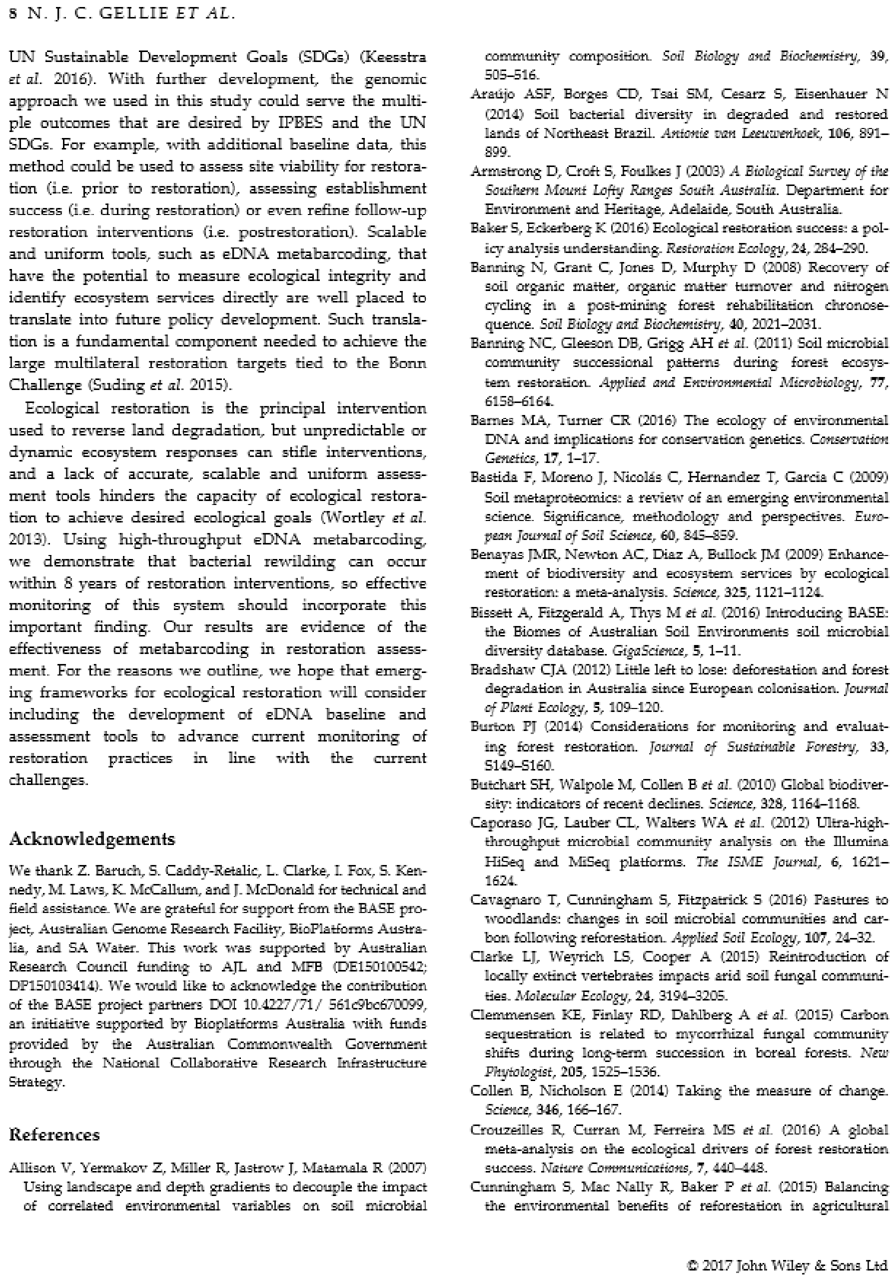
Organic matter turnover and nutrient cycling are often driven by the activity of soil microorganisms (Fierer *et al.* 2007; Banning *et al.* 2008). We observed shifts in the bacterial community with depth, which corresponds with a decrease in the general availability of macronutrients and organic carbon at depth. This seems likely to have occurred as a result of resource inputs being highly stratified in the soil column, where for example detritus accumulates on the surface and is more subject to wetting, drying and O_2 than would occur at depth (Allison *et al.* 2007). We found that concentrations of organic carbon in the top 10 cm of soil increased with age of restoration (see Table S2). However, this trend was reversed at lower depths, which may be due to a higher fraction of carbon occurring in only moderately labile or recalcitrant forms (i.e. organic molecules that can resist microbial decomposition) in soil below 20 cm (George *et al.* 2010). To determine the fraction of carbon (recalcitrant or otherwise) that occurred at these two horizons would require additional analyses (e.g. isotopic analysis of $\delta^{13}C$ and microfiltration of the soil).

The application of metabarcoding to ecological restoration is novel, and these findings are encouraging for establishing this method as a rapid, scalable and comprehensive tool to monitor the microbiome of restoration interventions. Banning *et al.* (2011) used 16S rRNA microarrays to demonstrate that bauxite mining significantly impacted on the soil bacterial community and that mine site rehabilitation facilitated the bacterial community to become similar to the adjacent remnant forest. These authors observed greatest recovery of bacterial community between 6 and 14 years, which is comparable to our findings of ex-pasture revegetation, where the greatest change occurred after 8 years of revegetation. With further development, metabarcoding has great potential to be an effective tool to monitor the efficacy of restoration interventions, which will be a timely extension of its diverse application and utility shown in allied areas of ecology (Thomsen & Willerslev 2015; Valentini *et al.* 2016).

Conclusions

Despite the rosy outlook our findings paint for using eDNA metabarcoding in restoration contexts, additional work is required to address some technical limitations of this approach. For example, exploring how to best control for technical issues such as artificial diversity introduced during PCR or sequencing steps requires careful consideration. The biogeography of soil microbiota and testing the consistency of soil bacterial responses to revegetation and other restoration interventions will also underpin its utility. Extending soil assessments to include the study of metaproteomics has potential to yield functional data about these changing communities that cannot be derived by the eDNA metabarcoding (Bastida *et al.* 2009). Improved understanding of the functional diversity in the microbiome is important to give greater understanding of important symbiosis and trophic interactions (e.g. changes in the rhizosphere during the restoration process, Requena *et al.* 2001).

A revision of the assessment of ecological restoration by the Intergovernmental Platform on Biodiversity and Ecosystem Services (IPBES) is due to be delivered in 2018 (i.e. the thematic assessment on land degradation and restoration – Deliverable 3bi). Identifying the functions of soils and the ecosystems that are connected to those functions is an important link in delivering many



UN Sustainable Development Goals (SDGs) (Keesstra *et al.* 2016). With further development, the genomic approach we used in this study could serve the multiple outcomes that are desired by IPBES and the UN SDGs. For example, with additional baseline data, this method could be used to assess site viability for restoration (i.e. prior to restoration), assessing establishment success (i.e. during restoration) or even refine follow-up restoration interventions (i.e. postrestoration). Scalable and uniform tools, such as eDNA metabarcoding, that have the potential to measure ecological integrity and identify ecosystem services directly are well placed to translate into future policy development. Such translation is a fundamental component needed to achieve the large multilateral restoration targets tied to the Bonn Challenge (Suding *et al.* 2015).

Ecological restoration is the principal intervention used to reverse land degradation, but unpredictable or dynamic ecosystem responses can stifle interventions, and a lack of accurate, scalable and uniform assessment tools hinders the capacity of ecological restoration to achieve desired ecological goals (Wortley *et al.* 2013). Using high-throughput eDNA metabarcoding, we demonstrate that bacterial rewilding can occur within 8 years of restoration interventions, so effective monitoring of this system should incorporate this important finding. Our results are evidence of the effectiveness of metabarcoding in restoration assessment. For the reasons we outline, we hope that emerging frameworks for ecological restoration will consider including the development of eDNA baseline and assessment tools to advance current monitoring of restoration practices in line with the current challenges.

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Data accessibility

All raw data have been deposited in the Sequence Read Archive (SRA) under the Bioproject ID PRJNA317932. Information on all SRA accessions related to this data set can also be found at (https://downloads.bioplatforms.com/metadata/base/amplicon/amplicons). All OTU pipelines can be found at (http://www.bioplatforms.com/soil-biodiversity/) under 'BASE protocols and Procedures'. OTU and soil chemistry data available from AEKOS Digital repository. OTU raw data matrix (Gellie *et al*. 2017a) doi: 10.4227/05/5878480a91885 and soil chemistry (Gellie *et al*. 2017b) doi: 10.4227/05/587d63e2dd056.

M.F.B., N.J.C.G. and A.J.L. designed the study. N.J.C.G., J.G.M. and M.F.B. ran the experiments and analysed the data. All authors discussed the results. M.F.B. and J.G.M. wrote the first draft of the manuscript, and N.J.C.G. and A.J.L. contributed substantially to revisions.

Supporting information

Additional supporting information may be found in the online version of this article.

Fig. S1 Principal coordinates analysis of dissimilarity of the restoration chronosequence sites based on Jaccard distance matrix of bacterial 16S rRNA OTU presence-absence.

Fig. S2 Principle coordinates analysis of restoration sites based on Bray-Curtis distance matrix of bacterial phyla rarefied abundances.

Fig. S3 Principle components analysis of restoration sites based on centred log-ratio transformation of bacterial phyla abundances.

Fig. S4 Principle components analysis of restoration sites based on centred log-ratio transformation of bacterial 16S rRNA OTU abundances.

Fig. S5 Principal coordinates analysis of dissimilarity of the restoration chronosequence sites based on soil physical and chemical characteristics.

Table S1 Mean (±SD) number of quality-filtered reads on raw data, OTU richness on raw data, Shannon's diversity index and evenness on rarefied data across the restoration chronosequence sites.

Table S2 Soil characteristic values across the restoration chronosequence sites.

Table S3 Pearson correlations between soil characteristics and Acidobacteria and Firmicutes, with + indicating a positive correlation and – indicating a negative correlation.

Table S4 Effect of restoration site and soil depth on diversity of bacteria phyla.

Supporting Information

Table S1 Mean (± SD) number of quality-filtered reads on raw data, OTU richness on raw data, Shannon's diversity index and evenness on rarefied data across the restoration chronosequence sites.

Site	Depth (cm)	Reads ± SD	OTUs ± SD	Shannon's diversity ± SD	Evenness ± SD	Effective species number ± SD
Cleared	0-10	66,010 ± 1,299	1,566 ± 45	5.82 ± 0.32	0.79 ± 0.04	416 ± 125
6 years ago	0-10	54,190 ± 7,772	1,508 ± 32	6.03 ± 0.15	0.82 ± 0.02	475 ± 71
7 years ago	0-10	51,844 ± 3,962	1,664 ± 38	6.23 ± 0.02	0.84 ± 0.01	588 ± 18
8 years ago	0-10	52,954 ± 8,710	1,653 ± 43	6.06 ± 0.15	0.82 ± 0.02	509 ± 81
10 years ago	0-10	74,068 ± 7,538	1,616 ± 172	5.87 ± 0.25	0.79 ± 0.02	413 ± 105
Remnant A	0-10	58,871 ± 7,995	1,574 ± 101	6.12 ± 0.14	0.83 ± 0.01	566 ± 92
Remnant B	0-10	56,762 ± 2,549	1,515 ± 56	5.99 ± 0.18	0.82 ± 0.03	498 ± 88
Remnant C	0-10	45,439 ± 18,008	1,382 ± 142	6.00 ± 0.24	0.83 ± 0.02	491 ± 115
Cleared	20-30	72,322 ± 10,009	2,184 ± 26	6.11 ± 0.12	0.81 ± 0.01	451 ± 53
6 years ago	20-30	71,636 ± 2,953	2,146 ± 140	6.11 ± 0.15	0.81 ± 0.01	452 ± 71
7 years ago	20-30	66,406 ± 14,519	2,187 ± 229	5.95 ± 0.42	0.79 ± 0.05	406 ± 156
8 years ago	20-30	60,362 ± 14,007	2,232 ± 183	6.26 ± 0.15	0.82 ± 0.01	527 ± 79
10 years ago	20-30	72,939 ± 9,574	2,154 ± 169	6.17 ± 0.07	0.82 ± 0.00	477 ± 35
Remnant A	20-30	67,699 ± 11,715	2,196 ± 154	6.20 ± 0.15	0.82 ± 0.02	499 ± 72
Remnant B	20-30	60,405 ± 19,200	1,926 ± 97	5.78 ± 0.20	0.77 ± 0.02	327 ± 61
Remnant C	20-30	46,906 ± 6,609	1,784 ± 240	5.90 ± 0.19	0.79 ± 0.01	370 ± 67

Table S2 Soil characteristic values across the restoration chronosequence sites.

Site	Depth (cm)	Nitrate (mg/Kg)	Phosphorus (mg/Kg)	Ammonium N (mg/Kg)	Sulphur (mg/Kg)	Organic C (%)	pH ($CaCl_2$)	pH (H_2O)	Soil moisture (%)
Cleared	0-10	16.00 ± 2.00	20.67 ± 4.41	6.67 ± 0.88	5.23 ± 0.13	3.15 ± 0.23	4.60 ± 0.06	5.60 ± 0.06	2.49 ± 0.11
6 years ago	0-10	7.67 ± 0.88	11.33 ± 0.67	10.33 ± 1.20	4.90 ± 0.35	2.98 ± 0.16	4.53 ± 0.03	5.63 ± 0.03	2.33 ± 0.23
7 years ago	0-10	7.33 ± 3.28	16.00 ± 1.15	7.00 ± 1.00	7.03 ± 0.68	4.87 ± 0.09	4.53 ± 0.03	5.67 ± 0.07	3.96 ± 0.34
8 years ago	0-10	0.83 ± 0.17	8.33 ± 1.86	12.67 ± 1.86	4.27 ± 0.46	3.03 ± 0.62	4.60 ± 0.10	5.80 ± 0.12	2.63 ± 0.33
10 years ago	0-10	3.00 ± 0.58	11.00 ± 1.00	7.00 ± 2.08	5.57 ± 0.52	4.15 ± 0.19	4.13 ± 0.03	5.30 ± 0.06	2.64 ± 0.22
Remnant A	0-10	1.17 ± 0.44	11.00 ± 2.08	12.33 ± 2.73	5.53 ± 0.87	4.27 ± 0.23	4.90 ± 0.38	6.10 ± 0.32	4.26 ± 1.04
Remnant B	0-10	3.00 ± 0.58	8.00 ± 0.00	6.33 ± 1.20	6.87 ± 1.11	3.74 ± 0.18	5.17 ± 0.19	6.17 ± 0.24	3.34 ± 0.35
Remnant C	0-10	9.33 ± 11.02	12.50 ± 4.50	11.50 ± 5.50	9.00 ± 3.00	4.05 ± 0.05	4.50 ± 0.10	5.65 ± 0.05	3.60 ± 1.40
Cleared	20-30	12.67 ± 3.79	17.00 ± 6.56	<1	2.40 ± 0.36	1.46 ± 0.28	4.73 ± 0.15	5.70 ± 0.26	4.66 ± 1.56
6 years ago	20-30	4.00 ± 1.73	7.33 ± 0.58	5.67 ± 3.51	2.50 ± 0.70	1.38 ± 0.14	4.57 ± 0.06	6.10 ± 0.17	3.55 ± 0.69
7 years ago	20-30	6.67 ± 3.06	6.67 ± 0.58	2.67 ± 0.58	3.73 ± 1.29	2.03 ± 0.52	4.57 ± 0.06	6.10 ± 0.10	2.41 ± 0.89
8 years ago	20-30	3.33 ± 1.53	3.33 ± 0.58	8.33 ± 3.21	2.43 ± 0.32	1.18 ± 0.02	4.60 ± 0.17	5.63 ± 0.06	1.78 ± 0.30
10 years ago	20-30	1.67 ± 0.58	4.00 ± 0.00	3.00 ± 1.00	2.47 ± 0.06	1.97 ± 0.25	4.40 ± 0.00	5.87 ± 0.15	2.59 ± 0.26
Remnant A	20-30	2.83 ± 2.84	4.33 ± 0.58	4.33 ± 0.58	3.50 ± 0.66	2.09 ± 0.56	4.83 ± 0.21	5.73 ± 0.15	3.82 ± 0.81
Remnant B	20-30	2.67 ± 0.58	4.33 ± 0.58	5.33 ± 4.93	3.83 ± 1.36	2.00 ± 0.46	4.83 ± 0.21	5.73 ± 0.12	4.16 ± 0.45
Remnant C	20-30	1.50 ± 0.71	5.00 ± 1.73	5.67 ± 1.53	3.97 ± 0.80	2.37 ± 0.60	4.47 ± 0.23	5.73 ± 0.15	2.93 ± 0.58

Table S3 Pearson correlations between soil characteristics and Acidobacteria and Firmicutes, with + indicating a positive correlation and - indicating a negative correlation.

Depth (cm)	Variable	Acidobacteria r^2	Firmicutes r^2
0-10	**Nitrate**	0.21 (-)*	0.15 (+)$^{\circ}$
0-10	**Phosphorous**	0.37 (-)**	0.13 (+)$^{\circ}$
0-10	**Organic carbon**	0.19 (+)*	0.24 (-)*
0-10	**pH $\mathrm{CaCl_2}$**	0.01 (+)^{ns}	0.08 (-)^{ns}
0-10	**pH $\mathrm{H_2O}$**	0.03 (+)^{ns}	0.11 (-)^{ns}
0-10	**Soil moisture**	0.12 (+)$^{\circ}$	0.28 (-)**
0-10	**Ammonium**	0.01 (+)^{ns}	0.03 (-)^{ns}
0-10	**Sulphur**	0.16 (+)$^{\circ}$	0.21 (-)*
20-30	**Nitrate**	0.42 (-)**	0.41 (+)**
20-30	**Phosphorous**	0.38 (-)**	0.48 (+)**
20-30	**Organic carbon**	0.17 (+)*	0.27 (-)**
20-30	**pH $\mathrm{CaCl_2}$**	0.01 (-)^{ns}	0.01 (-)^{ns}
20-30	**pH $\mathrm{H_2O}$**	0.11 (+)^{ns}	0.11 (-)^{ns}
20-30	**Soil moisture**	0.03 (-)^{ns}	0.06 (+)^{ns}
20-30	**Ammonium**	0.08 (+)^{ns}	0.08 (-)^{ns}
20-30	**Sulphur**	0.23 (+)*	0.24 (-)*

^{ns}, not significant, $^{\circ}P < 0.1$, $^{*}P < 0.05$, $^{**}P < 0.01$

Table S4. Effect of restoration site and soil depth on diversity of bacteria phyla

	Effective species number					Effective species number				
Phylum	**Site ($F_{7,32}$)**	**Site ($F_{7,32}$)**	**Site ($F_{7,32}$)**	**Site ($F_{7,32}$)**	**Site ($F_{7,32}$)**	**Site ($F_{7,32}$)**	**Direction of effect**	**Depth ($F_{1,32}$)**	**Direction of effect**	**Site x Depth ($F_{7,32}$)**
Acidobacteria	1.60 ^{ns}	1.60 ^{ns}	1.60 ^{ns}	1.60 ^{ns}	1.60 ^{ns}	1.57 ^{ns}		2.65 ^{ns}		1.42 ^{ns}
Actinobacteria	3.03 *	3.03 *	3.03 *	3.03 *	3.03 *	2.75 *	Decreasing	6.52 *	Decreasing	0.69 ^{ns}
Bacteroidetes	1.03 ^{ns}	1.03 ^{ns}	1.03 ^{ns}	1.03 ^{ns}	1.03 ^{ns}	1.00 ^{ns}		0.30 ^{ns}		0.54 ^{ns}
Chloroflexi	6.25 ***	6.25 ***	6.25 ***	6.25 ***	6.25 ***	8.51 ***	Variable	0.03 ^{ns}		1.91 ^{ns}
Firmicutes	5.48 ***	5.48 ***	5.48 ***	5.48 ***	5.48 ***	4.17 **	Variable	7.23 *	Increasing	1.36 ^{ns}
Gemmatimonadetes	4.58 **	4.58 **	4.58 **	4.58 **	4.58 **	3.74 **	Variable	0.77 ^{ns}		0.78 ^{ns}
Planctomycetes	1.99 ^{ns}	1.99 ^{ns}	1.99 ^{ns}	1.99 ^{ns}	1.99 ^{ns}	2.09 ^{ns}		2.74 ^{ns}		0.97 ^{ns}
Proteobacteria	2.79 *	2.79 *	2.79 *	2.79 *	2.79 *	2.08 ^{ns}		3.03 ^{ns}		1.01 ^{ns}
Verrucomicrobia	5.86 ***	5.86 ***	5.86 ***	5.86 ***	5.86 ***	5.34 ***	Decreasing	28.84 ***	Decreasing	1.41 ^{ns}
Rare	2.97 *	2.97 *	2.97 *	2.97 *	2.97 *	2.65 *	Variable	0.54 ^{ns}		1.44 ^{ns}

Permuted analysis of variance ^{ns}, not significant, *P < 0.05, **P < 0.01, ***P < 0.001

Figure S1

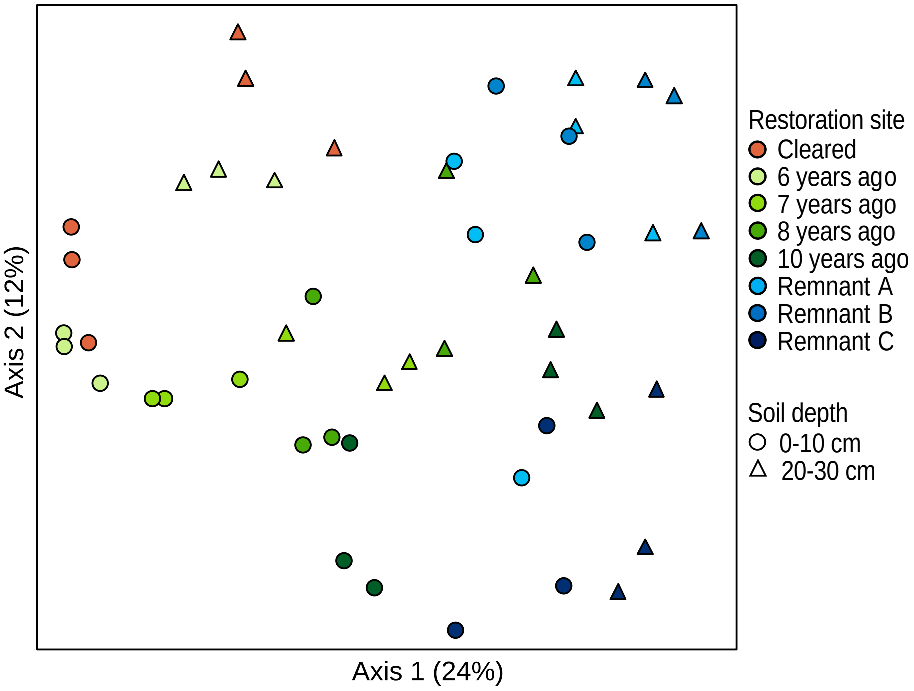

Figure S1. Principal coordinates analysis of dissimilarity of the restoration chronosequence sites based on Jaccard distance matrix of bacterial 16S rRNA OTU presence-absence.

Figure S2

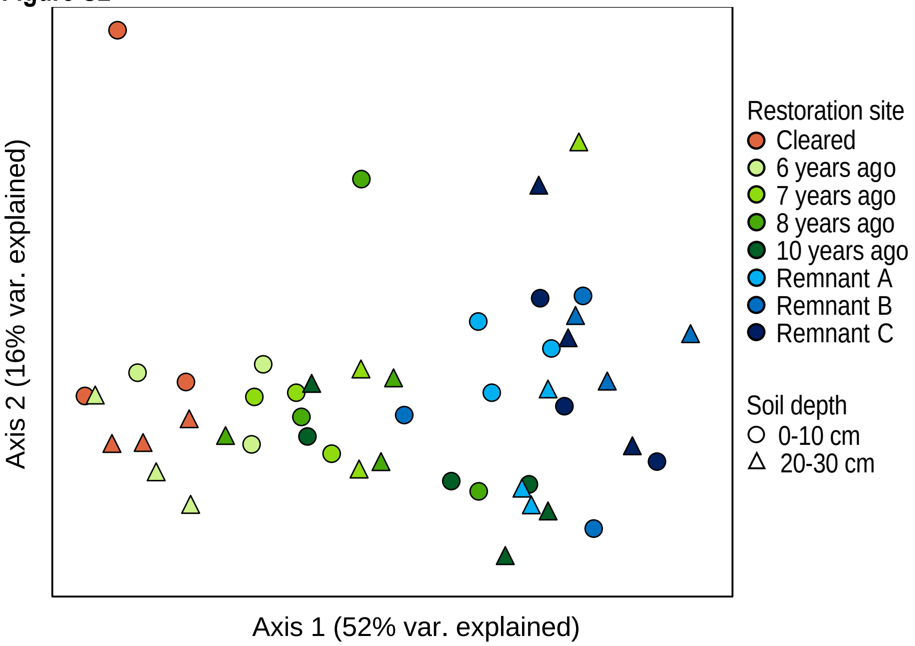

Figure S2. Principle coordinates analysis of restoration sites based on Bray-Curtis distance matrix of bacterial phyla rarefied abundances.

Figure S3

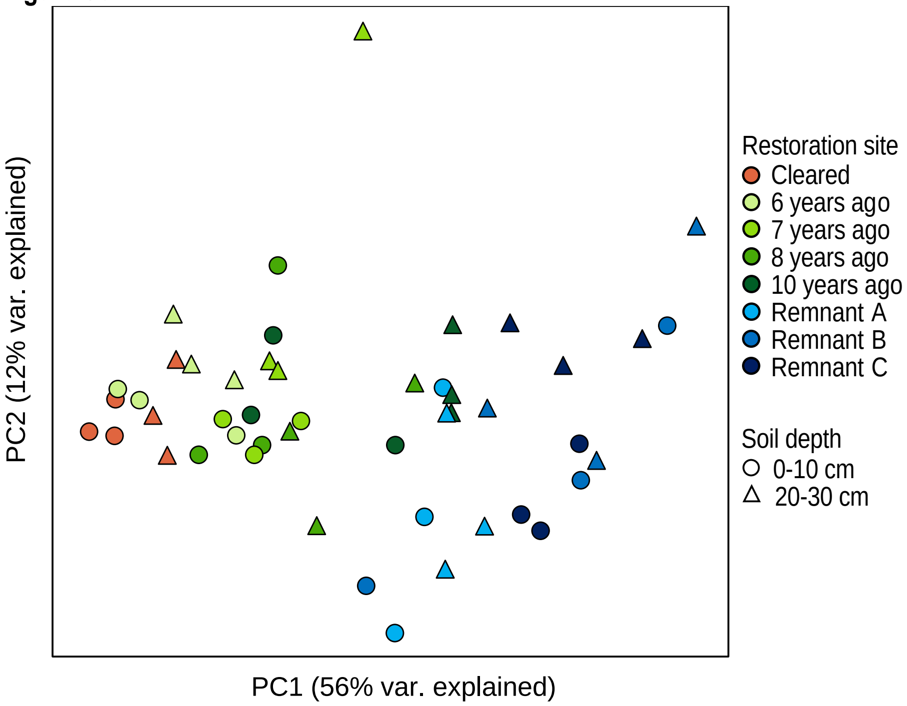

Figure S3. Principle components analysis of restoration sites based on centred log-ratio transformation of bacterial phyla abundances.

Figure S4

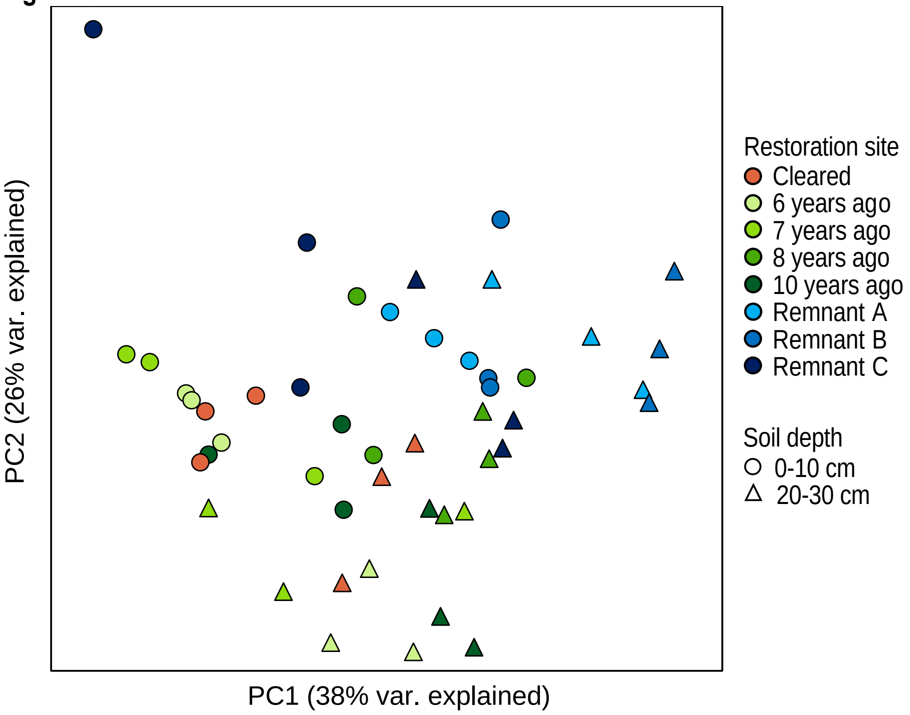

Figure S4. Principle components analysis of restoration sites based on centred log-ratio transformation of bacterial 16S rRNA OTU abundances.

Figure S5

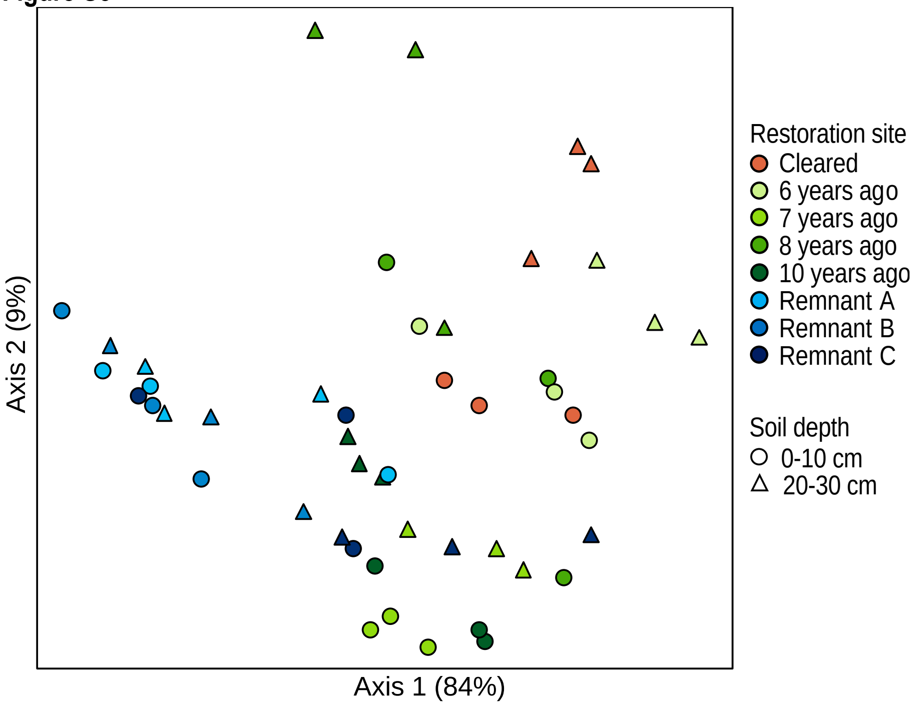

Figure S5. Principal coordinates analysis of dissimilarity of the restoration chronosequence sites based on soil physical and chemical characteristics.

CHAPTER 7: CONCLUSIONS

This thesis is presented as a portfolio of manuscripts that are either published, or pending submission to peer-reviewed international journals. The thesis chapters each include a discussion of the results obtained. Here in Chapter 7*,* the conclusion of this thesis, I put forward a cohesive synthesis of these works. I also build on the insight gained from new monitoring techniques shown in Chapter 6, and identify future directions of research provoked by the work contained in this thesis. I reveal the contribution this body of work has made towards bridging the knowledge gaps and developing best practice for restoration in a changing climate.

Synthesis of thesis

In Chapter 2, I introduced the concept that embedding experiments in restoration will advance restoration science and inform restoration practice. By applying this systematic approach to restoration projects I have advanced the understanding of the effect of provenance choice on restoration. It is apparent that remaining with the *status quo* of restoration practice (i.e. employing strictly local provenancing) can have significant negative consequences for restoration projects. Chapters 3, 4, and 5 largely support a conclusion for more dynamic provenancing practices but would also benefit from follow-up studies during later life stages. Collectively, these chapters amount to the study of six core-restoration species tested in 5 common gardens, from provenances spanning 2.5 degrees of latitude (*ca.* 460 km) in southern Australia. The results from these common garden studies give clear direction to our industry partners (e.g. Trees For Life, Greening Australia and SA Water) that arid to mesic transfer of seed is a low risk strategy to mitigate the negative effects of climate change. We capitalise on the substantial benefit offered by embedded experiments by revisiting restoration projects at latter life stages of the plants with research infrastructure available *in situ* (Chapter 3). Along with aiding the provenance selection process, we also present a timely and novel application to monitoring restoration projects by sequencing eDNA (Chapter 6). This genomics approach requires more development but holds great promise in reconciling a long standing restoration shortfall - access to a cost-effective, scalable restoration monitoring protocol.

Development of national standards for practice

Ambitious global restoration targets (e.g. The Bonn Challenge) have inspired huge investments in restoration, however the restoration community has had little information as to whether current restoration practices will meet long-term objectives. In Chapter 3, and 5 clear signals of maladaptation are identified in locally sourced plants used for restoration in southern Australia. These results have implications for restoration practices and suggest that reviewing current seed strategies could significantly improve restoration outcomes. For example, in Chapter 3 a study of *Eucalyptus leucoxylon*; 10% greater survival, 25% larger plants and up to 45% greater pathogen resistance could be achieved during establishment by incorporating a more diverse range of seed from warmer and dryer locations. Seed sourcing strategies which mix provenances from a combination of local and more distant locations are now gaining traction with restoration practitioners as a direct result of our studies (Appendix B). To this effect, I along with other authors contributed to the 2016 Society for Ecological Restoration Australasia: National Standards for Ecological Restoration Content (Appendix C). Our contribution appears in the guidelines as (Appendix 3; genetics, fragmentation, and climate change) and provides a pragmatic step forward towards achieving best practice restoration.

Adaptive management improvement

I have significantly contributed to simplifying adaptive management options for our stakeholders by qualifying and reporting on the performance of plant genetic resources *in situ*. Too often the funding cycles that enable restoration to initiate interventions are prohibitively short and lack oversight, resulting in a set and forget approach that does not harness or act on the knowledge contained in project outcomes. By embedding experiments and implementing the strategies outlined (i.e. the principal recommendations from Chapter 2) a science-practice interface has been forged with partners that has the agility to act decisively on evidence as it is produced. The modest setup costs of such a collaborative model has already paid dividends to our partners and has the ability to adapt to emerging restoration challenges by dynamically realigning an appropriate research focus when needed. Furthermore, an ability to act authoritatively with evidence based follow-up interventions is empowering and encourages partnerships. I suggest that fostering this joint stewardship between science and practice is an important advance in delivering optimal results to projects and goes a long way towards the development of best practice in this industry.

Collation, interpretation and distribution of results

Chapter 2 recognises that consolidating and networking the information obtained in experiments (such as provenance trials) is a necessary but all too often missing in restoration practice. To build on this observation and with the assistance of The University of Adelaide I helped convene the first National Provenance Synthesis Workshop in April 2016. The objective of the workshop was to gather world leaders in the field of restoration genetics and forestry science to synthesise provenance trial resources and derive evidence-based seed collection guidelines. Data from 153 trials (including published data from Chapters 3-5) spanning 47 years of provenance research in Australia have be collated and analysed. This workshop was an Australian first and a workgroup is currently exploring the potential of predicting provenance performance based on geographic distance, climate distance, and/or fragmentation of source pops which if successful will be a paradigm step forward to developing best practice in this industry.

Infrastructure development

The research conducted during this thesis also provides a secure foundation from which to stage further studies. By establishing trials that contain in excess of 5,000 plants we have created important research infrastructure. These trials have already progressed into teaching facilities, been used as demonstration sites and have the capacity to develop as long term studies where we can explore the biotic and abiotic factors effecting restoration at later life stages of these plants. Facilitating a temporal dimension of restoration performance is generally considered too costly but it remains widely recognised as a major gap by contributors to the restoration ecology literature. This infrastructure now allows us to explore patterns of selection that could perpetuate an adaptation lag to contemporary conditions, and important aspects of recruitment or phenological variation that cannot be assessed during early establishment (as was done in in Chapter 3). Furthermore, the methods we advocate for embedding experiments in restoration projects in

Chapter 2 and the eDNA assessment tool from Chapter 6 may also provide a platform to measure ecosystem service benefits to people as well as nature. This progressive approach to assessment has found popular acceptance with peers (Appendix D) and aligns well with multilateral policy development from the Intergovernmental Platform on Biodiversity and Ecosystem Services (IPBES) and the United Nations Sustainable Development Goals (SDGs) that are outlined in more detail in Chapter 6.

Formation of strategic provenance advice

In *Chapter 5* I argue for directional selection (e.g. arid to mesic) as a low risk mitigative management strategy to increase climate resilience in plantings. This finding is corroborated in Chapter 3 and 4 along with adjunct empirical studies I have been involved in during my candidature (Appendix E: Breed *et al.* 2016b and Aoppendix F: Baruch *et al.* 2016). Although, as has been stated in these chapters, when no genetic information is available precautionary principles of genetic risk management should apply to any translocation of genetic material. In fact this precaution is often used as the principal justification of local provenancing (e.g. the risk of outbreeding depression and maladaptation). Gaps in the information on genetic diversity and structure of Australian native plants used in restoration add to the hyperbole surrounding provenance choice. I was thus fortunate to have been invited to a workshop convened in Canberra (2014) that aimed to investigate the overarching population genetic parameters of Australian plants. The output of this workshop (Appendix G; Broadhurst *et al.* 2017) found some notable differences in population genetic parameters compared with global trends. The differences from global trends included a striking effect of disjunction and abundance in Australia and the unexpected result of higher genetic diversity residing in the eastern biome of Australia. The consequence of this important work is that we can now facilitate genetic predictions into conservation and restoration decision-making with better confidence than could ever be done before.

The trends that were found in the genetic diversity of Australian Flora (Broadhurst *et al.* 2017) have the potential to provide important evidence to support on-ground restoration decisions. However scientific literature does not always translate easily into policy or practice so interpretive guidelines are often a necessary interim step to help empower end-users. Discussions with land managers and policy makers about seed availability and choice at a workshop jointly convened by Trees for Life and the Environment Institute in March 2017 highlighted that the lack of the descriptive procedures was a barrier to them using alternative provenancing strategies in projects. To bridge this gap our research group has engaged with partners to produce project specific provenancing guidelines for mitigating the effects of climate change. The guidelines are combined in a pamphlet that presents background (Figure 1.) and a decision tree (Figure 2.) to help end-users choose seed recipes for their projects. Although still in development, once finalised we hope these guidelines will provide a useable tool to advise decision makers about the strategic management of genetic resources used for restoration.

Figure 1. Draft guidelines to incorporate climate change decisions into seed collection for restoration (Background and resources page 1)

SEED COLLECTION PROTOCOLS

Source plants: no more than 20% of seed should be harvested from an individual plant in a season. Avoid plants with obvious disease or pathogen occurrence that could affect seed fitness or contaminate collections. Collect from the entire seed on offer of the source plants.

Source population: aim to collect from a minimum of 50 plants in a population to increase the genetic diversity of the seed mix but try to avoid adjacent plants (Vitt et al 2010). Include size, age and a spatial stagger (e.g. central and peripheral source plants should be included even if they are smaller) to ensure a representative selection of the trait variation and spatial arrangement of the population on offer. Collect across years and curate the seed separately. If using restoration sites as seed production areas curate the seed separately.

SEED COLLECTION DATA STANDARDS

Recording information at the time of collection is a pivotal step in best practice. The collector's name, locality (GPS coordinates], date, property ownership and contact details along with the collection permit are standard (but minimum) collection protocols.

Contextual data that includes habitat and land use, basic soil type, description of the topography, and hydrology as well as community dominant species are critical components for ecogeographically matching provenances.

Additionally estimates of population size, age stagger, number of donor populations, and the amount of recruitment will be important qualifiers of bulked seed if seeds are not separated by maternal line.

Some species hybridise or are notoriously difficult to identify so field voucher specimens allow expert confirmation of species identification. Vouchering is also important if small or marginal populations are the only ones available, or if species are known to be self incompatible (Vitt et al 2010).

Furthermore, well curated tissue samples can become DNA vouchers of target seed sources allowing *post hoc* validation and research of provenancing strategies.

SPECIES DISTRIBUTION CHANGE PREDICTED WITH CLIMATE CHANGE

The figure above shows how the local climates we know in the Mediterranean biome of Australia are generally predicted to head poleward and upward (Klausmeyer, et al 2009). Desert and grassland expansion along with temperate species range contractions are predicted with warming and drier conditions that are expected for southern Australia in the 21st century.

South Australia has relatively low topography and three terminal peninsulas that may impede leading edge range expansion for species that cannot adapt fast enough to changing environmental conditions. A rule of thumb for selecting seed with respect to climate change is to select source populations from drier hotter conditions.

AVOID ISOLATED TREES

The main impact on seed quality in more isolated and fragmented trees is a tendency for higher inbreeding and also receiving pollen from fewer potential fathers (see figure below). These features lead to seedlings that have poorer germination success, grow less vigorously and react poorly to stressful conditions (e.g. droughts, heat waves). The advice is that isolated trees or those in highly fragmented habitat blocks should not be heavily relied on as a revegetation seed source.

The Figure above shows how land clearing and habitat fragmentation can impact on the quality of seed. Genetically similar trees are represented by colours. Pollination is indicated by lines that show failed pollination by coloured dotted lines followed by a red cross (-); reduced pollination by coloured dotted lines; normal pollination by solid coloured lines; a relative increase in pollination by coloured lines with a green plus (+).

INCORPORATING CLIMATE CHANGE DECISIONS INTO SEED COLLECTION FOR RESTORATION

Selecting the geographic origin – the provenance – of seed is one of the key decisions in restoration. The last decade has seen a vigorous debate over whether to use local or non-local seed for restoration. The use of local seed has been the preferred approach as it is expected to maintain local adaptation and avoid deleterious population effects, such as outbreeding depression. However, the impacts of habitat fragmentation and global climate change on the suitability of local seed has been the catalyst for the alternative strategies that we provide in this guide. Collaboration can improve the efficiency of restoration methods and supply adaptive management options that can contribute to the success of projects.

Invest in monitoring because monitoring projects is the best way to assess project success, determine if intervention is needed and refine establishment methods. The data can be compared with provenance information to gain an understanding on the relative success of different provenances. Data should be electronically databased to allow effective assessment.

Embed experiments during the project planning, design, implementation and monitoring phases. Researchers can design experiments and collect, collate and analyse data. This information can then be used to help generated updated guidelines. Most if not all ecological restoration researchers want, but do not get, the chance to work with the people who apply their field of study.

MONITORING

The type and extent of monitoring to undertake depends on the scale and aim of the project, the resources available and the planting method (tubestock/direct seeding). The project will entail the propagation and establishment of a number of different species, but not all species need to be monitored; the aim of monitoring is to determine if your chosen seed sourcing strategy for particular species was successful.

Basic. At the minimum, data should be collected, after the first summer, on the recipient location and description, GPS coordinates., species monitored, date, surveyor, survival rate per provenance and management actions recommended/implemented.

Intermediate. If time and resources permit, record (for each provenance) the life stage (juvenile/adult/senescent) and health (evidence of pathogens/insect attack; herbivory; stress: heat/ frost/ toxicity/ water) of each plant. In following years it would be useful to measure recruitment (no. of seedlings, life stage and health), to indicate success of establishment.

Ideal. You may wish to learn whether a self-sustaining system is establishing, by recording data such as plant height/width/canopy cover, presence and abundance of weeds, and any establishment by other unplanted indigenous plant species. Is the landscape functioning (are there cryptogams, leaf litter, invertebrates, birds and mammals)

RESOURCES

Climate change projections: http://www.climatechangeinaustralia.gov.au/

Comprehensive seed management: http://spatialwebapps.environment.sa.gov.au/naturemaps/?locale=en-us&viewer=naturemaps

National standards for restoration: http://www.seraustralasia.com

Native seed management: http://www.florabank.org.au/

Species distribution Australia: http://www.ala.org.au

Species distribution South Australia: http://spatialwebapps.environment.sa.gov.au/naturemaps/?locale=en-us&viewer=naturemaps

Stepwise climate ready restoration guide: Hancock N, Harris R, Broadhurst L & Hughes L (2016) Climate-ready revegetation. A guide for natural resource managers. *Macquarie Uni*

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Byrne M, Stone L & Millar MA (2011) Assessing genetic risk in revegetation. *J Applied Ecol* 48, 1365–1373

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Havens K, Vitt P, Still S, Kramer AT, Fant JB & Schatz K (2015) Seed sourcing for restoration in an era of climate change. *Natural Areas J* 35(1):122-133

Klausmeyer, KR, Shaw, MR. 2009. Climate change, habitat loss, protected areas and the climate adaptation potential of species in Mediterranean ecosystems worldwide. PLoS One 4:e6392.

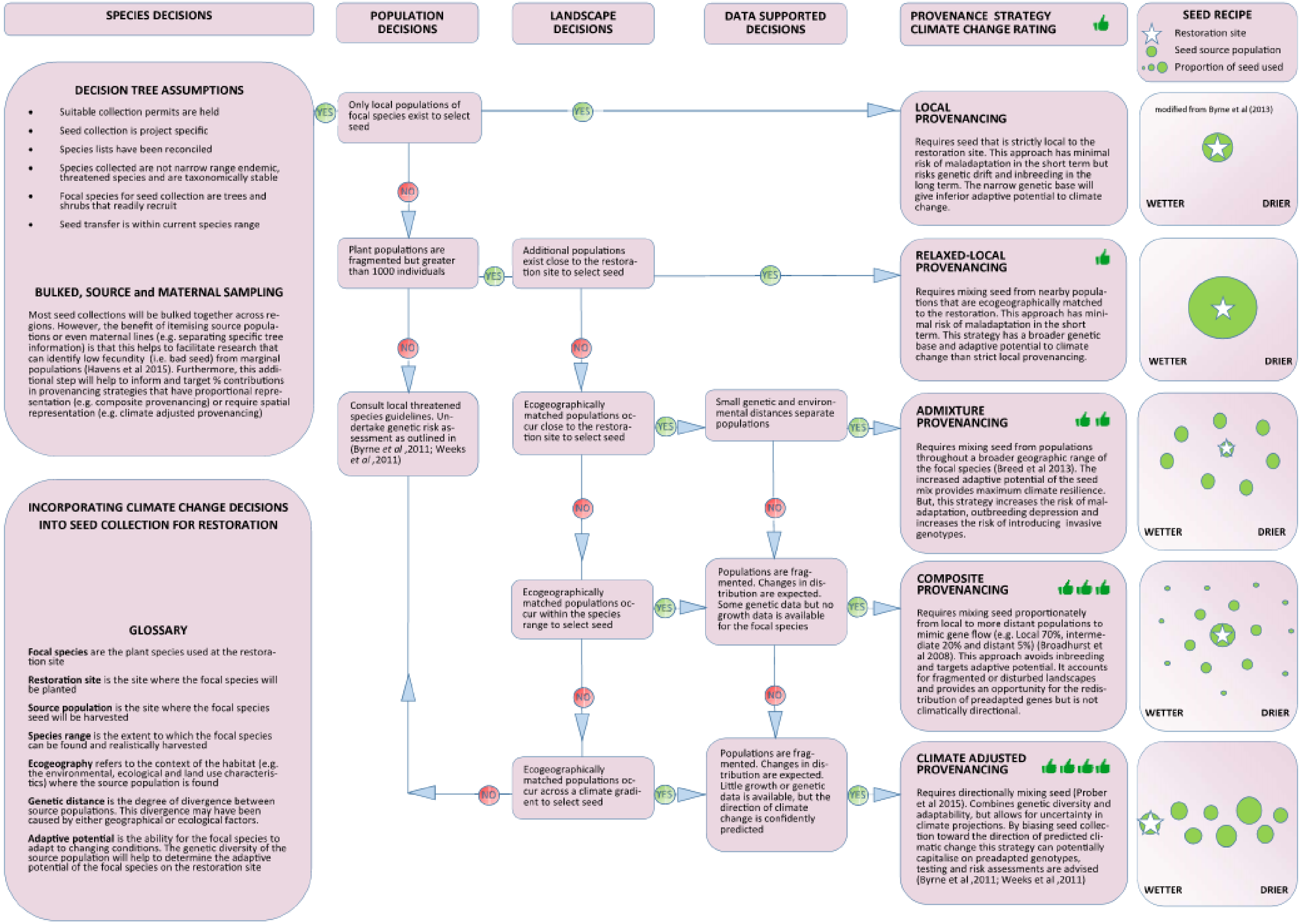
Prober SM, Byrne M, McLean EH, Steane DA, Potts BM, Vaillancourt RE & Stock WD (2015) Climate-adjusted provenancing: a strategy for climate-resilient ecological restoration. *Frontiers in Ecology & Evolution 3*

Ramalho CE, Byrne M & Yates CJ (2017) A Climate-Oriented Approach to Support Decision-Making for Seed Provenance in Ecological Restoration. *Frontiers in Ecology & Evolution* V5:95

Weeks AR, Sgro CM, Young AG, Frankham R, Mitchell NJ, Miller KA, Byrne M, Coates DJ, Eldridge MDB, Sunnucks P, Breed MF, James EA & Hoffmann AA (2011) Assessing the benefits and risks of translocations in changing environments: a genetic perspective. *Evolutionary Applications*, Blackwell Publishing, 709–725

Vitt, P, Havens, K, Kramer, AT, Sollenberger, D, Yates, E. 2010. Assisted migration of plants: Changes in latitudes, changes in attitudes. Biological Conservation 143:18-27.

Figure 2. Draft guidelines to incorporate climate change decisions into seed collection for restoration (Decision tree page 2)



DECISION TREE ASSUMPTIONS

- Suitable collection permits are held
- Seed collection is project specific
- Species lists have been reconciled
- Species collected are not narrow range endemic, threatened species and are taxonomically stable
- Focal species for seed collection are trees and shrubs that readily recruit
- Seed transfer is within current species range

BULKED, SOURCE and MATERNAL SAMPLING

Most seed collections will be bulked together across regions. However, the benefit of itemising source populations or even maternal lines (e.g. separating specific tree information) is that this helps to facilitate research that can identify low fecundity (i.e. bad seed) from marginal populations (Havens et al 2015). Furthermore, this additional step will help to inform and target % contributions in provenancing strategies that have proportional representation (e.g. composite provenancing) or require spatial representation (e.g. climate adjusted provenancing)

INCORPORATING CLIMATE CHANGE DECISIONS INTO SEED COLLECTION FOR RESTORATION

GLOSSARY

Focal species are the plant species used at the restoration site

Restoration site is the site where the focal species will be planted

Source population is the site where the focal species seed will be harvested

Species range is the extent to which the focal species can be found and realistically harvested

Ecogeography refers to the context of the habitat (e.g. the environmental, ecological and land use characteristics) where the source population is found

Genetic distance is the degree of divergence between source populations. This divergence may have been caused by either geographical or ecological factors.

Adaptive potential is the ability for the focal species to adapt to changing conditions. The genetic diversity of the source population will help to determine the adaptive potential of the focal species on the restoration site

LOCAL PROVENANCING

Requires seed that is strictly local to the restoration site. This approach has minimal risk of maladaptation in the short term but risks genetic drift and inbreeding in the long term. The narrow genetic base will give inferior adaptive potential to climate change.

RELAXED-LOCAL PROVENANCING 👍

Requires mixing seed from nearby populations that are ecogeographically matched to the restoration. This approach has minimal risk of maladaptation in the short term. This strategy has a broader genetic base and adaptive potential to climate change than strict local provenancing.

ADMIXTURE PROVENANCING 👍👍

Requires mixing seed from populations throughout a broader geographic range of the focal species (Breed et al 2013). The increased adaptive potential of the seed mix provides maximum climate resilience. But, this strategy increases the risk of maladaptation, outbreeding depression and increases the risk of introducing invasive genotypes.

COMPOSITE PROVENANCING 👍👍👍

Requires mixing seed proportionately from local to more distant populations to mimic gene flow (e.g. Local 70%, intermediate 20% and distant 5%) (Broadhurst et al 2008). This approach avoids inbreeding and targets adaptive potential. It accounts for fragmented or disturbed landscapes and provides an opportunity for the redistribution of preadapted genes but is not climatically directional.

CLIMATE ADJUSTED PROVENANCING 👍👍👍👍

Requires directionally mixing seed (Prober et al 2015). Combines genetic diversity and adaptability, but allows for uncertainty in climate projections. By biasing seed collection toward the direction of predicted climatic change this strategy can potentially capitalise on preadapted genotypes, testing and risk assessments are advised (Byrne et al ,2011; Weeks et al ,2011)

Next generation monitoring tools

Finding effective restoration monitoring tools that can assess site viability, monitor intervention efficacy and have the acuteness to prescribe follow-up actions has generally eluded the restoration industry. In Chapter 6 I provide a significant and innovative contribution to monitoring practices that may have the utility to serve the diversity of briefs required by the industry. The diversity and composition of the soil microbiome was investigated and I found that bacterial rewilding (i.e. return to a remnant state) occurs after 8 years of revegetation, however importantly this return does not have a linear relationship with time since restoration interventions. This published work was also the kernel for investigating the effect of restoration on alternate taxa (e.g. fungi, archaea) and has prompted collaborations with allied research groups looking at the effect of biodiversity and restoration on the human microbiome. Even with the traction we have attained in this field, additional work is required to address some of the technical limitations to this approach (e.g. PCR bias and poorly characterised taxonomic databases). This work is also planned for expansion to examine meta-proteomics of microbial communities to yield functional data about our focal taxon, taking us closer to accurate assessment of the effect of restoration on ecosystem services. The results from Chapter 6 suggest that best practice not only requires effective monitoring, but it should include the wider biodiversity of microbial communities for optimal outcomes, and that monitoring early performance (i.e. <8 years) will not accurately reflect the trajectory of community change.

Future directions

Local-provenancing has a strong historical grounding in forestry research and genecological studies and appeals to land managers due to its intuitiveness and logistical ease. However important findings in this thesis (e.g. local-maladaptation and sub-optimal plant performance) indicate that further investigation of this convention along with other core restoration practices (e.g. monitoring) are warranted.

First, the embedded experiments have proved their merit in this thesis but at this stage remain firmly in the domain of the researcher, I believe developing strategies to empower practitioners is a necessary evolution of practice. I envisage a modular design for embedded experiments in the future that can easily be added to projects by stakeholders. The units of experimentation should be simple, repeatable and inexpensive (see NutNet example Chapter 2). These units can be targeted and designed to explore the key knowledge gaps we identified in Chapter 2 (e.g. outcomes and co-benefits, community integration, financing, genetic resources, new technologies, policy and governance), and where necessary attend the abiotic and biotic site dependencies (see Chapter 5) that may be picked up in the new monitoring paradigm we suggest.

Second, we are only beginning to harvest the wealth of knowledge contained in past trials (see above *Collate, interpret and distribute results*) and this is particularly relevant for our partners but the data pipelines we are generating will have global appeal. I hope that I get the opportunity to progress this work further towards a coordinated network of strategic provenance advice aimed at the people restoring landscapes on my behalf anywhere on the planet.

Third, the eDNA monitoring we present in Chapter 6 in is in early stages of development but has substantial scope for improving practice. The ease of sampling means that sites can be remotely stratified and sampled for a modest cost with rudimentary tools by third parties (i.e. potentially even citizen scientists) following simple protocols. By using this technique we can add to established baseline information (see BASE project *Chapter 6*) to help predict restoration viability, prescribe initial treatments, generate *post hoc* assessments and potentially even rejuvenate failed restoration sites with inoculant microbiota. The utility of this method may lie in its broad application, but with future development it has the potential to acutely guide stakeholders where restoration dollars can be best spent.

Synopsis

I have identified broad-scale sub-optimal plant performance in southern Australia, exploited and re-purposed emerging technologies, and provided a suitable mechanism for the investigation of knowledge gaps in restoration. All of these elements have provided incremental steps towards best practice that is sympathetic to stakeholder needs. The extensions to this thesis that are highlighted in the sub-sections above are testimony that a systematic approach to bridging knowledge gaps is appropriate and bearing fruit for industry partners, but restoration is a complex task. The evidence I have presented suggests that even though static approaches to restoration are unlikely to meet the challenging new dynamics of restoration in the 21st century a cure-all panacea is unrealistic too. More so to achieve the millions of hectares forecasted in the coming decade's restoration practices will need to adapt and improve iteratively, through the effective use of science-practice synergies. A path towards networking the insights from embedded experiments has begun this process through the synthetic treatment of historical provenance trails, but the scale of current restoration projects requires this to be hastened and delivered globally.

So I will conclude my thesis with words that appear in Chapter 2, words that were initially crafted in anticipation of providing a way forward, but have since become an auspicious reprise in a thesis that has navigated me a little closer to developing best-practice.

> *"……..exploring the efficacy of restoration through embedded experiments and networking the results is a precautionary investment that will pay generational dividends".*
>
> *revision Frontiers in the Ecology and Environment)*

JOURNAL PUBLICATIONS DURING CANDIDATURE

2018

Yan, D., Mills, J.G., **Gellie, N.J**., Bissett, A., Lowe, A.J. and Breed, M.F. (2018). High-throughput eDNA monitoring of fungi to track functional recovery in ecological restoration. *Biological Conservation*, **217**, 113-120.

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DATA PUBLICATIONS DURING CANDIDATURE

2017

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Gellie, N.J.C., Mills, J.G., Breed, M.F., & Lowe, A.J. (2017b) Revegetation rewilds the soil bacterial microbiome of an old field. Part 2: Soil chemistry. AEKOS Data Submission. Version 2.0. doi: 10.4227/05/587d63e2dd056

2016

Baruch, Z., Christmas, M., Breed, M.F., Guerin, G.R., Caddy-Retalic, S., McDonald, J.T., Jardine, D.I., Leitch, E.J., **Gellie, N.J.C.**, Hill, K., McCallum, K., & Lowe, A.J. (2016a) Leaf trait associations with environmental variation in the wide-ranging shrub *Dodonaea viscosa* subsp. *angustissima* (Sapindaceae) Part 1: Latitude. AEKOS Data Submission. Version 1.0. doi: 10.4227/05/57C2343E4E9C2

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APPENDICES

APPENDIX A: Federated States of Degradia Multimedia*

Conceived: **Nick Gellie,** Martin Breed and Andrew Lowe
Script: **Nick Gellie,** Martin Breed, Corrin Baker and Andrew Lowe
Animation: Tullio Rossi
Voiceover: Verity Kingsmill
Sound production: **Nick Gellie** and Andrew Tokmakoff Bas3ment Studio, Adelaide Australia
Post production: Tullio Rossi
Production: The Environment Institute, The University of Adelaide

URL: https://www.youtube.com/watch?v=uhhb5MW5quI&feature=youtu.be

*This content was developed for promoting Chapter 2 but is embargoed for public release until the publication of the chapter

APPENDIX B

Breed MF, Lowe AJ. **Gellie NJC**, and P. Mortimer 2016. We're kidding ourselves if we think we can "reset" the earth's damaged ecosytems. *The Conversation* May 27, 2016.

https://theconversation.com/were-kidding-ourselves-if-we-think-we-can-reset-earths-damaged-ecosystems 59972

We're kidding ourselves if we think we can 'reset' Earth's damaged ecosystems

May 27, 2016 6.10am AEST

Earth is in a land degradation crisis. If we were to take the roughly one-third of the world's land that has been degraded from its natural state and combine it into a single entity, these "Federated States of Degradia" would have a landmass bigger than Russia and a population of more than 3 billion, largely consisting of the world's poorest and most marginalised people.

The extent and impact of land degradation have prompted many nations to propose ambitious targets for fixing the situation – restoring the wildlife and ecosystems harmed by processes such as desertification, salinisation and erosion, but also the unavoidable loss of habitat due to urbanisation and agricultural expansion.

In 2011, the Global Partnership on Forest and Landscape Restoration, a worldwide network of governments and action groups, proposed the Bonn Challenge, which aimed to restore 150 million hectares of degraded land by 2020.

This target was extended to 350 million ha by 2030 at the September 2014 UN climate summit in New York. And at last year's landmark Paris climate talks, African nations committed to a further 100 million ha of restoration by 2030.

These ambitious goals are essential to focus global effort on such significant challenges. But are they focused on the right outcomes?

For restoration projects, measuring success is crucial. Many projects use measures that are too simplistic, such as the number of trees planted or the number of plant stems per hectare. This may not reflect the actual successful functioning of the ecosystem.

Meanwhile, at the other end of the scale are projects that shoot for outcomes such as “improve ecosystem integrity” – meaningless motherhood statements for which success is too complex to quantify.

One response to this problem has been a widespread recommendation that restoration projects should aim to restore ecosystems back to the state they were in before degradation began. But we suggest that this baseline is a nostalgic aspiration, akin to restoring the “Garden of Eden”.

Beautiful, but not particularly realistic. Wenzel Peter/Wikimedia Commons

An unrealistic approach

Emulating pre-degradation habitats is unrealistic and prohibitively expensive, and does not acknowledge current and future environmental change. While a baseline that prescribes a list of pre-degradation species is a good place to start, it does not take into account the constantly changing nature of ecosystems.

Instead of a “Garden of Eden” baseline, we suggest that restoration projects should concentrate on establishing functional ecosystems that provide useful ecosystem services. This might be done by improving soil stability to counter erosion and desertification, or by planting deep-rooted species to maintain the water table and reduce dry land salinity, or by establishing wild pollinator habitats around pollinator-dependant crops such as apples, almonds and lucerne seed.

Natural ecosystems have always been in flux – albeit more so since humans came to dominate the planet. Species are constantly migrating, evolving and going extinct. Invasive species may be so prevalent and naturalised that they are impossibly costly to remove.

As a result, land allocated for restoration projects is often so altered from its pre-degradation state that it will no longer serve as habitat for the species that once lived there. Many local, native species can be prohibitively difficult to breed and release.

And present-day climate change may necessitate the use of non-local genotypes and even non-local native species to improve restoration outcomes. Newer, forward-thinking approaches may result in the generation of novel gene pools or even novel ecosystems.

Projects should focus on targets that are relevant to their overarching goals. For example, if a restoration project is established to improve pollination services, then the abundance and diversity of insect pollinators could be its metric of success. As we argue in correspondence to the science journal Nature, restoration should focus on helping to create functional, self-sustaining ecosystems that are resilient to climate change and provide measurable benefits to people as well as nature.

An excellent example of a successful, large-scale restoration project with targeted outcomes is Brazil’s ongoing Atlantic Forest Restoration Pact. This has committed to restoring 1 million hectares of Atlantic forest by 2020 and 15 million hectares by 2050.

This project has clear objectives. These include restoring local biodiversity (for conservation and human use, including timber and non-timber forest products); improving water quality for local communities; increasing carbon storage; and even creating seed orchards that can be either sustainably harvested or used to provide more seed for sowing as part of the restoration.

This project has clear social objectives as well as ecological ones. It has created new jobs and income opportunities. Local communities are contributing to seed collection and propagation, while the project gives landowners incentives to abide by laws against deforestation. For forests, this is the kind of pragmatic approach that will bear the most fruit

APPENDIX C

McDonald, T., Jonson, J. & Dixon, K.W., (2016) National standards for the practice of ecological restoration in Australia. *Restoration Ecology*, **24**, S4-S32.

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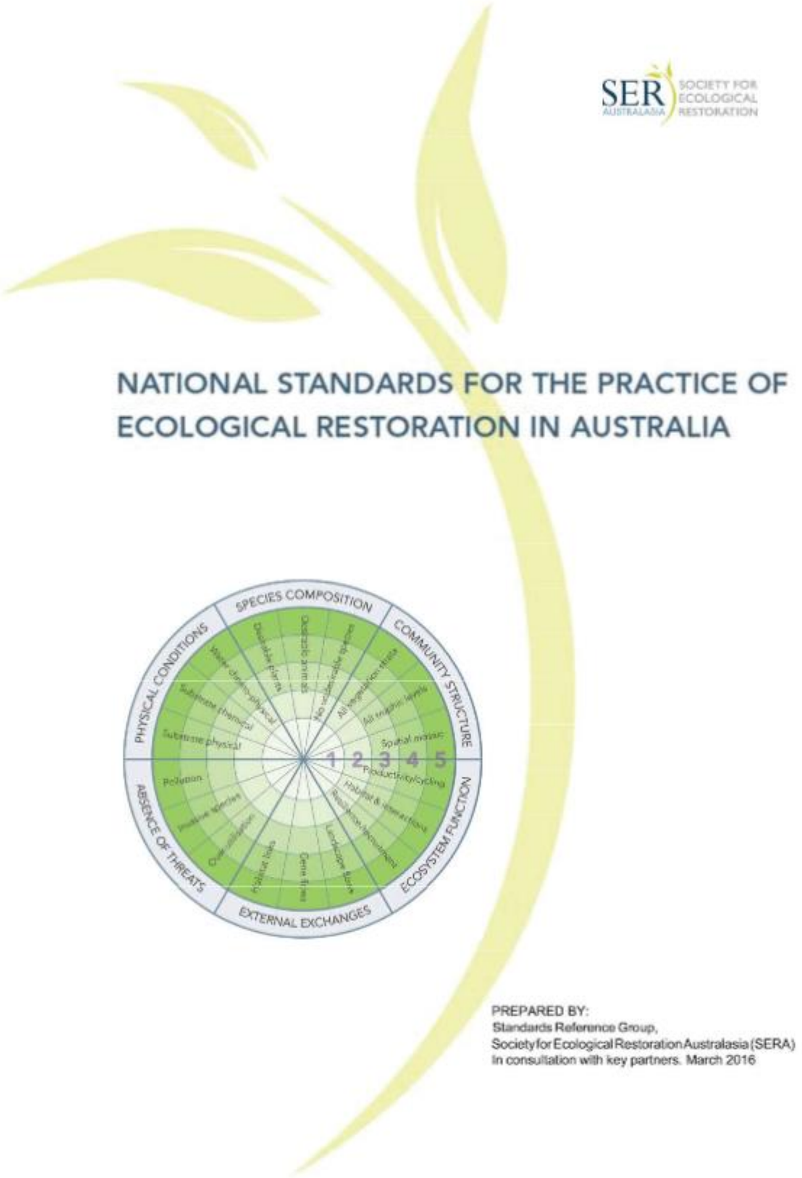
I contributed to (Appendix 3: Genetics, fragmentation, and climate change—implications for restoration and rehabilitation of local indigenous vegetation communities) of this document. The National standards for the practice of ecological restoration was conceived by The Society for Ecological Restoration *Australasia* (SERA) to raise the standard of restoration and rehabilitation practice across all sectors of the industry.



SER AUSTRALASIA SOCIETY FOR ECOLOGICAL RESTORATION

NATIONAL STANDARDS FOR THE PRACTICE OF ECOLOGICAL RESTORATION IN AUSTRALIA

PREPARED BY:
Standards Reference Group,
Society for Ecological Restoration Australasia (SERA)
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POLICY ARTICLE

National standards for the practice of ecological restoration in Australia

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EXECUTIVE SUMMARY

The contemporary call for restoration and rehabilitation comes at a critical point in our planet's history where human influence is all pervasive. Australia's long and relatively uninterrupted evolutionary past means the continent possesses ancient soils and exceptionally diverse and unique biota—yet its terrestrial and marine ecosystems carry a more recent legacy of extensive and continuing environmental degradation, particularly in urban, industrial, and production landscapes and aquatic environments. Anthropogenic climate change is superimposing further pressure on ecosystems, whose vulnerability to climate change is exacerbated by other causal factors including land clearing, overharvesting, fragmentation, inappropriate management, disease, and invasive species. Degradation is so severe in most cases that it will not be overcome without active and ecologically appropriate intervention including mitigation of these causal factors and reinstatement of indigenous biodiversity.

The practice of ecological restoration and rehabilitation seeks to transform humanity's role from one where we are the agents of degradation to one where we act as conservators and healers of indigenous ecosystems. It is in this context that the *National Standards for the Practice of Ecological Restoration in Australia* (the "Standards") has been prepared by the Society for Ecological Restoration Australasia (SERA) in collaboration with its 12 not-for-profit Partner and advisor organizations; all of whom, like SERA, are dedicated to effective conservation management of Australia's indigenous ecological communities.

This document identifies the need and purpose of ecological restoration and explains its relationship with other forms of environmental repair. The Standards identifies the principles underpinning restoration philosophies and methods, and outlines the steps required to plan, implement, monitor, and evaluate a restoration project to increase the likelihood of its success. The Standards are relevant to—and can be interpreted for—a wide spectrum of projects ranging from minimally resourced community projects to large-scale, well-funded industry or government projects.

SERA and its Partners have produced these Standards for adoption by community, industry, regulators/government, and land managers (including private landholders and managers of public lands at all levels of government) to raise the standard of restoration and rehabilitation practice across all sectors. The document provides a blueprint of principles and the standard that will aid voluntary as well as regulatory organizations in their efforts to encourage, measure, and audit ecologically appropriate environmental repair in all land and water ecosystems of Australia.

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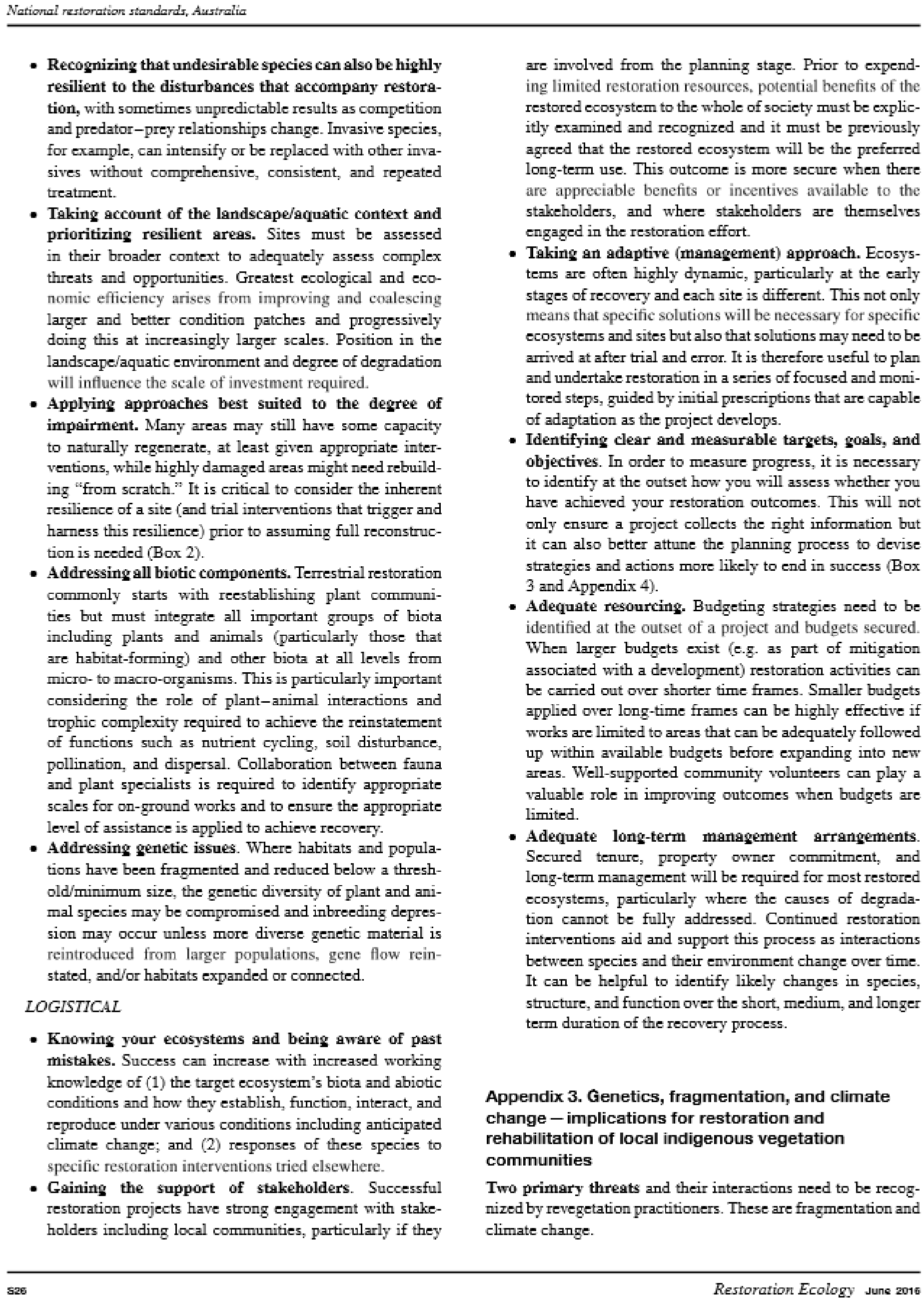

- **Recognizing that undesirable species can also be highly resilient to the disturbances that accompany restoration,** with sometimes unpredictable results as competition and predator–prey relationships change. Invasive species, for example, can intensify or be replaced with other invasives without comprehensive, consistent, and repeated treatment.
- **Taking account of the landscape/aquatic context and prioritizing resilient areas.** Sites must be assessed in their broader context to adequately assess complex threats and opportunities. Greatest ecological and economic efficiency arises from improving and coalescing larger and better condition patches and progressively doing this at increasingly larger scales. Position in the landscape/aquatic environment and degree of degradation will influence the scale of investment required.
- **Applying approaches best suited to the degree of impairment.** Many areas may still have some capacity to naturally regenerate, at least given appropriate interventions, while highly damaged areas might need rebuilding "from scratch." It is critical to consider the inherent resilience of a site (and trial interventions that trigger and harness this resilience) prior to assuming full reconstruction is needed (Box 2).
- **Addressing all biotic components.** Terrestrial restoration commonly starts with reestablishing plant communities but must integrate all important groups of biota including plants and animals (particularly those that are habitat-forming) and other biota at all levels from micro- to macro-organisms. This is particularly important considering the role of plant–animal interactions and trophic complexity required to achieve the reinstatement of functions such as nutrient cycling, soil disturbance, pollination, and dispersal. Collaboration between fauna and plant specialists is required to identify appropriate scales for on-ground works and to ensure the appropriate level of assistance is applied to achieve recovery.
- **Addressing genetic issues**. Where habitats and populations have been fragmented and reduced below a threshold/minimum size, the genetic diversity of plant and animal species may be compromised and inbreeding depression may occur unless more diverse genetic material is reintroduced from larger populations, gene flow reinstated, and/or habitats expanded or connected.

LOGISTICAL

- **Knowing your ecosystems and being aware of past mistakes.** Success can increase with increased working knowledge of (1) the target ecosystem's biota and abiotic conditions and how they establish, function, interact, and reproduce under various conditions including anticipated climate change; and (2) responses of these species to specific restoration interventions tried elsewhere.
- **Gaining the support of stakeholders**. Successful restoration projects have strong engagement with stakeholders including local communities, particularly if they are involved from the planning stage. Prior to expending limited restoration resources, potential benefits of the restored ecosystem to the whole of society must be explicitly examined and recognized and it must be previously agreed that the restored ecosystem will be the preferred long-term use. This outcome is more secure when there are appreciable benefits or incentives available to the stakeholders, and where stakeholders are themselves engaged in the restoration effort.
- **Taking an adaptive (management) approach.** Ecosystems are often highly dynamic, particularly at the early stages of recovery and each site is different. This not only means that specific solutions will be necessary for specific ecosystems and sites but also that solutions may need to be arrived at after trial and error. It is therefore useful to plan and undertake restoration in a series of focused and monitored steps, guided by initial prescriptions that are capable of adaptation as the project develops.
- **Identifying clear and measurable targets, goals, and objectives**. In order to measure progress, it is necessary to identify at the outset how you will assess whether you have achieved your restoration outcomes. This will not only ensure a project collects the right information but it can also better attune the planning process to devise strategies and actions more likely to end in success (Box 3 and Appendix 4).
- **Adequate resourcing.** Budgeting strategies need to be identified at the outset of a project and budgets secured. When larger budgets exist (e.g. as part of mitigation associated with a development) restoration activities can be carried out over shorter time frames. Smaller budgets applied over long-time frames can be highly effective if works are limited to areas that can be adequately followed up within available budgets before expanding into new areas. Well-supported community volunteers can play a valuable role in improving outcomes when budgets are limited.
- **Adequate long-term management arrangements**. Secured tenure, property owner commitment, and long-term management will be required for most restored ecosystems, particularly where the causes of degradation cannot be fully addressed. Continued restoration interventions aid and support this process as interactions between species and their environment change over time. It can be helpful to identify likely changes in species, structure, and function over the short, medium, and longer term duration of the recovery process.

Appendix 3. Genetics, fragmentation, and climate change — implications for restoration and rehabilitation of local indigenous vegetation communities

Two primary threats and their interactions need to be recognized by revegetation practitioners. These are fragmentation and climate change.

Effect of fragmentation on genetic diversity. The concept of confining seed collection to a "local provenance" area (to ensure local adaptation is maintained) has been widely adopted by plant-based restoration practitioners. However, the paradigm of collecting very close to the restoration site is no longer considered useful. Firstly, scientists agree that plant local adaptation is not as common as many believe. Secondly, many practitioners now understand that a "local" genotype may occur over wider areas (i.e. from 10s to 100s of km) depending on the species and its biology. However, in a largely cleared landscape, small fragments are at risk of elevated inbreeding when populations of a species drop below threshold numbers, which can be different for every species. As inbred seed may fail to reinstate functional and adaptable plant populations, in general it is best to collect seed from larger, higher density stands. This means that in fragmented landscapes where vegetation stands are smaller, less dense, and more isolated, collecting seed from wider distances and multiple sources will be necessary to capture sufficient genetic diversity to rebuild functional communities. This seed should be multiplied in regional seed production areas, however, to avoid overharvesting from remnants.

Climate change. Examination of Australian ecosystems shows that many indigenous species have endured ancestral extremes of climate well beyond predicted climate change scenarios. However, *accelerated* climate change is a serious emerging problem. Some species will be impaired by increasing ocean temperatures and acidity, and marine, freshwater, and terrestrial habitats will be lost in some locations due to sea level rise. Many river channels, lakes, and wetlands may also be affected by drying or its consequences such as increased salinity and cold-adapted species will be lost at colder, higher elevations where there is nowhere higher for them to migrate as climate warms. Indeed, even conservative global warming scenarios suggest that a wide range of local environments to which species may have adapted will change dramatically.

Although we cannot precisely predict the type and scale of risks that ecosystems face because only a small proportion of species has been individually studied, we know that some species may be lost from their current locations while others will colonize new areas, altering local species assemblages. We also know that the effect of climate change will be particularly strong when combined with high levels of fragmentation.

Some species may have sufficient inherent "adaptive plasticity" to persist as climates change, as has been demonstrated from translocation experiments and detailed pollen analysis of past environments. That is, an *individual* plant may be able to adjust its form by mechanisms such as reducing its leaf size, increasing leaf thickness, or altering flowering and emergence times. But in many cases, persistence may depend on a species' capacity for genetic selection or adaptation, which in turn depends on population size and the diversity of the genes available.

Species that have large, connected populations, a wide climatic range, naturally high dispersal characteristics and whose populations have many genes in common are likely to have a higher chance of genetically adapting to the new environments or migrating as their climate envelope moves. Conversely, species with low pollen and seed dispersal characteristics, that occur naturally in "islands" or "outliers" or that have been isolated through land clearing or river regulation, for example, may be less able to adapt or migrate in response to climate change (Box 5).

Implications for restoration and rehabilitation

Techniques and protocols are emerging to guide the collection of genetically diverse material to use in revegetation in order to enhance a species' adaptive potential. In extensive, intact indigenous habitats where species and populations are likely to have a greater capacity to adapt unaided because of high connectivity, interventions to enhance adaptive potential are unlikely to be needed. But where landscapes or waterscapes remain largely fragmented, interventions to assist genetic adaptation are expected to be beneficial. This means that, while the local gene pool still has potential to play a major role in adaptation, it is prudent to consider including at least a small amount of germplasm of the same species from a "future climate"—that is, a region with a climate similar to that which is predicted for the area being restored. Research is underway to test some of these new approaches and it is hoped that "rules of thumb" will eventually be developed. Meanwhile, researchers are designing protocols and proformas for appropriately documented and registered "citizen science" trials integrated into low-risk restoration settings. Participation in such trials will enable groups to actively test a range of recommendations on their sites while also optimizing opportunities for improved science and practice.

Tools for assessing climate-readiness in relation to genetics

Some tools are available to help restoration planners undertake what could be called "climate-readiness" analysis at the planning stage. Firstly, restoration practitioners are encouraged to seek out predictions of locations where ecosystems are likely to be affected by climate change. Secondly, practitioners are encouraged to liaise with researchers to gain a better understanding of predicted responses of species to both

Box 5. Climate envelope

The climate range in which a species currently exists can be referred to as its "climate envelope." During climate change this climate envelope is likely to uncouple from the current location in which the species exists and, where conditions become hotter, move further poleward or to higher elevations. This means that the species may be lost from the more equatorial extreme of the range and need more help to adapt as it, or its genotypes, move poleward or to higher elevations.
However, as precipitation is likely to change in less predictable ways, it is likely that the displacement of climate envelopes will be more complex.

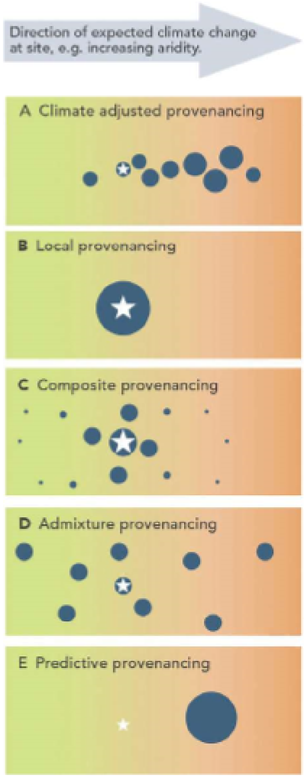

Figure 4. Provenancing strategies for revegetation (Reproduced here from Prober et al. 2015.) The star indicates the site to be revegetated, and the circles represent native populations used as germplasm sources. The size of the circles indicates the relative quantities of germplasm included from each population for use at the revegetation site. In the case of the climate-adjusted provenancing, the relative quantities of the germplasm from the various populations will depend upon factors such as genetic risks, and the rate and reliability of climate change projections. For simplicity, this represents the major direction of climate change in a single dimension (e.g. aridity, to combine influences of increasing temperature and decreasing rainfall), but multiple dimensions could be considered as required.

fragmentation and climate change and to identify the relative risks of a range of options relating to the deliberate movement of genetic material in restoration projects. (Genetic analysis can be undertaken by a range of research institutions and is increasingly affordable for practitioners. This cost reduction is increasing the numbers of species being studied while rapid improvements in the effectiveness and efficiency of genetic testing tools are also occurring.)

Web-based tools are also readily accessible for identifying whether the species currently occurring in the vicinity of your site will still be suited to climates predicted to occur at your site in the future. One of the most important of these is the Atlas of Living Australia website (www.ala.org.au) which can help practitioners identify the natural geographic range of a species and whether it may have the potential to tolerate the conditions predicted to occur under climate change scenarios which themselves are mapped on the website www.climatechangeinaustralia.gov.au. An explanation of how these tools can be combined is found in Booth et al. (2012).

Proposed propagule sourcing strategies to build climate-readiness into restoration through ensuring genetic diversity include: *composite provenancing* Broadhurst et al. 2008), *admixture provenancing* (Breed et al. 2013), *predictive provenancing* (e.g. Crowe & Parker 2008), and *climate adjusted provenancing* (Prober et al. 2015; Fig. 4). Application of any such models should be undertaken within a risk management framework that considers the potential negative effects of inbreeding and outbreeding depression, interpreted in a manner clearly understood by practitioners. It should also include long-term monitoring (i.e. at least a decade) to enable lessons learned to be captured for both restoration and climate science.

Practitioners designing planting lists need to bear in mind, however, that it is impossible to be certain of the changes that will occur. Different species will respond to climate change in different ways and at the moment there is no easy way to predict this. Furthermore, temperature and rainfall are not the only important predictors. A range of physical (e.g. soils) and biological factors (e.g. dispersal)—which themselves may or may not be affected by a changing climate—can also have important roles in influencing the distribution of a species. While some caution will always be required, a balanced approach in fragmented areas would see the restoration plan specify the use of locally occurring species (preferring germplasm from larger populations, even if somewhat more distant) and where advised, formally trialling the inclusion of some germplasm from 'future climate' locations. Such a combined approach—coupled with optimizing connectivity to the extent possible—is likely to improve opportunities for natural adaptation should it be required.

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APPENDIX D

Gellie N. 2016. The maladapted future is here – it's just not evenly distributed*. *Biodiversity Revolution Blog* https://biodiversityrevolution.wordpress.com/2016/11/23/the-maladapted-future-is-here-its-just-not-evenly-distributed/

The maladapted future is here – it's just not evenly distributed*

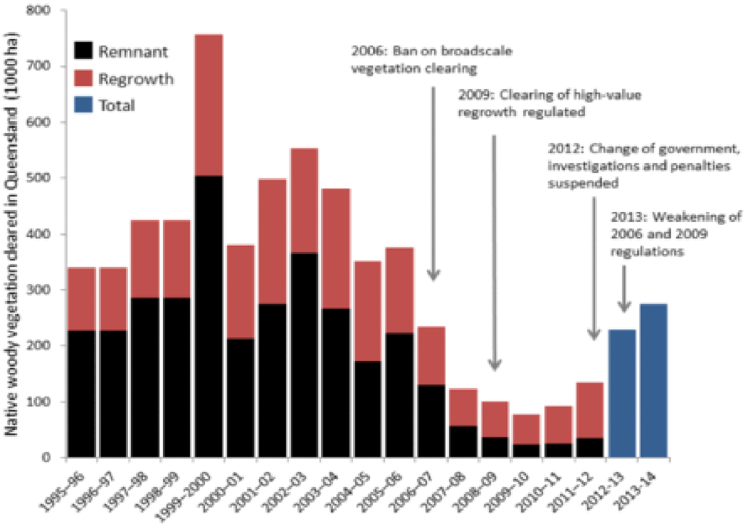
Posted on November 23, 2016 by Nick Gellie

Why do we need to take urgent account of current climate maladaptation in ecological restoration?

* paraphrased quote from cyberpunk essayist, William Gibson

I am sitting in my office writing this blog barely 100 m away from where Professor Tim Flannery would have written sections of his best seller The Future Eaters. It was published while he was Director of the South Australian Museum more than a decade ago. Flannery's book describes the actions of destructive colonial settlers as "maladaptative" to the delicate Australian environment.

The preferred method of land clearing for agriculture in Australia has been to pull a ships anchor chain behind two bull-dozers and by any measure can be considered a maladaptive way to manage the land. Photo credit: Angela Wylie



Habitat clearance in Queensland is again on the increase and is now at similar levels to when The Future eaters was first published. Credit The Conversation. The Future Eaters became an iconoclastic call to arms to preserve Australia's natural heritage, and its message remains as relevant today as it was back when it was published.

Thoughts of this book have recently resurfaced with me following the publication of our article in the journal Biological Conservation, with another past director of the South Australian Museum, Professor Andrew Lowe. Together with our colleagues we found more evidence of maladaptation. But unlike the overt results from the mechanised wave of European clearance, the subject of our maladaptation is virtually blind to the naked eye, exists in common trees species and provides a whole new set of challenges for biodiversity management.

Wikipedia defines Maladaptation (/ˌmælædæpˈteɪʃən/) as a trait that is (or has become) more harmful than helpful, in contrast with an adaptation, which is more helpful than harmful. All organisms, from bacteria to humans, display maladaptive and adaptive traits.

This definition is fine for a general context, but for our study we need to consider maladaptation from an evolutionary perspective. Where maladaptation is the poor growth or survival of some plants relative to others in a set environment. Environment and climate are important predictors of plant growth and survival and determine where a plant can or cannot grow. But environment is not the only driver, genetic issues can also influence maladaptation.

Importance of maladaptation for restoration?

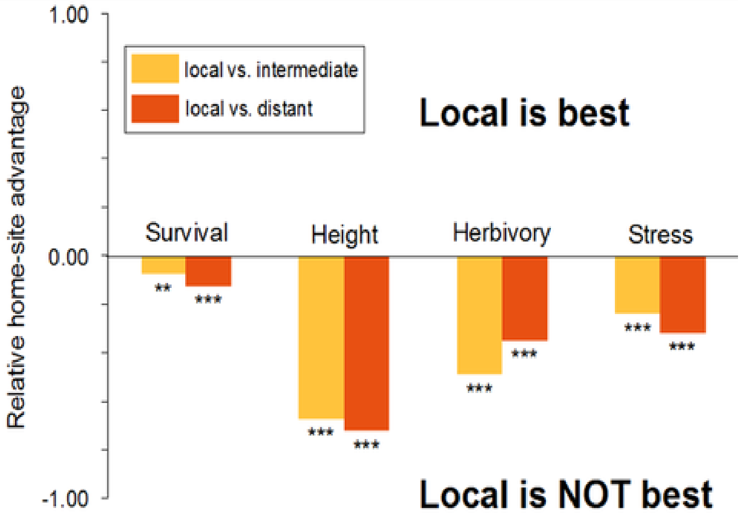
Globally there are now important initiatives to reverse habitat clearing and degradation and to rebuild biodiverse systems. However with the rapid rate of recent climate change, choosing where to source seed from for revegetation has become a complex and unresolved step in the practice of restoration.

The distribution of plants is largely predicted to shift poleward and upward as global temperatures increase due to climate change. These shifts will have particularly dire consequences for some plants (e.g. forest dieback), resulting in the creation of new ecosystems that have no historical equivalent. Restoration practices, rather than accepting the magnitude and velocity of these changes, often take a nostalgic view of what the landscape should look like in the future. Reinstating these past landscapes and disregarding their maladaptation could hamper our ability to cultivate resilient function into the degraded systems where restoration is being practiced.

Our study

In our recent paper we looked at how the origin of seed used for restoration affects the growth and survival of plants in restoration projects.

To test this idea, we selected plants that were local (within 5km), intermediate (20kms) and distant (45kms) to a site that was being restored in southern Australia. The local plants were from the highest rainfall area of the study, with intermediate and distant plants from successively drier habitats. We grew approximately 1500 plants from seed collected from these 3 sources together at a single site, known in scientific terminology as a common garden. If the local plants are well adapted (and not maladapted) to the site, then we would expect them to outperform plants from other more distant sources. However, this expectation was not borne out by our findings (see below). Rather, we found that local plants had the highest death rates, grew slowest and showed signs of increased invertebrate attack and stress, all signs of maladaptation. By contrast the plants from warmer dryer sources survived and grew the best.



Maladaptation as indicated by a lack of home-site advantage for local plants compared to those from other better adapted locations, when survival, height, levels of herbivory and stress are compared. If the graphs dip below the line it indicates that the local provenance is performing less well than others, i.e. local is not best, **P < 0.01, ***P < 0.001

These results have implications for restoration practices and would suggest that reviewing current seed strategies could significantly improve restoration outcomes. For example at the site in our study, up to 10% greater survival, up to 25% larger plants and up to 45% more pathogen resistance could be achieved during establishment by incorporating a more diverse range of seed from warmer and dryer locations. These new seed sourcing strategies which mix provenances from a combination of local and more distant locations (known as composite or predictive provenancing) are now gaining traction with restoration practitioners.

A practical way forward

We identified maladaptation in our landscape because we intensively investigated it. However it may not be so easily observed on casual inspection, as the impacts can be subtle and nuanced. In this system we now have sufficient scientific evidence to take an evidence based approach to restoration practices. Augmenting seed mixes beyond simple local sourcing strategies would provide substantial benefits to this project. Like Flannery's narrative the options available to restoration practitioners to mitigate maladaptation are not without controversy but sticking with the status quo (sourcing local seed only) will have important suboptimal ramifications, as shown in our results.

Our work also emphasises that we need to take an integrated approach to gathering evidence. This can be done by embedding experiments directly into restoration projects. It provides a cost effective way to develop adaptive management options with restoration stakeholders and hopefully lessen the impact of issues like maladaptation.

I sentimentally enjoy my trees in the ground not in museums or art galleries (see below), but this does not require me to commit to a nostalgic view of the landscape. Rather I understand that restored plant communities need to be persistent, and that this persistence may be challenged by the results we found. If, as William Gibson contends, “the future is already here”, and maladaptation is a fragment of this future, then we are obliged to find practical solutions to mitigate its consequences.

Eucalyptus 2013 was conceived by Cai Guo-Qiang as a quintessential element of the Australian landscape, transposed into the Gallery of Modern Art like a vast readymade.31 m spotted gum Photo credit Sydney Morning Herald.

This post, by Nick Gellie, discusses the recent paper ‘Local maladaptation in a foundation tree species: implications for restoration‘ by himself and co-authors Martin Breed, Nikki Thurgate, Shaun Kennedy, and Andrew Lowe published recently in the journal *Biological Conservation*.

APPENDIX E

Breed M.F., **Gellie N.J.C**., & Lowe, A.J. (2016) Height differences in two eucalypt provenances with contrasting levels of aridity. *Restoration Ecology* **24**,471-478.

Abstract: Huge investments are fed into repairing the world's degraded land, placing unparalleled pressure on delivering large quantities of quality seed. One of the most pressing issues is to identify which region to collect seed from and specifically whether local seed has a home-site advantage, particularly given the pressures of climate change. Recent theoretical recommendations have supported supplementing local seed with seed transferred in an arid-to-mesic direction to improve climate resilience of plantings. We tested this recommendation by establishing a reciprocal transplant trial in June 2010 of two seed provenances with contrasting aridity of *Eucalyptus socialis*, a tree widely used for restoration in Southern Australia. We recorded survival and height over 5 years. The years 2010 and 2011 were particularly wet years at both sites (>1.8 times historical rainfall), but the years 2012–2015 were consistent with long-term rainfall trends, with the arid site receiving 12–48% less annual rainfall than the mesic site. Only the arid provenance showed a home-site advantage, and only for height after the two wet years followed by the three drier years. Provenances had similar levels of survival at both sites and did equally well at the mesic site. These results only provide initial evidence to support the recommendation that restoration plantings aiming to incorporate climate resilience should include arid-to-mesic transferred seed. Further work is needed to fully explore potential confounding site-specific effects. Supplementing locally collected seed with arid-to-mesic transferred seed could be important to increase climate resilience of plantings and demands further studies to explore its costs versus benefits.

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RESEARCH ARTICLE

Height differences in two eucalypt provenances with contrasting levels of aridity

Martin F. Breed[1], Nicholas J. C. Gellie[1], Andrew J. Lowe[1,2]

Huge investments are fed into repairing the world's degraded land, placing unparalleled pressure on delivering large quantities of quality seed. One of the most pressing issues is to identify which region to collect seed from and specifically whether local seed has a home-site advantage, particularly given the pressures of climate change. Recent theoretical recommendations have supported supplementing local seed with seed transferred in an arid-to-mesic direction to improve climate resilience of plantings. We tested this recommendation by establishing a reciprocal transplant trial in June 2010 of two seed provenances with contrasting aridity of *Eucalyptus socialis*, a tree widely used for restoration in Southern Australia. We recorded survival and height over 5 years. The years 2010 and 2011 were particularly wet years at both sites (>1.8 times historical rainfall), but the years 2012–2015 were consistent with long-term rainfall trends, with the arid site receiving 12–48% less annual rainfall than the mesic site. Only the arid provenance showed a home-site advantage, and only for height after the two wet years followed by the three drier years. Provenances had similar levels of survival at both sites and did equally well at the mesic site. These results only provide initial evidence to support the recommendation that restoration plantings aiming to incorporate climate resilience should include arid-to-mesic transferred seed. Further work is needed to fully explore potential confounding site-specific effects. Supplementing locally collected seeds with arid-to-mesic transferred seed could be important to increase climate resilience of plantings and demands further studies to explore its costs versus benefits.

Key words: climate change, local adaptation, plant genetic resources, provenance trial, transplant experiment

Implications for Practice

- Using locally sourced seed—local provenancing—is commonplace in restoration and is selected without thoroughly testing alternative provenancing strategies.
- We only found limited support for local provenancing, which suggests that alternative provenancing strategies may be more appropriate.
- We recommend that alternative provenancing strategies should be further explored in restoration practice as they may provide improved restoration outcomes.

Introduction

Ambitious global restoration targets have inspired huge investments in restoration. For example, the aspirational goal of Land Degradation Neutrality was put forward during the Rio+20 UN Conference on Sustainable Development; its goal is to restore circa 2 billion hectares by 2030. In Australia, the focus of our study, the federal government has committed AUD $2.55 billion for emissions reduction, including large-scale restoration plantings (Australian Government 2014). Much of this investment is being directed to restoration of marginal agricultural land, such as the semi-arid rangelands (http://www.environment.gov.au/land/green-army/projects).

The enormous scale of restoration puts huge pressure on supply of quality seed. How to deliver large quantities of seed without significantly impacting native vegetation is a serious challenge and has been discussed elsewhere (Broadhurst et al. 2008, 2015). In this study, we focus on the quality of seed where there are two main issues to consider: the regional scale of where to collect seed (i.e. local vs. nonlocal) and the impacts of fragmentation (i.e. site-specific issues). We will focus on regional-scale issues of where to collect seed, as recent work has already demonstrated the potential impact of fragmentation on seed quality (Eckert et al. 2010; Vranckx et al. 2011; Breed et al. 2015; Lowe et al. 2015).

The most fundamental issue when considering which region to collect seed from is whether the local seed shows local adaptation, that is a home-site advantage. There has been a recent push to advance the experimental evidence of local adaptation for species commonly used in restoration, as this information will help build the empirical foundation of seed collecting for restoration (Breed et al. 2013; Jones 2013; Thomas et al. 2014). Developing experimental evidence of local adaptation

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Author contributions: MFB, AJL designed the experiments; MFB, NJCG analyzed the data; MFB rote the first draft of the manuscript; all authors contributed substantially to revisions.

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is a topic of great concern to land managers today due to the risks and uncertainty caused by climate change and its potential impact on restoration success (Harris et al. 2006; Chazdon 2008; Havens et al. 2015). A number of seed collecting strategies have been proposed to increase the climate resilience of restoration plantings to combat the uncertainty introduced by climate change (Broadhurst et al. 2008; Sgrò et al. 2011; Breed et al. 2013; Prober et al. 2015). These strategies have largely encouraged the augmentation of adaptive potential by mixing populations (e.g. admixture and composite provenancing), or the supplementation of local seed with seed from more arid environments in attempt to match extant populations with future conditions (e.g. predictive and climate-adjusted provenancing). However, little data from manipulative experimental work are available to assess the feasibility and potential risks/benefits of these strategies.

Previous studies on plants have shown that local adaptation is common, though not ubiquitous. Reciprocal adaptation (i.e. two seed sources each show a local advantage at home but a disadvantage at away sites) was observed in 45% of plant studies reviewed by Leimu and Fischer (2008), and local adaptation was found in 71% on studies of plants and animals in the meta-analysis by Hereford (2009). Leimu and Fischer (2008) found evidence that small populations may lack the genetic variation required to adapt to local conditions, as small populations experience elevated levels of genetic drift which constrains the genetic basis of adaptation (Young et al. 1996). Hereford (2009) found that local adaptation was most often detected when environmental differences between parental sites were greater (including climate stress; Parachnowitsch 2013). However, identifying the drivers of local adaptation is hampered by the lack of reporting, particularly of negative or unusual results (Godefroid et al. 2011; Suding 2011). Furthermore, most previous work on plant local adaptation has been conducted on model or agricultural/forestry systems, and it is only now with the global effort to restore degraded land, that a shift in focal species is needed.

Integrating scientific experiments into broadscale restoration projects provide many opportunities to help build its evidence-based foundation. An approach that holds great promise is to embed reciprocal transplant experiments into restoration plantings (Breed et al. 2013). Reciprocal transplant experiments are one of the most effective ways to determine whether plant populations display local adaptation (Kawecki & Ebert 2004), and can be incorporated into large-scale restoration projects with minimal cost (Breed et al. 2013). Furthermore, as adaptation to one habitat may cause lower fitness in other habitats, causing a fitness trade-off or a cost of adaptation, reciprocal transplant experiments provide a direct exploration and quantification of these costs (Kawecki & Ebert 2004; Hereford 2009).

In this study, we established a reciprocal transplant experiment between two populations of *Eucalyptus socialis* ssp. *socialis* (F. Muell. ex Miq.; hereafter *E. socialis*) on two private conservation sanctuaries that differed markedly in aridity in the winter of 2010 to address the following questions: (1) do seed provenances differ in height between these trial sites? and if so, (2) are differences consistent with a home-site advantage? (3) are differences consistent across the 5 years of the trial? and (4) does variation in height through time relate to aridity? As aridity is expected to be a strong agent of selection in eucalypts (Steane et al. 2014; Booth et al. 2015), it should present a challenge to plants at the more arid site. Unless there are large costs to plants performing well under arid conditions, we expect that adaptation will only manifest in the arid site. In this article, we discuss the implications of finding evidence indicative of aridity adaptation to seed collecting for restoration under climate change, plus we explore the opportunities for improving restoration science by integrating experiments into commercial restoration projects.

Methods

Study Species

Eucalyptus socialis is a multistemmed, sclerophyllous tree common throughout the sand and sand-over-limestone soils of the Murray-Darling Basin, Southern Australia (Parsons 1969; Nicolle 1997). *Eucalyptus socialis* generally grows from 2 to 8 m high, it has small white hermaphroditic flowers (diameter of mature flowers with reflexed stamens, <15 mm) and is pollinated primarily by small insects and, to a lesser degree, by birds and small marsupials (Slee et al. 2006; Morrant et al. 2010). Data from closely related eucalypts suggest that *E. socialis* probably has a late-acting self-incompatibility mechanism, resulting in mixed mating to preferential outcrossing (t_m generally >0.70) (Horsley & Johnson 2007), which is supported by published data on *E. socialis* mating system (Breed et al. 2015). Serotinous fruits (i.e. seeds released in response to an environmental trigger) are held over numerous years, with drying triggering seed release. Seeds are small (<2 mm diameter) and gravity dispersed. Based on published data on the ecologically similar *Eucalyptus incrassata* and our own field observations of *E. socialis*, ants generally exhaust soil seed banks, except during particularly heavy seed release such as post fire (Wellington & Noble 1985*a*, 1985*b*).

Populations and Seed Collection

To capture aridity differences that might contribute to height, we chose two populations of *E. socialis* in the Murray-Darling Basin (Fig. 1). The southwest, less arid population was at Yookamurra Sanctuary (34.52°S, 139.47°E; hereafter Yookamurra) and the more arid population was at Scotia Sanctuary (33.22°S, 141.15°E; hereafter Scotia). Scotia is circa 200 km northeast of Yookamurra and they are both characterized by similar sandy soils over limestone. Climate data indicate that Scotia is more arid than Yookamurra (aridity index: Scotia = 0.15, Yookamurra = 0.25; aridity index = mean annual precipitation/potential evapotranspiration; rainfall: Scotia = 238 mm, Yookamurra = 278 mm; Williams et al. 2010; Williams et al. 2012; Table S1). These two locations represent the central range of the climatic envelope of *E. socialis* ssp. *socialis* (http://spatial.ala.org.au/; Fig. S1).

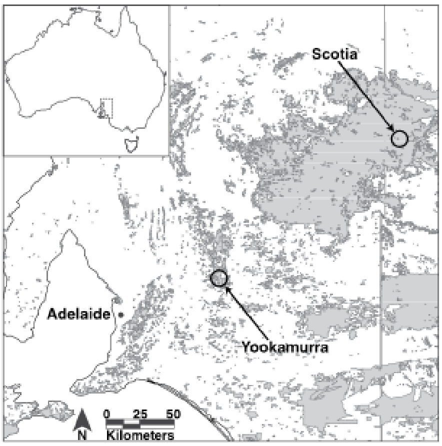

Figure 1. Map showing the locations of the reciprocal transplant experiment, Yookamurra and Scotia. Shading indicates remnant vegetation, and the inset map shows the study location in Australia.

We collected open-pollinated seeds from greater than 100 mature fruits across the canopies of 19 trees at Scotia and 20 trees at Yookamurra in January 2010. The mean height of these mother trees was 6.58 m ± 1.07 SD at Yookamurra and 4.72 m ± 1.60 SD at Scotia. We avoided sampling nearest neighbors (>20 m distance between trees) to minimize the risk of sampling the same mother tree or close relatives. Fruits from each mother tree were processed separately. Fruits were dried for circa 2–3 weeks in individual plastic containers under greenhouse conditions. Dried fruits were then shaken in these plastic containers to encourage seed release. These seeds were then stored for circa 1 week in paper envelopes until sowing in February 2010 (details described below).

Reciprocal Transplant Trial

To assess height differences between Scotia and Yookamurra, we established a reciprocal transplant experiment in 2010 within 10 km of both provenances. Scotia and Yookamurra are part of an extensive belt of mostly intact mallee woodland (i.e. eucalypt dominated, Mediterranean scrub) in semi-arid to arid Southern Australia. This reciprocal transplant experiment was part of restoration projects undertaken on the privately owned and managed properties.

Twenty replicates (i.e. pots) of circa 20 seeds from each mother tree were sown in February 2010 onto saturated native potting mix. Germination was conducted under glasshouse conditions in Adelaide, South Australia (34.92°S, 138.61°E). Seedlings were moved to the full-sun nursery at the Mt Lofty Botanic Gardens, South Australia (34.99°S, 138.72°E) after 4 weeks in glasshouse conditions. Family cohorts of seedlings were grown in crates that were shifted and rotated weekly to avoid confounding effects of location in glasshouse/nursery. The most central seedling within each pot was chosen, and noncentral superfluous seedlings were removed over the subsequent weeks prior to planting to minimize selection on seedling fitness.

Plantings took place at Scotia and Yookamurra in June 2010. We used a randomized complete block design (Addelman 1969), with seedlings from each mother tree present at least once in a random location in each of 10 rows per site. We had approximately 60% seedling mortality in the nursery prior to planting (provenance sample sizes at planting: $n_{\text{Yookamurra}} = 247$; $n_{\text{Scotia}} = 229$; Table S2). Planting sites were prepared by rotary hoeing to remove residual surface vegetation, parallel rip-lines were drawn through at 3 m intervals, and seedlings were spaced at 3 m intervals. No manual watering or fertilization took place at the time of planting or thereafter. A 200 × 200 × 500 mm tree guard (Global Land Repairs, Fyshwick, Australia) surrounded each seedling to protect it against vertebrate herbivores (e.g. rabbits, kangaroos).

Height and Survival Data

In November 2010 (5 months after planting), May 2011 (11 months after planting), and February 2015 (4 years 9 months after planting), we measured the aboveground stem height of all plants (distance from ground to stem apex; hereafter height) and plant survival. No plants had developed a multi-stemmed habitat at any stage of this study. Weed control was not conducted after planting, and greater biomass of weeds was observed at Yookamurra than Scotia, particularly at the final assessment period (i.e. in February 2015) (data not shown).

Observing height, particularly over the time periods of this study, should allow an examination of differences that likely affect growth in later life. However, using height as a fitness proxy has limitations, as it does not include plant investments belowground, which may influence the observed patterns, and it does not directly estimate the plant's contribution to the next generation. However, height remains commonly measured in provenance trials to gain insight into plant performance in a given environment and we expect that it positively correlates with biomass and/or resource acquisitions and therefore also with plant lifespan and/or reproductive success (O'Brien et al. 2007).

To indicate the magnitude and direction of the advantage of local provenances, we calculated the relative home-site advantage for height, by dividing the difference between the home versus away mean height value by the home value.

Data Analysis

We used general linear mixed effects models in the package nlme v. 3.1-120 (Pinheiro et al. 2015) in R v. 3.0.2 (R Project for Statistical Computing, http://www.r-project.org) to assess height between *E. socialis* provenances in the two habitats for each sampling interval (i.e. 2010, 2011, and 2015). When we observed a significant provenance × site effect, we explored the

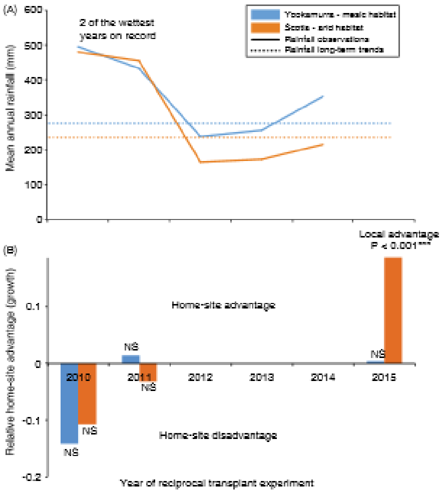

Figure 2. Comparisons between local height and rainfall during the period of the reciprocal transplant trial, with the more mesic Yookamurra in blue and the more arid Scotia in orange. (A) Solid lines show mean annual rainfall for the duration of the reciprocal transplant experiment (2010–2014), and horizontal dotted lines show long-term mean annual rainfall (1997–2009). (B) Relative home-site advantage measured for each sampling period with statistical significance of provenance differences indicated.

effects of provenance within each site further with a more simple model that excluded site effects.

Plant provenance and trial site were treated as fixed effects, and family (i.e. mother tree) was treated as a random effect nested within provenance. A binomial distribution with a logit link function was used for survival data and a Gaussian distribution with no link function was fitted for height data. Planting row was not included in models because model residuals showed no spatial autocorrelation and not including row in the model reduced model parameterisation. Fitted model residuals were visually assessed for normality and were normally distributed in each case. We had too few surviving individuals within families (Table S2) to confidently estimate trait heritability.

Results

Climate Variation

During the first two years of the reciprocal transplant experiment (2010 and 2011), mean annual rainfall was the highest on record at both locations (>430 mm at both Scotia and Yookamurra; >1.8 times historical mean annual rainfall; Fig. 2). The years 2012, 2013, and 2014 were similar to historical rainfall trends, with Yookamurra receiving 12–48% more rainfall than Scotia.

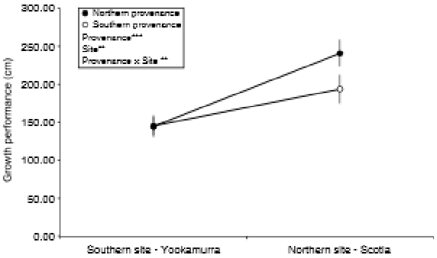

Figure 3. Mean height ± 95% confidence intervals (cm) of both provenances at both sites in the fifth year of the reciprocal transplant experiment.

Table 1. General linear mixed effects models of *Eucalyptus socialis* height explained by the fixed effects in these models across the three sampling periods of the reciprocal transplant trial established in June 2010.

Variable	*Effect Size ± SE (cm)*	*t Value*	*p Value*
2015 height			
Site	95.89 ± 11.87	8.08	<0.001
Provenance	46.92 ± 13.22	3.55	<0.01
Provenance × site	47.18 ± 16.32	2.89	<0.01
2011 height			
Site	23.04 ± 4.84	4.76	<0.001
Provenance	2.35 ± 5.46	0.43	0.67
Provenance × site	1.76 ± 6.62	0.27	0.79
2010 height			
Site	8.99 ± 2.02	4.45	<0.001
Provenance	0.02 ± 2.22	0.01	0.99
Provenance × site	2.29 ± 2.72	0.84	0.40

Variation in Performance

During the higher rainfall years, 2010 and 2011, neither population showed a local advantage for height at Scotia (Scotia in 2010: Scotia = 13.1 cm ± 1.3 SE vs. Yookamurra 14.5 cm ± 1.4 SE; Scotia in 2011: Scotia = 78.4 cm ± 2.7 SE vs. Yookamurra 80.9 cm ± 2.6 SE) or Yookamurra (Yookamurra in 2010: Scotia = 15.3 cm ± 0.9 SE vs. Yookamurra 13.4 cm ± 0.9 SE; Yookamurra in 2011: Scotia = 55.4 cm ± 2.0 SE vs. Yookamurra 56.2 cm ± 1.7 SE). However, we observed a home-site advantage for height of the more arid Scotia sourced plants in 2015, the fifth year of the reciprocal transplant experiment (Scotia = 241 cm ± 9 SE vs. Yookamurra 194 cm ± 10 SE; Figs. 2 & 3; Tables 1, 2, & S3). No local advantage was observed at Yookamurra in this year (Scotia = 144 cm ± 7 SE vs. Yookamurra 145 cm ± 6 SE). Height in the fifth year by Scotia and Yookamurra provenances was circa 51 and circa 41% of the height of local mother trees at Scotia, and both provenances were circa 22% of the height of local mother trees at Yookamurra. No marked differences in provenance survival were observed in any year at either site (Tables 2 & S2).

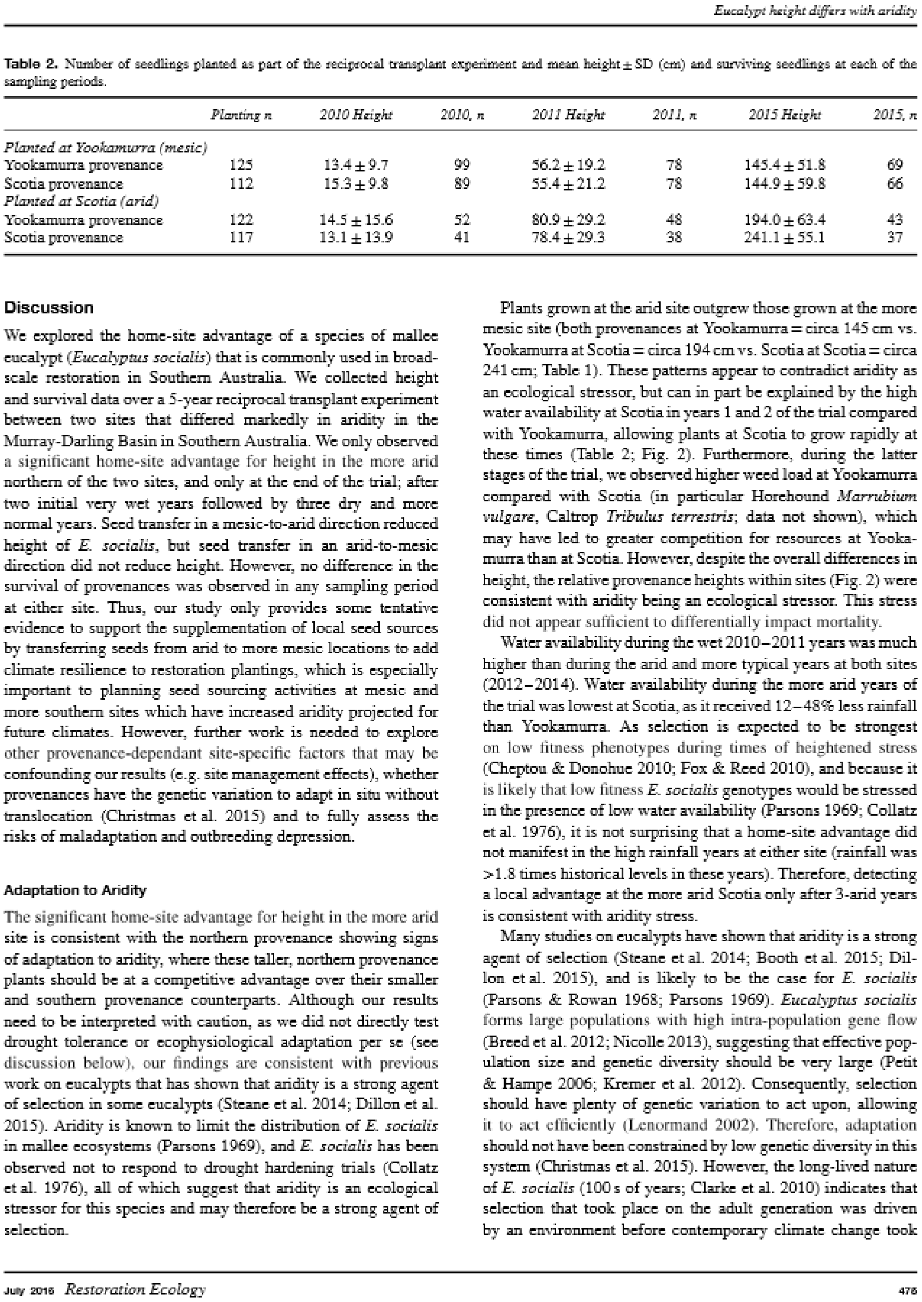

Table 2. Number of seedlings planted as part of the reciprocal transplant experiment and mean height ± SD (cm) and surviving seedlings at each of the sampling periods.

	Planting n	*2010 Height*	*2010, n*	*2011 Height*	*2011, n*	*2015 Height*	*2015, n*
Planted at Yookamurra (mesic)							
Yookamurra provenance	125	13.4 ± 9.7	99	56.2 ± 19.2	78	145.4 ± 51.8	69
Scotia provenance	112	15.3 ± 9.8	89	55.4 ± 21.2	78	144.9 ± 59.8	66
Planted at Scotia (arid)							
Yookamurra provenance	122	14.5 ± 15.6	52	80.9 ± 29.2	48	194.0 ± 63.4	43
Scotia provenance	117	13.1 ± 13.9	41	78.4 ± 29.3	38	241.1 ± 55.1	37

Discussion

We explored the home-site advantage of a species of mallee eucalypt (*Eucalyptus socialis*) that is commonly used in broad-scale restoration in Southern Australia. We collected height and survival data over a 5-year reciprocal transplant experiment between two sites that differed markedly in aridity in the Murray-Darling Basin in Southern Australia. We only observed a significant home-site advantage for height in the more arid northern of the two sites, and only at the end of the trial; after two initial very wet years followed by three dry and more normal years. Seed transfer in a mesic-to-arid direction reduced height of *E. socialis*, but seed transfer in an arid-to-mesic direction did not reduce height. However, no difference in the survival of provenances was observed in any sampling period at either site. Thus, our study only provides some tentative evidence to support the supplementation of local seed sources by transferring seeds from arid to more mesic locations to add climate resilience to restoration plantings, which is especially important to planning seed sourcing activities at mesic and more southern sites which have increased aridity projected for future climates. However, further work is needed to explore other provenance-dependant site-specific factors that may be confounding our results (e.g. site management effects), whether provenances have the genetic variation to adapt in situ without translocation (Christmas et al. 2015) and to fully assess the risks of maladaptation and outbreeding depression.

Adaptation to Aridity

The significant home-site advantage for height in the more arid site is consistent with the northern provenance showing signs of adaptation to aridity, where these taller, northern provenance plants should be at a competitive advantage over their smaller and southern provenance counterparts. Although our results need to be interpreted with caution, as we did not directly test drought tolerance or ecophysiological adaptation per se (see discussion below), our findings are consistent with previous work on eucalypts that has shown that aridity is a strong agent of selection in some eucalypts (Steane et al. 2014; Dillon et al. 2015). Aridity is known to limit the distribution of *E. socialis* in mallee ecosystems (Parsons 1969), and *E. socialis* has been observed not to respond to drought hardening trials (Collatz et al. 1976), all of which suggest that aridity is an ecological stressor for this species and may therefore be a strong agent of selection.

Plants grown at the arid site outgrew those grown at the more mesic site (both provenances at Yookamurra = circa 145 cm vs. Yookamurra at Scotia = circa 194 cm vs. Scotia at Scotia = circa 241 cm; Table 1). These patterns appear to contradict aridity as an ecological stressor, but can in part be explained by the high water availability at Scotia in years 1 and 2 of the trial compared with Yookamurra, allowing plants at Scotia to grow rapidly at these times (Table 2; Fig. 2). Furthermore, during the latter stages of the trial, we observed higher weed load at Yookamurra compared with Scotia (in particular Horehound *Marrubium vulgare*, Caltrop *Tribulus terrestris*; data not shown), which may have led to greater competition for resources at Yookamurra than at Scotia. However, despite the overall differences in height, the relative provenance heights within sites (Fig. 2) were consistent with aridity being an ecological stressor. This stress did not appear sufficient to differentially impact mortality.

Water availability during the wet 2010–2011 years was much higher than during the arid and more typical years at both sites (2012–2014). Water availability during the more arid years of the trial was lowest at Scotia, as it received 12–48% less rainfall than Yookamurra. As selection is expected to be strongest on low fitness phenotypes during times of heightened stress (Cheptou & Donohue 2010; Fox & Reed 2010), and because it is likely that low fitness *E. socialis* genotypes would be stressed in the presence of low water availability (Parsons 1969; Collatz et al. 1976), it is not surprising that a home-site advantage did not manifest in the high rainfall years at either site (rainfall was >1.8 times historical levels in these years). Therefore, detecting a local advantage at the more arid Scotia only after 3-arid years is consistent with aridity stress.

Many studies on eucalypts have shown that aridity is a strong agent of selection (Steane et al. 2014; Booth et al. 2015; Dillon et al. 2015), and is likely to be the case for *E. socialis* (Parsons & Rowan 1968; Parsons 1969). *Eucalyptus socialis* forms large populations with high intra-population gene flow (Breed et al. 2012; Nicolle 2013), suggesting that effective population size and genetic diversity should be very large (Petit & Hampe 2006; Kremer et al. 2012). Consequently, selection should have plenty of genetic variation to act upon, allowing it to act efficiently (Lenormand 2002). Therefore, adaptation should not have been constrained by low genetic diversity in this system (Christmas et al. 2015). However, the long-lived nature of *E. socialis* (100 s of years; Clarke et al. 2010) indicates that selection that took place on the adult generation was driven by an environment before contemporary climate change took

effect, possibly resulting in an adaptation lag to the rapid climate change occurring today (Kremer et al. 2012). To further explore the extent of climate adaptation lag in *E. socialis*, it is imperative to extend monitoring of this trial to extend into the future, with a particular focus on differential recruitment between provenances. As aridity increases with climate change, an adaptation lag should manifest by the arid provenance out-doing the mesic provenance at the mesic site in years to come.

Challenges with Tree Provenance Trials

Deciding on the performance traits to measure in provenance trials of long-lived trees that reflect fitness is a nontrivial exercise, and traditional fitness components (reproductive success and lifespan) are impractical to measure (Petit & Hampe 2006). Furthermore, most tree species used in restoration are nonmodel species, and do not usually have large trait or genomic resources available. Consequently, practical performance traits are needed.

Aboveground plant height was the performance trait used in this study, and is a commonly measured trait in provenance trials related to restoration (O'Brien et al. 2007). When this trait is used as a fitness proxy, it is assumed that it positively correlates with biomass and/or resource acquisitions, and therefore also with plant lifespan and/or reproductive success. However, it does have limitations because plant height itself is unlikely to be under direct selection, as height reflects a complex interaction of many genes and traits. However, its complex nature also makes it a useful trait to observe, as it increases the probability of detecting differences due to selection, since the summed action of selection on many genes and traits will manifest as height, whereas selection on an individual gene or trait would risk going undetected if their role in aridity adaptation was small or absent (Rockman 2012; Anderson et al. 2014). Indeed in our case, follow-up studies should explore phenotypic and genotypic target(s) of selection in more detail. For example, it would be useful to explore functional ecological traits (e.g. δ^{13}C signatures, specific leaf area, wood density, and so on), reproductive traits (e.g. phenology, fruit/seed production), and genetic signatures of selection to more fully understand the aridity adaptation.

Our results must also be interpreted within their limited geographic range of material and sites used in this study. Additional work will be needed to confirm the generalized nature of aridity adaptation in *E. socialis* by assessing more provenances at more sites. Other time- or provenance-dependant factors than aridity may have contributed to the local advantage we observed at Scotia. We can rule out mating system differences between provenances as previous work on these provenances has shown that mating systems do not differ between the two provenances (Breed et al. 2015). However, time- or aridity-dependant provenance-environment interactions may also be contributing to the patterns observed. For example, the planting sites may have abiotic or biotic differences that impact the performance of provenances at later life stages or only under arid conditions (e.g. soil microbial activity). In addition, as discussed above, future work is needed to tease apart the climatic differences between sites from other site-specific factors, such as site management, edaphic, or competition differences.

Management Implications

Eucalyptus socialis is heavily relied upon for restoration in the mallee, where its utility of drought resistance in shallow soils over limestone is beneficial (Nicolle 2013), but climate models suggest that this region is likely to undergo significant increases in aridity (CSIRO & BOM 2014). Restoration projects that use *E. socialis* could actively transfer seeds from more arid provenances to their location of interest to supplement local seed collection to potentially increase climate resilience of plantings. This approach is not without risks, as transferring seeds across ecological gradients may increase the risk of maladaptation (Hereford 2009) and outbreeding depression (Frankham et al. 2011; Weeks et al. 2011). Indeed, these risks could potentially be overcome by increasing planting density of local seed. However, the concept of seed transfer across aridity gradients such as this has repeatedly put forward from theoretical points of view (Broadhurst et al. 2008; Sgrò et al. 2011; Weeks et al. 2011; Breed et al. 2013; Prober et al. 2015), but few studies have helped assess these management recommendations with empirical data. Further studies are needed to explore the consistency of our findings by exploring additional fitness traits over longer periods of time (e.g. biomass estimates, and recruitment), plus it would be fruitful to explore the generality of this trend in this and other regions.

The trials studied here were established on land owned and managed by Australian Wildlife Conservancy, which is a private organization that was interested in re-establishing vegetation on areas cleared prior to land purchase. Implementing the reciprocal transplant trial used in this study on their property imposed minimal costs to the organization, as it was consistent with their land management plans, and the modest costs of setting up the trial and monitoring was met by the researchers. We believe that this model of collaboration meets the needs of land managers/owners, the restoration community, and researchers. Given the projected scale of worldwide landscape restoration, there is an unparalleled opportunity to embed field experiments within restoration projects, to further our understanding of restoration processes and ecosystem science.

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Supporting Information

The following information may be found in the online version of this article:

Table S1. Annual mean aridity index and precipitation data at Yookamurra and Scotia.
Table S2. Survival for each family for each sampling period.
Table S3. Relative home-site advantage for height for each sampling period of the reciprocal transplant trial.
Figure S1. Climate variation between the two provenances compared to climate envelope of study species.

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APPENDIX F

Baruch, Z., Christmas, M., Breed, M.F., Guerin, G.R., Caddy-Retalic, S., McDonald, J.T., Jardine, D.I., Leitch, E.J., **Gellie, N.J.C.**, Hill, K., McCallum, K., & Lowe, A.J. (2016) Leaf trait associations with environmental variation in the wide-ranging shrub Dodonaea viscosa subsp. angustissima (Sapindaceae). *Austral Ecology*. doi:10.1111/aec.12474.

Abstract: Intra-species variation in specific leaf area (SLA) and leaf area (LA) provides mechanistic insight into the persistence and function of plants, including their likely success under climate change and their suitability for revegetation. We measured SLA and LA in 101 Australian populations of the perennial shrub Dodonaea viscosa (L.) Jacq. subsp. angustissima (narrow-leaf hop-bush) (Sapindaceae). Populations were located across about a 1000 km north–south gradient, with climate grading from arid desert to mesic Mediterranean. We also measured leaves from 11 populations across an elevational gradient (300–800 m asl), where aridity and temperature decrease with elevation. We used regression and principal component analyses to relate leaf traits to the abiotic environment. SLA displayed clinal variation, increasing from north to south and correlated with latitude and the first principal component of joint environmental variables. Both SLA and LA correlated positively with most climatic and edaphic variables. Across latitude, LA showed more variability than SLA. Changes in leaf density and thickness may have caused the relative stability of SLA. Only LA decreased with elevation. The absence of a SLA response to elevation could be a consequence of abiotic conditions that favour low SLA at both ends of the elevational gradient. We demonstrated that the widely distributed narrow-leaf hop-bush shows considerable variability in LA and SLA, which allows it to persist in a broad environmental envelope. As this shrub is widely used for revegetation in Australia, South America and the Asia-Pacific region, our results are consistent with the notion that seed used to revegetate mesic environments could be sourced from more arid areas to increase seed suitability to future climate change.

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Leaf trait associations with environmental variation in the wide-ranging shrub *Dodonaea viscosa* subsp. *angustissima* (Sapindaceae)

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Abstract Intra-species variation in specific leaf area (SLA) and leaf area (LA) provides mechanistic insight into the persistence and function of plants, including their likely success under climate change and their suitability for revegetation. We measured SLA and LA in 101 Australian populations of the perennial shrub *Dodonaea viscosa* (L.) Jacq. subsp. *angustissima* (narrow-leaf hop-bush) (Sapindaceae). Populations were located across about a 1000 km north–south gradient, with climate grading from arid desert to mesic Mediterranean. We also measured leaves from 11 populations across an elevational gradient (300–800 m asl), where aridity and temperature decrease with elevation. We used regression and principal component analyses to relate leaf traits to the abiotic environment. SLA displayed clinal variation, increasing from north to south and correlated with latitude and the first principal component of joint environmental variables. Both SLA and LA correlated positively with most climatic and edaphic variables. Across latitude, LA showed more variability than SLA. Changes in leaf density and thickness may have caused the relative stability of SLA. Only LA decreased with elevation. The absence of a SLA response to elevation could be a consequence of abiotic conditions that favour low SLA at both ends of the elevational gradient. We demonstrated that the widely distributed narrow-leaf hop-bush shows considerable variability in LA and SLA, which allows it to persist in a broad environmental envelope. As this shrub is widely used for revegetation in Australia, South America and the Asia-Pacific region, our results are consistent with the notion that seed used to revegetate mesic environments could be sourced from more arid areas to increase seed suitability to future climate change.

Key words: *Dodonaea viscosa* subsp. *angustissima*, elevational gradient, intra-specific trait variation, latitudinal gradient, leaf area, specific leaf area.

INTRODUCTION

Variability in functional traits within and among populations allows plants to persist across a broad range of environmental conditions. By virtue of expanding niche breadth, intra-specific functional trait variation, caused by local adaptation and plasticity, is assumed to result in wider species distributions (Ramirez-Valiente *et al.* 2010; Bolnick *et al.* 2011). Consequently, intra-specific functional trait variation influences the assembly, dynamics and function of local communities (Cornwell & Ackerly 2009; Violle *et al.* 2012; Kunstler *et al.* 2015; Siefert *et al.* 2015; Escudero & Valladares 2016; Funk *et al.* 2017). The assessment of functional traits across environmental clines provides insight into the mechanisms and drivers of trait variation, as well as into the persistence and function of widespread species (Hulshof *et al.* 2013; Carlson *et al.* 2016). Inference can be made of the likely success of those species under future climate change and their suitability for revegetation projects (Laughlin 2014). However, most studies explore trait variation in few populations, along short geographic and environmental distances, and often with many co-varying environmental parameters (e.g. spatial, edaphic and climatic variation). Limited sampling and co-varying environmental parameters make it difficult to determine the specific abiotic driver(s) of trait variation and limit the utility of those studies.

Specific leaf area (SLA, equivalent to leaf area per unit mass) and leaf area (LA) are traits known to vary in response to plant habitat as these traits influence heat and gas exchange with the atmosphere (Westoby *et al.* 2002; Díaz *et al.* 2016). Functionally, SLA indicates how much leaf surface is produced by one unit of

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leaf biomass, which closely correlates with photosynthetic and growth rates, resource use efficiency, leaf life span and cost of construction (Wright *et al.* 2004; Poorter *et al.* 2009; Kunstler *et al.* 2015). The impact of intra-specific variation in SLA expands to ecosystem function by influencing productivity, litter breakdown and nutrient recycling (de Bello *et al.* 2010). The multiple roles of SLA, its ease of measurement and the availability of a large worldwide database (Kattge *et al.* 2011) make it a preferred trait for research in functional ecology. LA influences leaf temperature regulation and transpiration rate through its effect on the boundary layer thickness, and consequently also impacts on leaf heat and water balance (Díaz *et al.* 2016). For numerous species with contrasting life forms and from different biomes, SLA and LA correlate well with abiotic stresses such as drought, nutrient availability and insolation (Poorter *et al.* 2009).

Australia's wide variation in aridity and substrates, coupled with the presence of common species whose natural distribution spans these gradients, makes the continent an excellent natural laboratory in which to test the processes influencing intra-specific plant trait variation. The perennial shrub *Dodonaea viscosa* (L.) Jacq. subsp. *angustissima*. (DC.) JG West (West 1984) (Sapindaceae) (hereafter Dodonaea) is appropriate for our study because it is distributed from the hot, arid centre of the continent to the temperate Mediterranean zone on the southern coast, and also at all elevations of the Flinders and Mt Lofty Ranges in South Australia. This wide distribution suggests Dodonaea should have substantial variability in SLA and LA, which is supported by accounts of phenotypic clines in leaf width (Guerin *et al.* 2012), stomatal density (Hill *et al.* 2014) and allele frequencies of genes associated with water use efficiency (Christmas 2015; Christmas *et al.* 2016) in this species. It is possible that Dodonaea is now being pushed to adapt to climate change at a rate that may exceed its adaptive potential. Despite this threat, its extensive range, apparent plasticity and ability to pioneer degraded sites suggest that it may have more capacity to adapt to changing environmental conditions than other native shrubs (Booth *et al.* 1996). Dodonaea is a ruderal species able to grow on disturbed or eroded soils, and is often employed for restoration and soil stabilization in Australia (Monie *et al.* 2013; Pickup *et al.* 2013) and overseas (Groenendijk *et al.* 2005; Bonfil & Trejo 2010; Ammondt *et al.* 2013; Yelenik *et al.* 2015). As such, intra-specific trait variation could be a useful basis on which to decide on seed sources for restoration by increasing the likelihood of planting success and resilience under climate change (Laughlin 2014). Here, we intensively sample Dodonaea across a broad environmental and spatial range to identify the likely drivers of two important leaf traits. We couple this analysis with leaf trait analysis of populations distributed over altitudinal gradients, representing a short linear distance but with steep environmental variation, effectively decoupling geographic distance from environmental distance. We aim to answer the following questions: (i) what is the range of Dodonaea's SLA and LA responses to its environment? (ii) which abiotic variables associate with these leaf traits? and (iii) do latitude and elevation have similar effects on SLA and LA?

METHODS

Study species and populations

Dodonaea (narrow-leaf hop-bush) is a 1–4 m tall woody shrub, with upright, narrow, tough and sticky leaves covered by reflective wax. It is widely distributed throughout the southern half of Australia, predominantly on well-drained soils. Locally, it forms sparse-to-dense cover in shrublands and in open woodlands as a recognizable shrub layer (Hyde & Playfair 1997; Foulkes & Gillen 2000; Lang *et al.* 2003; Brandle 2010).

We analysed SLA and LA from two data sets. The first comprised samples from 101 populations across about a 1000-km non-linear latitudinal sequence (23.6–35.9°S), mainly focused in the Northern Territory and South Australia (Fig. 1). Climatic and edaphic variables for each population site include mean annual precipitation, the aridity index ranging from 0 (most arid) to 1 (least arid), air temperature, solar radiation, soil pre-European nitrogen concentration and phosphorous content, clay percentage and bulk density. Environmental data were sourced from the Atlas of Living Australia at 0.01° (~1 km) resolution (http://www.ala.org.au; accessed 15 February 2016) (Williams *et al.* 2012). From north to south, there are gradual decreases in solar radiation and temperature with parallel increases in rainfall and soil fertility. The annual mean aridity index (annual rainfall / potential or pan evaporation) integrates water stress condition and decreases southwards. Latitude and discrete abiotic variables such as aridity, rainfall, mean temperature and soil N and P quantities relate linearly from 23°S to about 30°S, but further southwards this relationship becomes exponential (data not shown).

The second data set comprised 11 populations sampled at 50 m elevation intervals between 300 to 800 m asl, in the Heysen Range (31.31° S; 138.57° E; Fig. 1) (Guerin *et al.* 2012). We obtained the low elevation climate records from the Atlas of Living Australia (http://www.ala.org.au; accessed 15 February 2016) (Williams *et al.* 2012) (mean air temperature = 16.0°C; mean annual rainfall = 300 mm). Climatic data were unavailable for the highest elevation, but considering the average elevation lapse rate, we estimated mean temperature and mean annual rainfall to be 12.0°C and 500 mm, respectively, with both temperature and aridity decreasing at higher elevations.

Trait measurements

We measured LA and SLA on either recently collected (fresh) or dried, preserved leaves. Samples came from

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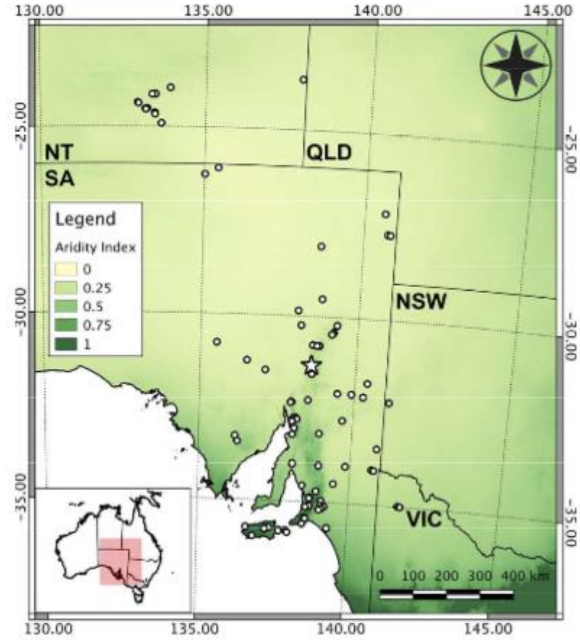

Fig. 1. Location of the sampled populations of *Dodonaea viscosa* subsp. *angustissima*. Shades represent the aridity index (range: 0–1; more to less arid). The star symbol indicates the elevation gradient site. Insolation, temperature range and aridity increase northwards, whereas rainfall and soil N and P amounts increase southwards.

one to five individuals per population and we analysed five undamaged leaves per individual. We followed standard procedures for field sampling and preservation of fresh leaves (Pérez-Harguindeguy *et al.* 2013). We scanned the leaves and measured their area with ImageJ (Rasband 2011), before oven drying at 65°C for 48 h, then weighing and calculating SLA. To account for the area contraction of preserved leaves, which made direct comparison with fresh samples impossible, we calculated a shrinkage factor by measuring fresh and dry LAs from two populations: one from a mesic site (Mt. Bryan 33.33°S; 139.05°E) and the other from an arid site (Andamooka 30.47°S; 137.15° E). Leaves from the northern and more arid site showed less shrinkage (15.6 ± 2.4%; $n = 20$) than those from the wetter southern site (23.4 ± 4.3%; $n = 25$) ($F_{(1,42)} = 50.2$; $P < 0.001$). The mean shrinkage (20.1 ± 5.3%) is consistent with published values (Torrez *et al.* 2013; Queenborough & Porras 2014). Consequently, all LAs were converted into a fresh basis by: Fresh LA = Dry LA / 0.201 before SLA was calculated and used in further analysis. By including preserved leaves, we expanded the data available for this study several fold.

Data analysis

For both data sets, we correlated SLA and LA and each trait with the abiotic variables listed above. In addition, and to obtain an integrated response of leaf traits to multiple physical variables, we employed principal component analysis (PCA) to ordinate the population sites within the environmental space using PC-Ord V6 (McCune & Mefford 2011). We then regressed the coordinates generated by PCA along its main variance axis to latitude, SLA and LA using the lm function in R v. 3.3.1 (R Core Team 2016). Due to a southern bias in our sampling along the latitudinal gradient, we carried out bootstrap resampling with 10 000 iterations using the boot function in the R package v. 1.3-18 (Canty & Ripley 2016) to obtain the confidence intervals for regression coefficients.

RESULTS

SLA significantly increased southwards but LA did not show a significant correlation with latitude

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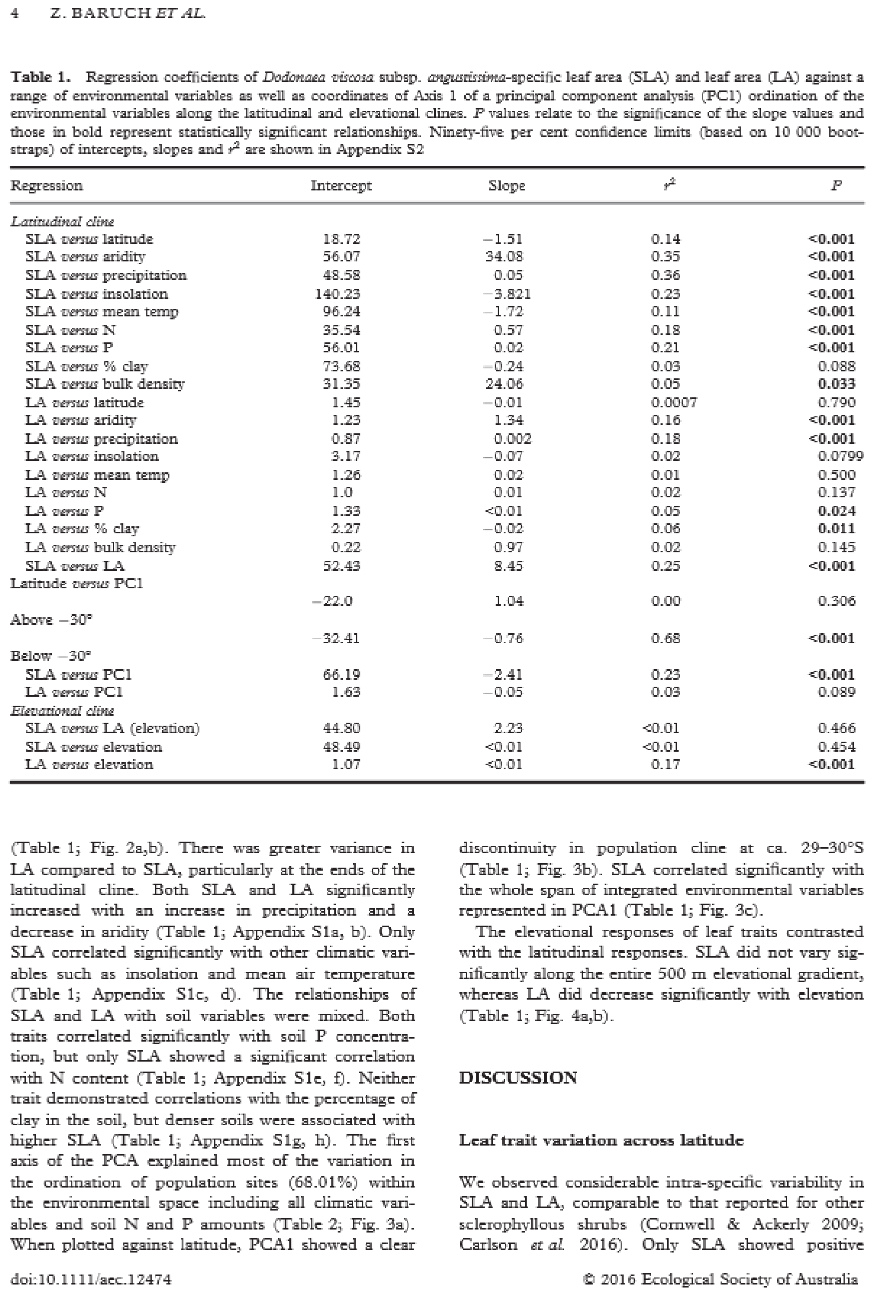

Table 1. Regression coefficients of *Dodonaea viscosa* subsp. *angustissima*-specific leaf area (SLA) and leaf area (LA) against a range of environmental variables as well as coordinates of Axis 1 of a principal component analysis (PC1) ordination of the environmental variables along the latitudinal and elevational clines. *P* values relate to the significance of the slope values and those in bold represent statistically significant relationships. Ninety-five per cent confidence limits (based on 10 000 bootstraps) of intercepts, slopes and r^2 are shown in Appendix S2

Regression	Intercept	Slope	r^2	*P*
Latitudinal cline				
SLA *versus* latitude	18.72	−1.51	0.14	**<0.001**
SLA *versus* aridity	56.07	34.08	0.35	**<0.001**
SLA *versus* precipitation	48.58	0.05	0.36	**<0.001**
SLA *versus* insolation	140.23	−3.821	0.23	**<0.001**
SLA *versus* mean temp	96.24	−1.72	0.11	**<0.001**
SLA *versus* N	35.54	0.57	0.18	**<0.001**
SLA *versus* P	56.01	0.02	0.21	**<0.001**
SLA *versus* % clay	73.68	−0.24	0.03	0.088
SLA *versus* bulk density	31.35	24.06	0.05	**0.033**
LA *versus* latitude	1.45	−0.01	0.0007	0.790
LA *versus* aridity	1.23	1.34	0.16	**<0.001**
LA *versus* precipitation	0.87	0.002	0.18	**<0.001**
LA *versus* insolation	3.17	−0.07	0.02	0.0799
LA *versus* mean temp	1.26	0.02	0.01	0.500
LA *versus* N	1.0	0.01	0.02	0.137
LA *versus* P	1.33	<0.01	0.05	**0.024**
LA *versus* % clay	2.27	−0.02	0.06	**0.011**
LA *versus* bulk density	0.22	0.97	0.02	0.145
SLA *versus* LA	52.43	8.45	0.25	**<0.001**
Latitude *versus* PC1				
	−22.0	1.04	0.00	0.306
Above −30°				
	−32.41	−0.76	0.68	**<0.001**
Below −30°				
SLA *versus* PC1	66.19	−2.41	0.23	**<0.001**
LA *versus* PC1	1.63	−0.05	0.03	0.089
Elevational cline				
SLA *versus* LA (elevation)	44.80	2.23	<0.01	0.466
SLA *versus* elevation	48.49	<0.01	<0.01	0.454
LA *versus* elevation	1.07	<0.01	0.17	**<0.001**

(Table 1; Fig. 2a,b). There was greater variance in LA compared to SLA, particularly at the ends of the latitudinal cline. Both SLA and LA significantly increased with an increase in precipitation and a decrease in aridity (Table 1; Appendix S1a, b). Only SLA correlated significantly with other climatic variables such as insolation and mean air temperature (Table 1; Appendix S1c, d). The relationships of SLA and LA with soil variables were mixed. Both traits correlated significantly with soil P concentration, but only SLA showed a significant correlation with N content (Table 1; Appendix S1e, f). Neither trait demonstrated correlations with the percentage of clay in the soil, but denser soils were associated with higher SLA (Table 1; Appendix S1g, h). The first axis of the PCA explained most of the variation in the ordination of population sites (68.01%) within the environmental space including all climatic variables and soil N and P amounts (Table 2; Fig. 3a). When plotted against latitude, PCA1 showed a clear discontinuity in population cline at ca. 29–30°S (Table 1; Fig. 3b). SLA correlated significantly with the whole span of integrated environmental variables represented in PCA1 (Table 1; Fig. 3c).

The elevational responses of leaf traits contrasted with the latitudinal responses. SLA did not vary significantly along the entire 500 m elevational gradient, whereas LA did decrease significantly with elevation (Table 1; Fig. 4a,b).

DISCUSSION

Leaf trait variation across latitude

We observed considerable intra-specific variability in SLA and LA, comparable to that reported for other sclerophyllous shrubs (Cornwell & Ackerly 2009; Carlson *et al.* 2016). Only SLA showed positive

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clinal variation along the sampled latitudinal gradient, increasing from north to south. The responses of SLA and LA to the abiotic variables across the gradient differed, indicating that the mathematical relationship between SLA and LA did not translate into similar responses. We suggest that these different responses were due to their separate and specific roles in leaf function. Both traits decreased with increasing aridity, but the response of SLA was more pronounced. Furthermore, only SLA responded to air temperature and soil N and P amounts, which are predictable and common responses in sclerophyllous shrubs (Fonseca *et al.* 2000; Ackerly *et al.* 2002; Poorter *et al.* 2009). Soil fertility (as measured by N and P soil amount) was positively associated with SLA, but not with LA. These findings correspond to the tendency that perennial sclerophyllous shrubs growing on oligotrophic soils, such as the northern Dodonaea populations with their low SLA, exhibit conservative strategies that minimize nutrient loss by producing more robust, long-lived leaves (Fonseca *et al.* 2000; Wright *et al.* 2004; Ordoñez *et al.* 2009; Poorter *et al.* 2009).

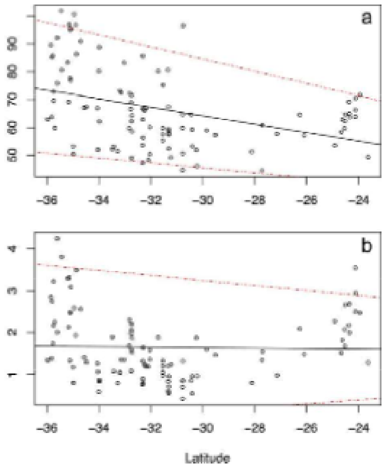

Fig. 2. Leaf trait responses of *Dodonaea viscosa* subsp. *angustissima* to the latitudinal gradient. Linear regressions between (a) SLA and (b) LA and latitude. Coefficients and statistical significance are shown in Table 1. Symbols represent the average for each population. In all panels, broken lines indicate 95% confidence limits.

The variability in LA across the latitudinal gradient was higher than SLA, which attests to the higher plasticity of LA reported in other shrubs (Carlson *et al.* 2016). Coordinated changes in leaf tissue anatomy related to leaf density and thickness may have played a role in the relative stability of SLA in Dodonaea (Witkowski & Lamont 1991; Poorter *et al.* 2009; Villar *et al.* 2013). In evergreen woody species, SLA is influenced more by leaf density in terms of less intercellular air spaces and smaller cells (Villar *et al.* 2013), but confirmation for Dodonaea requires further investigation.

The latitudinal trends of both traits across our sampling area appear to be driven by the main north–south environmental gradients. However, when integrated into a single PCA axis of variation, two patterns stand out. Firstly, the environmental disjunction between northern (23.5–30°S) and southern (30–35°S) sites becomes obvious. We suggest that the change from summer-dominant or non-seasonal rains in the north to a defined winter rain regime in the south, or the putatively high soil salinity near Lake Eyre at the disjunction latitude, may have caused the

Table 2. Pearson correlations with coordinates of Axes 1 and 2 of the PCA ordination of abiotic variables along the latitudinal cline. Regression coefficients in bold represent highly correlated environmental variables. Included is the percentage of the total variance explained by each axis. High correlation coefficients are shown in bold lettering

	PC1		PC2	
Axis	*r*	*r*²	*r*	*r*²
Aridity index	**−0.92**	**0.84**	0.05	0.00
Annual Mean Rainfall (mm)	**−0.93**	**0.86**	0.12	0.02
Solar radiation (MJ m^{-2} day^{-1})	**0.95**	**0.91**	0.12	0.02
Temperature (mean) (°C)	**0.88**	**0.77**	−0.01	0.00
Temperature (range) (°C)	**0.94**	**0.89**	0.07	0.00
Soil nitrogen concentration (mg kg H_20^{-1})	**−0.87**	**0.75**	0.11	0.01
Soil phosphorus content (kg ha^{-1})	**−0.94**	**0.88**	0.07	0.00
Clay (%)	0.22	0.05	**−0.88**	**0.77**
Soil bulk density (g cm^{-3})	−0.43	0.18	**−0.78**	**0.61**
% Variance explained		68.01		15.89

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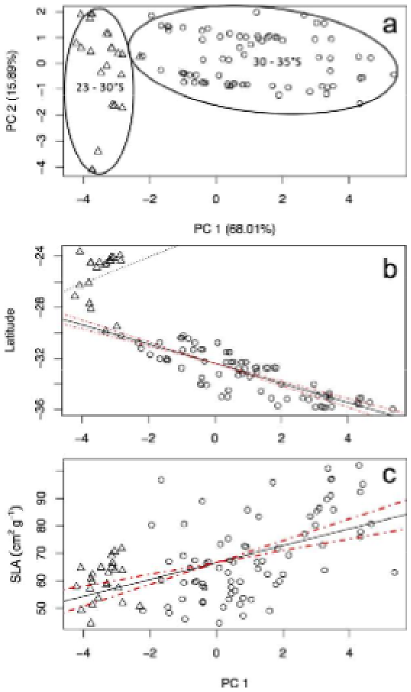

Fig. 3. (a) Principal component analysis (PCA) of the environmental variables shown in Table 2. In parentheses is the percentage of total variation explained by each PC axis. (b) Linear regression between latitude and the coordinates along Axis 1 of PCA. Solid line indicates regression for samples below −30°; dotted line indicates (insignificant) regression for samples above −30°. (c) Linear regression between *Dodonaea viscosa* subsp. *angustissima* SLAs and the coordinates of PCA along Axis 1. In all panels, circles represent the southern (30–35°S) populations and triangles represent the northern (23–29°S) populations. In panels (b) and (c) the red broken lines indicate 95% confidence limits and the coefficients and statistical significance are shown in Table 1.

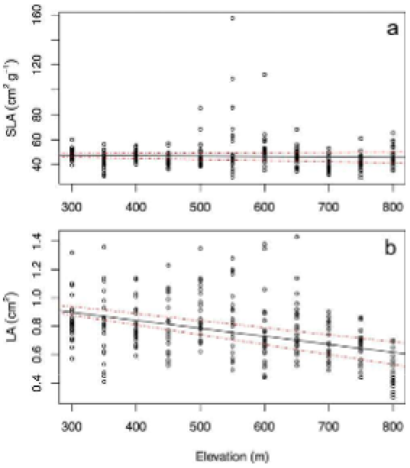

Fig. 4. Linear regression between *Dodonaea viscosa* subsp. *angustissima* (a) SLA and (b) elevation. Symbols represent values for individual leaves. In all panels, the broken lines indicate 95% confidence limits and the coefficients and statistical significance are shown in Table 1.

observed discontinuity. Secondly, the SLA cline does not show any apparent disjunction, which is more difficult to explain as we would have expected that leaf traits would also respond to the environmental discontinuity. More intensive sampling at the discontinuity latitude may clarify this point.

It is important to note that the approach we have taken here did not allow us to test the responses of SLA and LA to specific abiotic factors. Many of the abiotic variables co-varied along the gradient. We, therefore, cannot disentangle the relative contributions of the specific environmental variables we consider to direct (or indirect) effects on leaf traits. As such, we took a principal component analysis approach to account for these correlations, reducing redundancy in the abiotic data and addressing statistical issues associated with multiple testing. The associations we have identified do hint at causative responses to environment, but further testing (e.g. controlled glasshouse trials) of the effects of specific abiotic factors on leaf traits in Dodonaea is required.

Leaf trait variation across elevation

SLA and LA reacted differently to the parallel changes in decreasing temperature and increasing precipitation taking place from low to high elevation. Reduced LA at higher elevations and under lower temperature has been previously shown in Dodonaea (Guerin *et al.* 2012) and in other species (Gratani *et al.* 2012; Kichenin *et al.* 2013; Pescador *et al.* 2015). However, the absence of an SLA response is challenging to explain. We suggest that it could have arisen because there are forces that favour low SLA at both ends of the elevation gradient. At higher elevation, low LA causes low SLA. At low elevation, higher aridity reduces SLA. However, similar conditions prevail along the latitudinal range we sampled, where SLA responded linearly to the complex environmental gradient. To account for this discrepancy, we suggest that the elevational range we sampled (500 m) is too short to generate population differences in SLA due to unimpeded gene flow. Also, the combined effects of increased UV radiation and wind velocity at higher altitude might influence SLA. Again, the mechanism involved in this type of SLA homeostasis likely implicates simultaneous changes in leaf density and thickness. The elevational stability of SLA suggests that maintaining SLA within narrow limits may be critical to the life history of Dodonaea. Similar trends were reported for the sclerophyllous shrub *Protea repens* in South Africa and in Mediterranean highlands (Pescador *et al.* 2015; Carlson *et al.* 2016). Common garden trials or growing Dodonaea under controlled conditions with simultaneous measurements of leaf thickness and density could reveal the mechanism behind the stability of SLA.

CONCLUSIONS

Dodonaea shows substantial variability in SLA and LA, as a consequence of it responding to a wide variety of environmental demands throughout its extensive range and helping to facilitate its presence in multiple community assemblages. The relative stability of SLA compared to LA is probably the result of trade-offs imposed by the web of close relationships with other important life-history traits. Common garden experiments and growth trials under controlled conditions are needed to understand the effects of abiotic forces on these anatomical and ecophysiological relationships. The covariance of climate and soils with latitude makes it difficult to separate the individual effects of each of these variables on SLA and LA responses. In addition, it is difficult to explain the absence of an SLA response to changes in climate with elevation in contrast to its clear response to changes in climate across latitudes. A combination of inherent SLA stability, small inter-population distances along the slope, increased UV radiation and wind velocity at higher elevations might be responsible for this disparity in SLA response. As *D. viscosa* subspecies are widely employed for revegetation, our results are consistent with the notion that, if differences in leaf traits are genetically based, seed from populations from more arid areas could be used to revegetate mesic environments to increase seed suitability to climate change (Breed *et al.* 2013).

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DATA ARCHIVING

All leaf trait data have been published online via the AEKOS data repository and can be accessed via the following links: Latitudinal data set: http://www.aekos.org.au/dataset/262394; DOI: 10.4227/05/57C2343E4E9C2. Altitudinal data set: http://www.aekos.org.au/dataset/264061; DOI: 10.4227/05/57C396637EE56

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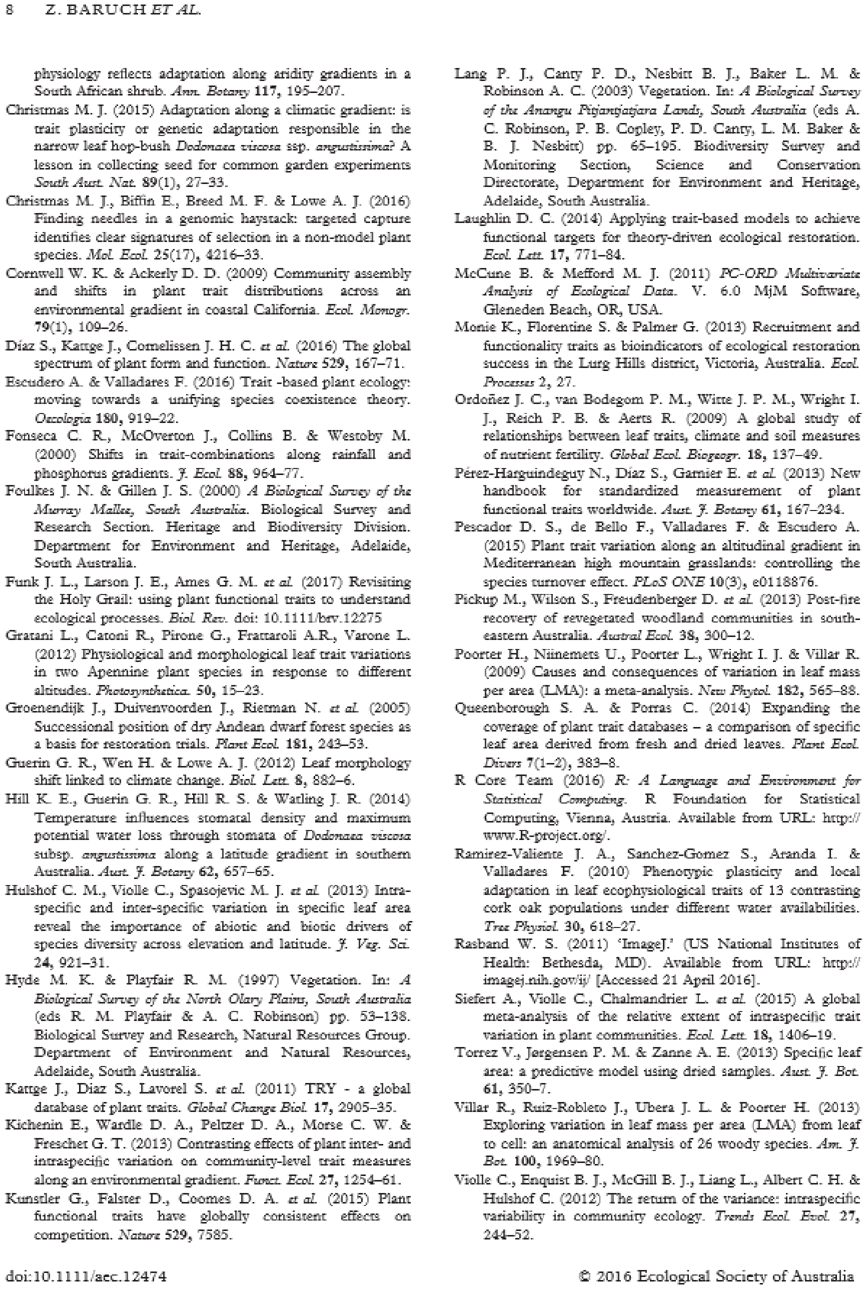

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Appendix S1. Linear regressions of Dodonaea leaf traits SLA and LA with eight environmental variables
Appendix S2. Regression coefficients of Dodonaea specific leaf area (SLA) and leaf area (LA) against a range of environmental variables

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APPENDIX G

Broadhurst, L., Breed, M., Lowe, A., Bragg, J., Catullo, R., Coates, D., Encinas-Viso, F., **Gellie, N.**, James E., Krauss, S., Potts, B., Rossetto, M., Shephard M., & Byrne M. (2017) Genetic diversity and structure of the Australian flora. *Diversity and Distributions*, **23**, 41-52.

Abstract

Aim To investigate the relationships between species attributes and genetic parameters in Australian plant species and to determine the associations in relation to predictions from population theory and previous global analyses.
Location Continent of Australia.
Methods We assembled a dataset of all known population genetic analyses of Australian plants based on neutral markers and catalogued them according to key species attributes, including range, abundance, range disjunction, biome and growth form; and genetic parameters, mean number of alleles per locus, observed and expected heterozygosity and population differentiation. We determined relationships between species attributes and genetic parameters using a maximum-likelihood, multimodel inference approach.
Results We found many associations that were consistent with predictions. Species attributes with greatest effect on genetic diversity were range size, growth form, abundance and biome. The most important attributes influencing genetic differentiation were range disjunction and abundance. We found unexpected results in the effects of biome and growth form on genetic diversity with greater diversity in the eastern biome of Australia, and lower diversity in shrubs compared to trees.
Main conclusions Our analysis of genetic diversity of Australian plants showed associations consistent with predictions based on population genetics theory, with strong effects of range size, abundance and growth form. We identified a striking effect of range disjunction on population genetic differentiation, an effect that has received little attention in the literature. We also found some notable differences to global predictions, which were most likely explained by confounding effects across variables. This highlights that caution is needed when extrapolating trends from global analyses to regional floras. Identifying associations between species attributes and patterns of genetic diversity enables broadscale predictions to facilitate the inclusion of genetic considerations into conservation decision-making.

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Diversity and Distributions, (Diversity Distrib.) (2017) **23**, 41–52

BIODIVERSITY RESEARCH

Genetic diversity and structure of the Australian flora

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ABSTRACT

Aim To investigate the relationships between species attributes and genetic parameters in Australian plant species and to determine the associations in relation to predictions from population theory and previous global analyses.

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Methods We assembled a dataset of all known population genetic analyses of Australian plants based on neutral markers and catalogued them according to key species attributes, including range, abundance, range disjunction, biome and growth form; and genetic parameters, mean number of alleles per locus, observed and expected heterozygosity and population differentiation. We determined relationships between species attributes and genetic parameters using a maximum-likelihood, multimodel inference approach.

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Main conclusions Our analysis of genetic diversity of Australian plants showed associations consistent with predictions based on population genetics theory, with strong effects of range size, abundance and growth form. We identified a striking effect of range disjunction on population genetic differentiation, an effect that has received little attention in the literature. We also found some notable differences to global predictions, which were most likely explained by confounding effects across variables. This highlights that caution is needed when extrapolating trends from global analyses to regional floras. Identifying associations between species attributes and patterns of genetic diversity enables broadscale predictions to facilitate the inclusion of genetic considerations into conservation decision-making.

Keywords
biome, conservation, disjunction, genetic differentiation, genetic diversity, life history.

INTRODUCTION

Levels of genetic diversity within and among populations have important consequences for the evolutionary trajectories of species and for the function and composition of ecological communities (Hughes *et al.*, 2008). Genetic diversity influences functional trait variation, recovery of populations following disturbance, species interactions, community structure and nutrient and energy fluxes (Whitham *et al.*, 2006; Hughes *et al.*, 2008; Bell & Gonzalez, 2009).

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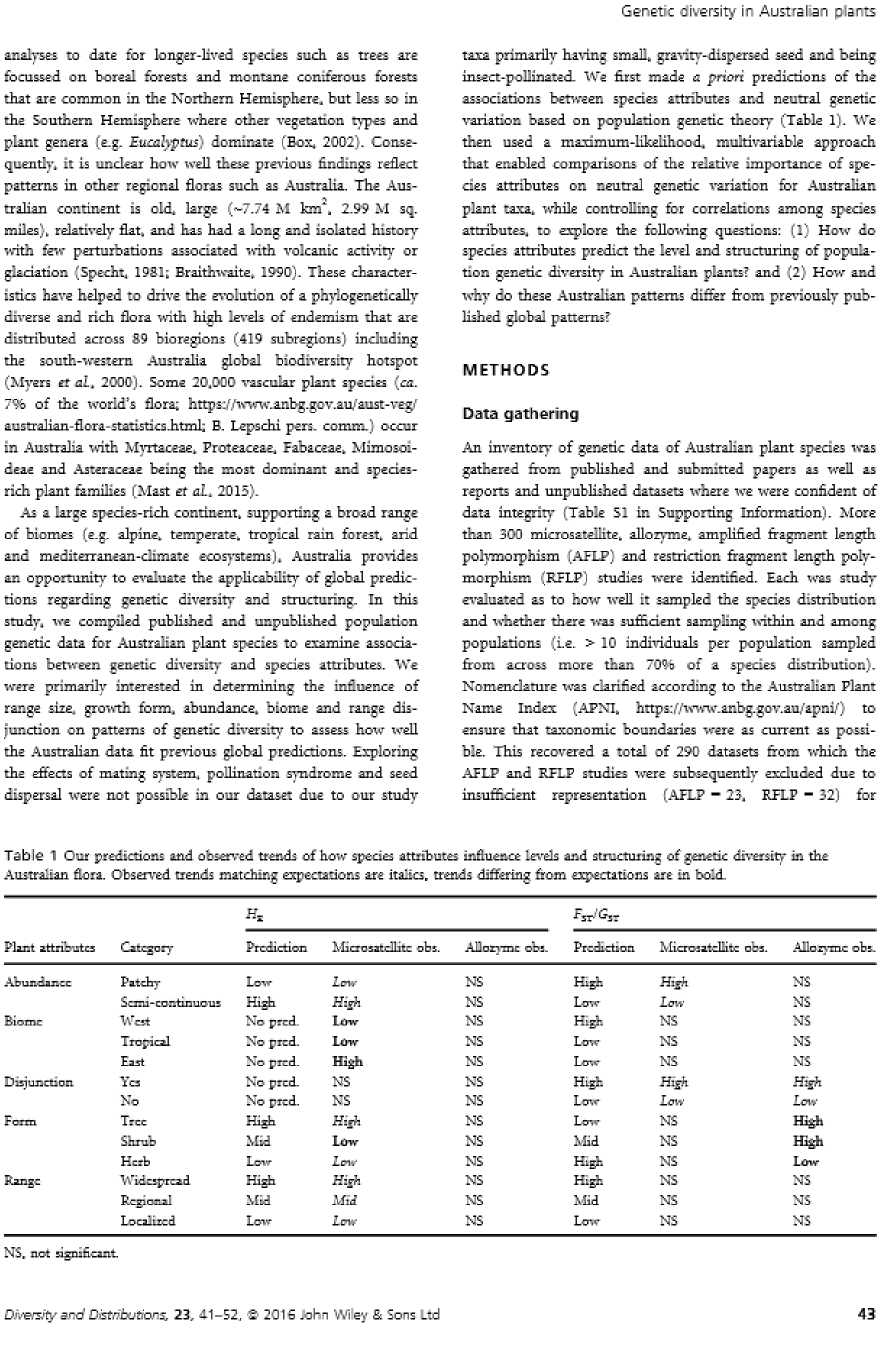
Consequently, understanding how genetic diversity is distributed in time and space is critical for managing biodiversity over broad spatial scales (e.g. responses to climate change) within biologically realistic time frames (i.e. decadal and longer) and helping to guide investments into on-ground actions (e.g. restoration). A major goal of multi-species meta-analyses in conservation biology is the identification of predictable biological patterns that can be used to guide the development of conservation and restoration frameworks. Identifying predictive associations between genetic diversity and explanatory variables that are easily measured is highly advantageous, given that the resources available for studying genetic diversity are finite.

Both adaptive and neutral evolutionary processes shape the distribution of genetic variation within species. While knowledge of the genetic variation underlying past adaptation and potentially available for future adaptation is an ideal for conservation and restoration biology, assessing this variation is both time-consuming and resource intensive (e.g. common garden or transplant studies). Such assessments often require large-scale and often long-term quantitative genetic studies, with the validation of associations between functional traits and fitness being challenging (Rockman, 2012). Consequently, for the majority of species of interest in conservation, we must continue to largely rely on putatively neutral genetic variation to link molecular variants with functional traits. In plants, neutral genetic variation is influenced by a range of life-history, geographic and demographic attributes, such as growth form, range size and abundance (Hamrick & Godt, 1996). Understanding associations between these species attributes and the level and structuring of neutral genetic diversity can help build generalizations to guide conservation and restoration decisions, especially for plant species where little or no information exists. These generalizations would be useful in several areas of conservation biology including: (1) informing the current debate in restoration genetics on the importance of genetic diversity in seed sources and the genetic connectivity of restored and remnant populations (Broadhurst *et al.*, 2008; Breed *et al.*, 2013); (2) providing guidance for the application of risk and management frameworks in conservation and restoration (Byrne *et al.*, 2011a; Ottewell *et al.*, 2016); (3) planning to meet the enormous global scale of restoration in the coming decades (Perring *et al.*, 2015; Suding *et al.*, 2015); (4) facilitating the inclusion of demographic processes (e.g. source–sink dynamics, refugia) into the next generation of species distribution models (Bellard *et al.*, 2012; Pauls *et al.*, 2013; Catullo *et al.*, 2015); (5) the incorporation of genetic factors into population viability modelling (Pierson *et al.*, 2015); (6) identifying groups of species to be prioritized for assisted management strategies (Rossetto *et al.*, 2015; Christmas *et al.*, 2016); and (7) developing guidelines for the management of small populations of threatened species (Frankham, 2015).

Developing broad principles to meet any or all of these objectives is complex and rests on the premise that generalized patterns of genetic diversity actually exist. Several studies have examined the partitioning of neutral genetic variation by species attributes to produce generalized findings that have been argued to be globally relevant (Hamrick *et al.*, 1979; Loveless & Hamrick, 1984; Hamrick & Godt, 1989, 1996; Gitzendanner & Soltis, 2000; Nybom & Bartish, 2000; Nybom, 2004; Duminil *et al.*, 2007). These studies have indicated that range size, growth form and mating system are some of the most important predictors of species' genetic diversity. Widespread species presumably maintain more diversity due to lower genetic drift in large, stable meta-populations than species with narrower distributions (Hamrick & Godt, 1989). Range size has also been found to be correlated with plant mating systems, with the distribution of self-pollinating species being up to two times larger than their outcrossed sister species (Grossenbacher *et al.*, 2015). Self-pollinating species are predicted to be better colonizers than those that outcross as reproductive assurance can facilitate geographic range expansion (Baker, 1955; Stebbins, 1957; Pannell, 2015). Plant form and generation time are predicted to influence genetic diversity because species with shorter generation times are expected to have smaller neighbourhoods, which promotes population isolation, whereas genetic diversity should decay more slowly in longer-lived species (Loveless & Hamrick, 1984), although annuals with large population sizes may not experience this effect. Age-related fecundity and overlapping generations also homogenize long-lived populations (Kuparinen *et al.*, 2010). Reproductive strategy may influence genetic diversity as inbreeding tends to homogenize genotypes and increase population differentiation, while outcrossing enforces pollen dispersal, increasing the likelihood that long-distance gene flow will reduce population divergence. Many plant species have a mixed mating system, although this may include a preference for outcrossed pollen [e.g. eucalypts (Griffin *et al.*, 1987; Byrne, 2008)], while low genotype diversity is often characteristic of clonal species (Millar *et al.*, 2010; Binks *et al.*, 2015). Short dispersal distances should promote differentiation, whereas regular, long-distance dispersal should promote population homogenization (Loveless & Hamrick, 1984).

The importance of identifying associations between species attributes and their genetic diversity is highlighted by the strong influence of population size, genetic variation and inbreeding on plant population fitness and future viability (Spielman *et al.*, 2004; Leimu *et al.*, 2006). While previous reviews (Hamrick *et al.*, 1979; Loveless & Hamrick, 1984; Hamrick & Godt, 1989, 1996; Gitzendanner & Soltis, 2000; Nybom & Bartish, 2000; Nybom, 2004; Duminil *et al.*, 2007) provide insights that could help guide conservation and restoration actions, their relevance in the Australian context has not been explored. These previous reviews focussed largely on northern temperate and Neotropical species, as relatively few Southern Hemisphere species were available for inclusion at the time. Distinct taxonomic and compositional differences in vegetation also exist between the Northern and Southern Hemispheres. Many of the genetic diversity



analyses to date for longer-lived species such as trees are focussed on boreal forests and montane coniferous forests that are common in the Northern Hemisphere, but less so in the Southern Hemisphere where other vegetation types and plant genera (e.g. *Eucalyptus*) dominate (Box, 2002). Consequently, it is unclear how well these previous findings reflect patterns in other regional floras such as Australia. The Australian continent is old, large (~7.74 M km^2, 2.99 M sq. miles), relatively flat, and has had a long and isolated history with few perturbations associated with volcanic activity or glaciation (Specht, 1981; Braithwaite, 1990). These characteristics have helped to drive the evolution of a phylogenetically diverse and rich flora with high levels of endemism that are distributed across 89 bioregions (419 subregions) including the south-western Australia global biodiversity hotspot (Myers *et al.*, 2000). Some 20,000 vascular plant species (*ca.* 7% of the world's flora; https://www.anbg.gov.au/aust-veg/australian-flora-statistics.html; B. Lepschi pers. comm.) occur in Australia with Myrtaceae, Proteaceae, Fabaceae, Mimosoideae and Asteraceae being the most dominant and species-rich plant families (Mast *et al.*, 2015).

As a large species-rich continent, supporting a broad range of biomes (e.g. alpine, temperate, tropical rain forest, arid and mediterranean-climate ecosystems), Australia provides an opportunity to evaluate the applicability of global predictions regarding genetic diversity and structuring. In this study, we compiled published and unpublished population genetic data for Australian plant species to examine associations between genetic diversity and species attributes. We were primarily interested in determining the influence of range size, growth form, abundance, biome and range disjunction on patterns of genetic diversity to assess how well the Australian data fit previous global predictions. Exploring the effects of mating system, pollination syndrome and seed dispersal were not possible in our dataset due to our study taxa primarily having small, gravity-dispersed seed and being insect-pollinated. We first made *a priori* predictions of the associations between species attributes and neutral genetic variation based on population genetic theory (Table 1). We then used a maximum-likelihood, multivariable approach that enabled comparisons of the relative importance of species attributes on neutral genetic variation for Australian plant taxa, while controlling for correlations among species attributes, to explore the following questions: (1) How do species attributes predict the level and structuring of population genetic diversity in Australian plants? and (2) How and why do these Australian patterns differ from previously published global patterns?

METHODS

Data gathering

An inventory of genetic data of Australian plant species was gathered from published and submitted papers as well as reports and unpublished datasets where we were confident of data integrity (Table S1 in Supporting Information). More than 300 microsatellite, allozyme, amplified fragment length polymorphism (AFLP) and restriction fragment length polymorphism (RFLP) studies were identified. Each was study evaluated as to how well it sampled the species distribution and whether there was sufficient sampling within and among populations (i.e. > 10 individuals per population sampled from across more than 70% of a species distribution). Nomenclature was clarified according to the Australian Plant Name Index (APNI, https://www.anbg.gov.au/apni/) to ensure that taxonomic boundaries were as current as possible. This recovered a total of 290 datasets from which the AFLP and RFLP studies were subsequently excluded due to insufficient representation (AFLP = 23, RFLP = 32) for

Table 1 Our predictions and observed trends of how species attributes influence levels and structuring of genetic diversity in the Australian flora. Observed trends matching expectations are italics, trends differing from expectations are in bold.

		H_E			F_{ST}/G_{ST}		
Plant attributes	Category	Prediction	Microsatellite obs.	Allozyme obs.	Prediction	Microsatellite obs.	Allozyme obs.
Abundance	Patchy	Low	*Low*	NS	High	*High*	NS
	Semi-continuous	High	*High*	NS	Low	*Low*	NS
Biome	West	No pred.	**Low**	NS	High	NS	NS
	Tropical	No pred.	**Low**	NS	Low	NS	NS
	East	No pred.	**High**	NS	Low	NS	NS
Disjunction	Yes	No pred.	NS	NS	High	*High*	*High*
	No	No pred.	NS	NS	Low	*Low*	*Low*
Form	Tree	High	*High*	NS	Low	NS	**High**
	Shrub	Mid	**Low**	NS	Mid	NS	**High**
	Herb	Low	*Low*	NS	High	NS	**Low**
Range	Widespread	High	*High*	NS	High	NS	NS
	Regional	Mid	*Mid*	NS	Mid	NS	NS
	Localized	Low	*Low*	NS	Low	NS	NS

NS, not significant.

meaningful analysis. There were few studies of polyploid species, and so, these were removed as the genetic values were not directly comparable with those of diploid species. Multiple studies of the same species were retained if different markers were used or if recognized subspecies or ecotypes were examined. A similar number of microsatellite ($n = 118$) and allozyme ($n = 117$) studies were retained for analysis across 235 taxa. The compiled dataset highlighted some pronounced imbalances in studies of Australian plants. For example, there was a significant bias towards eucalypts (25% of the studies) and the Myrtaceae more generally (35%) that subsequently influenced data associated with pollination syndromes and seed dispersal. In addition, studies from the western biome were dominated by rare and disjunct species reflecting the evolutionary drivers associated with this biodiversity hotspot (Hopper, 2009), and the largely conservation-orientated focus of researchers in this region.

We classified species according to several attributes using agreed data standards (Table 2). Species were classified according to the total size of their range area (Range); how populations were distributed within the species range (Abundance); the level of disjunction in the distribution of populations across the range (Disjunction); the predominant biome within which the species occurred (Biome); and growth form (Form), with the class 'Herb' referring to herbaceous perennials only as there were no data for annual species, as these are not common in the Australian flora due to its evolutionary history (Byrne *et al.*, 2008b, 2011b). We also characterized the mating system, pollination syndrome and seed dispersal mechanism of the species as these variables have been shown to significantly influence patterns of genetic diversity (Hamrick & Godt, 1996). However, we were unable to analyse the influence of these variables on genetic parameters due to the biased and non-balanced expression of traits exhibited in the species investigated, where the vast majority of species in the dataset were animal-pollinated with a mixed mating system. Most species were also characterized by gravity-dispersed seed, with other classes of seed dispersal having sample sizes too small for effective analysis.

For each species, we collected species-level genetic summary statistics from each study including the mean number of alleles per locus (A, $n = 225$), expected and observed heterozygosity (H_E, $n = 219$; H_O, $n = 202$) and population differentiation (G_{ST} and F_{ST}, $n = 155$), which were used as the response variables for our data analysis. We treated all microsatellite studies in one class, although genetic diversity levels in microsatellite studies based on species specific loci have been found to be higher than those on based on cross-species amplification (Primmer *et al.*, 1996; Barbará *et al.*, 2007). While F_{ST} describes the amount of genetic variation that can be explained by population structure and G_{ST} quantifies the genetic divergence among populations, there are similarities between the two measures (Hartl & Clark, 2007) and in practice G_{ST} is equal to F_{ST} (Nei, 1977). Consequently, we included both of these measures as estimates of differentiation, herein denoted as 'F_{ST}', as has been carried out elsewhere (Gitzendanner & Soltis, 2000).

Data analysis

To explore the redundancy and structure among the variables, we used principal component analysis (PCA) for the continuous genetic response variables and multiple correspondence analysis (MCA) for the categorical species attributes in the FACTOMINER package (Husson *et al.*, 2014) in R v.3.0.2 (R Core Team, 2015). We then used general linear models in a maximum-likelihood, multimodel inference framework in R to test for our hypothesized relationships between the predictor variables (i.e. species attributes; Range, Distribution, Abundance, Biome, Form) and the genetic

Table 2 Species attributes and genetic parameters assessed in this study.

Variables	Category	Classification	Description	Reference
Plant attributes	Range	Predictor	Widespread = > 600 km in one direction; regional = 150–600 km; localized = small, localized, < 100 km	Moran & Hopper (1987)
	Abundance	Predictor	Semi-continuous or patchy. Describes the pattern of population distribution within the species range	
	Disjunction	Predictor	Yes or No. Describes whether populations in species with semi-continuous distributions are very discrete and unlikely to be interacting, that is whether divergent lineages are likely to have evolved	
	Biome	Predictor	Eastern, western or tropical Australia	Olson *et al.* (2001)
	Form	Predictor	Tree, shrub, herb (perennial)	
Genetic parameters	Marker	Covariate	Microsatellite, allozyme	
	A	Response	Mean number of alleles	Hartl & Clark (2007)
	H_E	Response	Expected heterozygosity	Hartl & Clark (2007)
	F_{ST}	Response	Population differentiation	Wright (1951), Nei (1973)

response variables (A, H_O, H_E, F_{ST}). Firstly, we ran an exhaustive set of additive models for each response variable to determine which predictor variables were most important in explaining variation in the response variables. We estimated Akaike information criterion corrected for small sample sizes (AIC_c) and Akaike weights ($_w$AIC) for each model (Burnham & Andersen, 2002). To select predictor variables of greatest importance to each response variable, we derived the index of the relative importance of predictor variable i (AIC_i), the sum of Akaike weights for all models that included parameter i (Burnham & Anderson, 2002). A high AIC_i implies parameter i was more important in predicting variation in the response variable j than parameters with a lower AIC_i (i.e. a predictor variable with AIC_i approaching 1 suggests that this parameter has great importance).

We conducted nested ANOVAs to explore the phylogenetic signal in each genetic response variable using the LME4 package (Bates *et al.*, 2014) in R. Taxonomic levels (order, family, genus) were nested random effects within higher levels. Unlike previous studies (e.g. Duminil *et al.*, 2007), we detected only a weak phylogenetic signal in the genetic response variables [Table 3; sum of phylogenetic effects in our case was $\leq$ 40%, whereas it was > 75% in Duminil *et al.* (2007)]. Indeed, when genetic marker type was included in these models, marker type explained much more variance than the sum of taxonomic levels in all analyses (Table 3). The higher allelic diversity detected with microsatellites compared to allozymes is likely to strongly influence diversity parameters, but would not be as strong an effect on values of differentiation, although Hedrick (1999) has shown that differentiation will be underestimated in loci, such as microsatellites, with very high H_E values. To maintain statistical power and to avoid overparameterization of models predicting genetic variables, we chose to include genetic marker type as a covariate in all models to avoid any confounding effects.

In all linear models, we used Box–Cox transformations (Box & Cox, 1964) of the response variables to meet the assumption of normality of residuals, testing the normality of residuals of models with Shapiro–Wilk tests (Shapiro & Wilk, 1965).

RESULTS

As in previous studies (e.g. Barrett *et al.*, 2005), we found great redundancy in the three genetic diversity response variables (A, H_O and H_E; Fig. 1a) and therefore chose to explore variation in expected heterozygosity (H_E) only as it had the weakest phylogenetic signal (Table 3). Life-history predictor variables had more complex structure. The first two dimensions of a PCA explained 35% of the variation among these variables (Fig. 1b), and therefore, all were included in subsequent analyses.

We found differences for population genetic diversity (H_E) in microsatellite-based studies with Abundance (semi-continuous versus patchy: mean H_E = 0.73 vs. 0.58; Table 4; AIC_i = 0.98; Tables 6 and 7), Form (tree versus shrub versus herb: mean H_E = 0.69 vs. 0.60 vs. 0.63; Table 4; AIC_i = 0.86; Tables 6 and 7), Range (widespread versus regional versus localized: mean H_E = 0.70 vs. 0.64 vs. 0.59; Table 4; AIC_i = 0.91; Tables 6 and 7) and Biome (east versus west versus tropical: mean H_E = 0.72 vs. 0.60 vs. 0.60; Table 4; AIC_i = 0.99; Tables 6 and 7). Despite a similar number of allozyme studies, no trends were detectable for this marker type. Disjunction also had no detectable effect on genetic diversity for either marker class. Differences in genetic diversity were observed among the three Australian biomes for microsatellite studies, with greater H_E in the eastern biome than either the western or tropical biomes.

Comparisons of our genetic diversity (H_E) data to estimates derived from global analysis revealed that for most categories, allozyme-derived values were generally higher or similar for Australian species. The exception to this finding

Table 3 Variance explained by taxonomic levels and genetic marker on the genetic response variables.

Taxonomic level	A (%)	H_E (%)	F_{ST} (%)
Marker	78.95	88.25	14.56
Order	2.26	1.43	35.33
Family nested in order	0.00	0.00	0.00
Genus nested in family	6.39	2.32	0.00

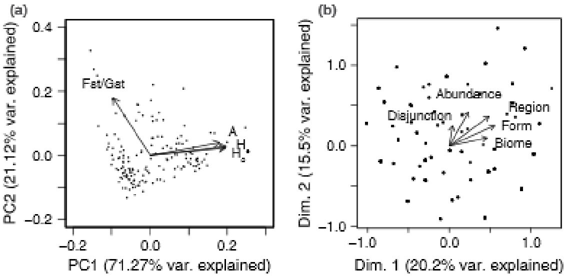


Figure 1 Variation explained in the datasets by the response variables. (a) Principal components analysis of genetic response variables from 155 Australian plant studies and (b) multiple correspondence analysis of life-history predictor variables from 254 Australian plant studies. Species with missing data were excluded from both analyses. Arrows represent the eigenvectors of the different variables included in the analyses.

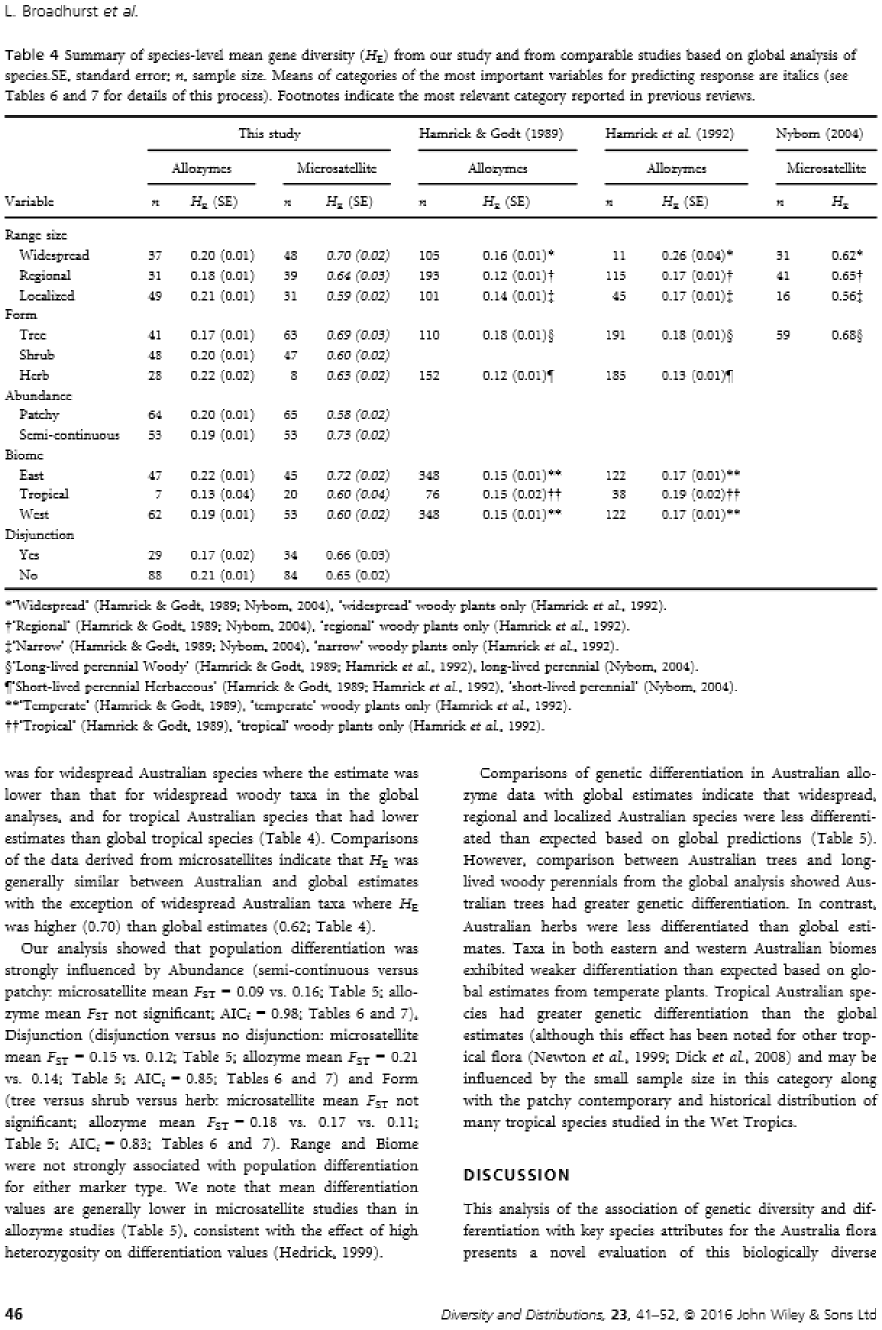


Table 4 Summary of species-level mean gene diversity (H_E) from our study and from comparable studies based on global analysis of species.SE, standard error; *n*, sample size. Means of categories of the most important variables for predicting response are italics (see Tables 6 and 7 for details of this process). Footnotes indicate the most relevant category reported in previous reviews.

	This study				Hamrick & Godt (1989)		Hamrick *et al.* (1992)		Nybom (2004)	
	Allozymes		Microsatellite		Allozymes		Allozymes		Microsatellite	
Variable	*n*	H_E (SE)	*n*	H_E (SE)	*n*	H_E (SE)	*n*	H_E (SE)	*n*	H_E
Range size										
Widespread	37	0.20 (0.01)	48	*0.70 (0.02)*	105	0.16 (0.01)*	11	0.26 (0.04)*	31	0.62*
Regional	31	0.18 (0.01)	39	*0.64 (0.03)*	193	0.12 (0.01)†	115	0.17 (0.01)†	41	0.65†
Localized	49	0.21 (0.01)	31	*0.59 (0.02)*	101	0.14 (0.01)‡	45	0.17 (0.01)‡	16	0.56‡
Form										
Tree	41	0.17 (0.01)	63	*0.69 (0.03)*	110	0.18 (0.01)§	191	0.18 (0.01)§	59	0.68§
Shrub	48	0.20 (0.01)	47	*0.60 (0.02)*						
Herb	28	0.22 (0.02)	8	*0.63 (0.02)*	152	0.12 (0.01)¶	185	0.13 (0.01)¶		
Abundance										
Patchy	64	0.20 (0.01)	65	*0.58 (0.02)*						
Semi-continuous	53	0.19 (0.01)	53	*0.73 (0.02)*						
Biome										
East	47	0.22 (0.01)	45	*0.72 (0.02)*	348	0.15 (0.01)**	122	0.17 (0.01)**		
Tropical	7	0.13 (0.04)	20	*0.60 (0.04)*	76	0.15 (0.02)††	38	0.19 (0.02)††		
West	62	0.19 (0.01)	53	*0.60 (0.02)*	348	0.15 (0.01)**	122	0.17 (0.01)**		
Disjunction										
Yes	29	0.17 (0.02)	34	0.66 (0.03)						
No	88	0.21 (0.01)	84	0.65 (0.02)						

*'Widespread' (Hamrick & Godt, 1989; Nybom, 2004), 'widespread' woody plants only (Hamrick *et al.*, 1992).

†'Regional' (Hamrick & Godt, 1989; Nybom, 2004), 'regional' woody plants only (Hamrick *et al.*, 1992).

‡'Narrow' (Hamrick & Godt, 1989; Nybom, 2004), 'narrow' woody plants only (Hamrick *et al.*, 1992).

§'Long-lived perennial Woody' (Hamrick & Godt, 1989; Hamrick *et al.*, 1992), long-lived perennial (Nybom, 2004).

¶'Short-lived perennial Herbaceous' (Hamrick & Godt, 1989; Hamrick *et al.*, 1992), 'short-lived perennial' (Nybom, 2004).

**'Temperate' (Hamrick & Godt, 1989), 'temperate' woody plants only (Hamrick *et al.*, 1992).

††'Tropical' (Hamrick & Godt, 1989), 'tropical' woody plants only (Hamrick *et al.*, 1992).

was for widespread Australian species where the estimate was lower than that for widespread woody taxa in the global analyses, and for tropical Australian species that had lower estimates than global tropical species (Table 4). Comparisons of the data derived from microsatellites indicate that H_E was generally similar between Australian and global estimates with the exception of widespread Australian taxa where H_E was higher (0.70) than global estimates (0.62; Table 4).

Our analysis showed that population differentiation was strongly influenced by Abundance (semi-continuous versus patchy: microsatellite mean $F_{ST} = 0.09$ vs. 0.16; Table 5; allozyme mean F_{ST} not significant; $AIC_i = 0.98$; Tables 6 and 7), Disjunction (disjunction versus no disjunction: microsatellite mean $F_{ST} = 0.15$ vs. 0.12; Table 5; allozyme mean $F_{ST} = 0.21$ vs. 0.14; Table 5; $AIC_i = 0.85$; Tables 6 and 7) and Form (tree versus shrub versus herb: microsatellite mean F_{ST} not significant; allozyme mean $F_{ST} = 0.18$ vs. 0.17 vs. 0.11; Table 5; $AIC_i = 0.83$; Tables 6 and 7). Range and Biome were not strongly associated with population differentiation for either marker type. We note that mean differentiation values are generally lower in microsatellite studies than in allozyme studies (Table 5), consistent with the effect of high heterozygosity on differentiation values (Hedrick, 1999).

Comparisons of genetic differentiation in Australian allozyme data with global estimates indicate that widespread, regional and localized Australian species were less differentiated than expected based on global predictions (Table 5). However, comparison between Australian trees and long-lived woody perennials from the global analysis showed Australian trees had greater genetic differentiation. In contrast, Australian herbs were less differentiated than global estimates. Taxa in both eastern and western Australian biomes exhibited weaker differentiation than expected based on global estimates from temperate plants. Tropical Australian species had greater genetic differentiation than the global estimates (although this effect has been noted for other tropical flora (Newton *et al.*, 1999; Dick *et al.*, 2008) and may be influenced by the small sample size in this category along with the patchy contemporary and historical distribution of many tropical species studied in the Wet Tropics.

DISCUSSION

This analysis of the association of genetic diversity and differentiation with key species attributes for the Australia flora presents a novel evaluation of this biologically diverse

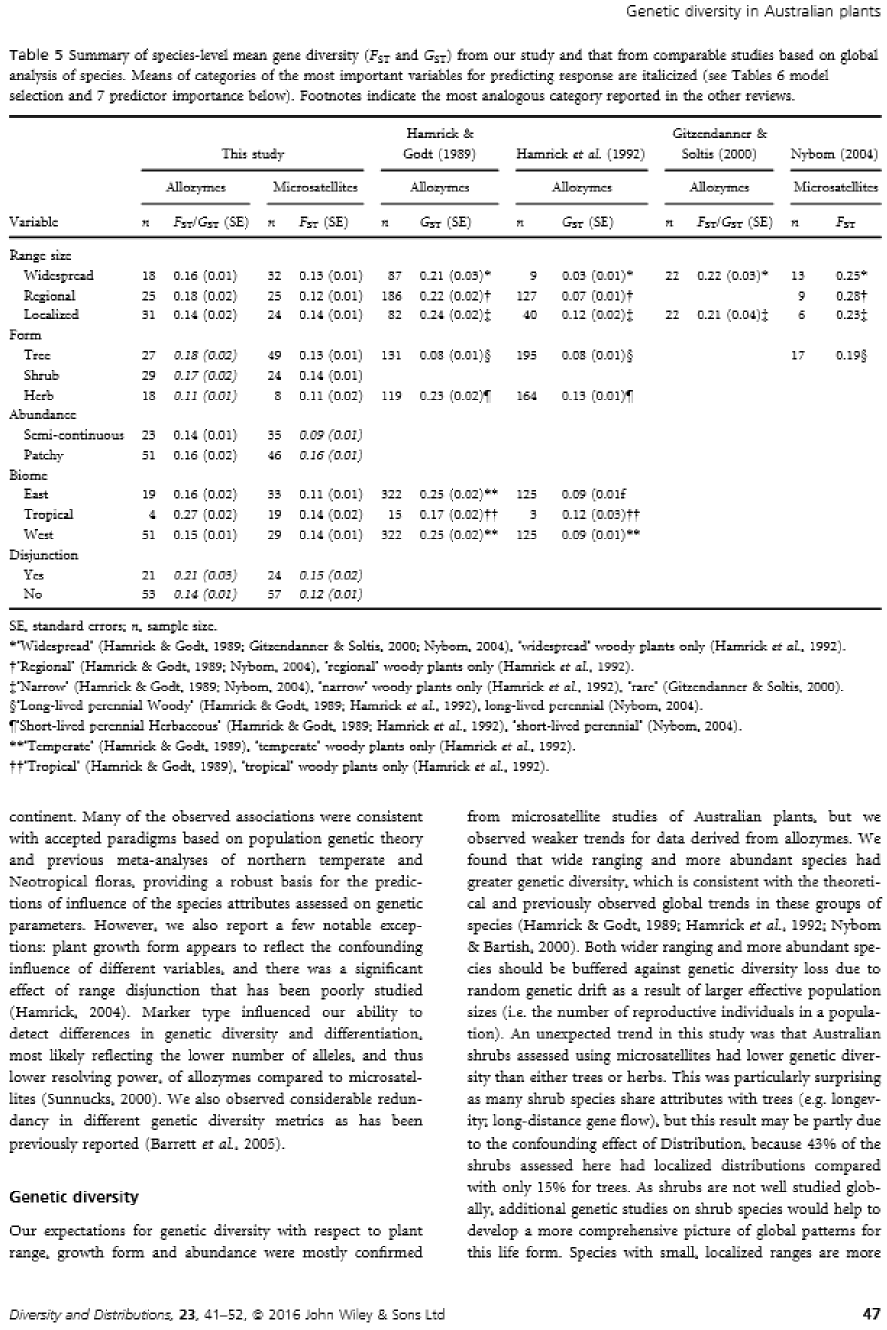


Table 5 Summary of species-level mean gene diversity (F_{ST} and G_{ST}) from our study and that from comparable studies based on global analysis of species. Means of categories of the most important variables for predicting response are italicized (see Tables 6 model selection and 7 predictor importance below). Footnotes indicate the most analogous category reported in the other reviews.

	This study				Hamrick & Godt (1989)		Hamrick *et al.* (1992)		Gitzendanner & Soltis (2000)		Nybom (2004)	
	Allozymes		Microsatellites		Allozymes		Allozymes		Allozymes		Microsatellites	
Variable	*n*	F_{ST}/G_{ST} (SE)	*n*	F_{ST} (SE)	*n*	G_{ST} (SE)	*n*	G_{ST} (SE)	*n*	F_{ST}/G_{ST} (SE)	*n*	F_{ST}
Range size												
Widespread	18	0.16 (0.01)	32	0.13 (0.01)	87	0.21 (0.03)*	9	0.03 (0.01)*	22	0.22 (0.03)*	13	0.25*
Regional	25	0.18 (0.02)	25	0.12 (0.01)	186	0.22 (0.02)†	127	0.07 (0.01)†			9	0.28†
Localized	31	0.14 (0.02)	24	0.14 (0.01)	82	0.24 (0.02)‡	40	0.12 (0.02)‡	22	0.21 (0.04)‡	6	0.23‡
Form												
Tree	27	*0.18 (0.02)*	49	0.13 (0.01)	131	0.08 (0.01)§	195	0.08 (0.01)§			17	0.19§
Shrub	29	*0.17 (0.02)*	24	0.14 (0.01)								
Herb	18	*0.11 (0.01)*	8	0.11 (0.02)	119	0.23 (0.02)¶	164	0.13 (0.01)¶				
Abundance												
Semi-continuous	23	0.14 (0.01)	35	*0.09 (0.01)*								
Patchy	51	0.16 (0.02)	46	*0.16 (0.01)*								
Biome												
East	19	0.16 (0.02)	33	0.11 (0.01)	322	0.25 (0.02)**	125	0.09 (0.01f				
Tropical	4	0.27 (0.02)	19	0.14 (0.02)	15	0.17 (0.02)††	3	0.12 (0.03)††				
West	51	0.15 (0.01)	29	0.14 (0.01)	322	0.25 (0.02)**	125	0.09 (0.01)**				
Disjunction												
Yes	21	*0.21 (0.03)*	24	*0.15 (0.02)*								
No	53	*0.14 (0.01)*	57	*0.12 (0.01)*								

SE, standard errors; *n*, sample size.

*'Widespread' (Hamrick & Godt, 1989; Gitzendanner & Soltis, 2000; Nybom, 2004), 'widespread' woody plants only (Hamrick *et al.*, 1992).

†'Regional' (Hamrick & Godt, 1989; Nybom, 2004), 'regional' woody plants only (Hamrick *et al.*, 1992).

‡'Narrow' (Hamrick & Godt, 1989; Nybom, 2004), 'narrow' woody plants only (Hamrick *et al.*, 1992), 'rare' (Gitzendanner & Soltis, 2000).

§'Long-lived perennial Woody' (Hamrick & Godt, 1989; Hamrick *et al.*, 1992), long-lived perennial (Nybom, 2004).

¶'Short-lived perennial Herbaceous' (Hamrick & Godt, 1989; Hamrick *et al.*, 1992), 'short-lived perennial' (Nybom, 2004).

**'Temperate' (Hamrick & Godt, 1989), 'temperate' woody plants only (Hamrick *et al.*, 1992).

††'Tropical' (Hamrick & Godt, 1989), 'tropical' woody plants only (Hamrick *et al.*, 1992).

continent. Many of the observed associations were consistent with accepted paradigms based on population genetic theory and previous meta-analyses of northern temperate and Neotropical floras, providing a robust basis for the predictions of influence of the species attributes assessed on genetic parameters. However, we also report a few notable exceptions: plant growth form appears to reflect the confounding influence of different variables, and there was a significant effect of range disjunction that has been poorly studied (Hamrick, 2004). Marker type influenced our ability to detect differences in genetic diversity and differentiation, most likely reflecting the lower number of alleles, and thus lower resolving power, of allozymes compared to microsatellites (Sunnucks, 2000). We also observed considerable redundancy in different genetic diversity metrics as has been previously reported (Barrett *et al.*, 2005).

Genetic diversity

Our expectations for genetic diversity with respect to plant range, growth form and abundance were mostly confirmed from microsatellite studies of Australian plants, but we observed weaker trends for data derived from allozymes. We found that wide ranging and more abundant species had greater genetic diversity, which is consistent with the theoretical and previously observed global trends in these groups of species (Hamrick & Godt, 1989; Hamrick *et al.*, 1992; Nybom & Bartish, 2000). Both wider ranging and more abundant species should be buffered against genetic diversity loss due to random genetic drift as a result of larger effective population sizes (i.e. the number of reproductive individuals in a population). An unexpected trend in this study was that Australian shrubs assessed using microsatellites had lower genetic diversity than either trees or herbs. This was particularly surprising as many shrub species share attributes with trees (e.g. longevity; long-distance gene flow), but this result may be partly due to the confounding effect of Distribution, because 43% of the shrubs assessed here had localized distributions compared with only 15% for trees. As shrubs are not well studied globally, additional genetic studies on shrub species would help to develop a more comprehensive picture of global patterns for this life form. Species with small, localized ranges are more

Table 6 General linear models of species attributes predicting population genetic response variables (H_E, expected heterozygosity; F_{ST}/G_{ST}, population differentiation). % DE, per cent deviance explained by the model; ΔAIC_c, indicator of difference between model Akaike 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) or with ΔAIC_c < 4 are shown.

Model	% DE	ΔAIC_c	$_{w}$AIC	*k*
Expected heterozygosity (H_E)				
H_E – Marker + Abundance + Form + Biome	81.06	0.00	0.46	7
H_E – Marker + Abundance + Disjunction + Form + Biome	81.08	1.94	0.17	8
H_E – Marker + Abundance + Form + Range + Biome	81.24	2.19	0.15	9
Population differentiation (F_{ST})				
F_{ST} – Marker + Abundance + Disjunction + Form	16.10	0.00	0.45	6
F_{ST} – Marker + Abundance + Disjunction + Form + Biome	17.16	2.50	0.13	8
F_{ST} – Marker + Abundance + Disjunction + Form + Range	16.87	3.04	0.10	8
F_{ST} – Marker + Abundance + Form	13.01	3.40	0.08	5

Table 7 The relative importance of each species attributes in predicting population genetic response variables (H_E, expected heterozygosity; F_{ST}/G_{ST}, population differentiation). 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 (see Burnham & Andersen, 2002 pp. 167–169), with the most important variables italics in both cases.

Response variable	Predictor variable	AIC_i
H_E	*Abundance*	0.98
	Biome	0.99
	Disjunction	0.27
	Form	0.86
	Range	0.91
F_{ST}	*Abundance*	0.98
	Biome	0.19
	Disjunction	0.85
	Form	0.83
	Range	0.18

likely to be influenced by the effects of genetic drift reducing genetic diversity and may explain our results.

Our observation of differences in the three Australian biomes, with greatest diversity in the eastern biome than in the western or tropical biome, was also unexpected. The differences in H_E may be explained by a combination of confounding effects of other life-history attributes and historical biogeographic factors of the three regions. The studies conducted in the eastern biome had a greater proportion of trees (62%) compared to those in the west (30%), as well as a greater proportion of species with widespread distribution (east 45% vs. west 26%); both of these attributes were also correlated with greater genetic diversity. Historical biogeographic factors may also have influenced our result as the impacts of increasing aridification and climate cycles over two million years during the Pleistocene led to expansion of the arid zone and contraction of tropical and mesic environments to the edges of the continent; this effect was more pronounced in the western mesic and northern tropical regions than in the eastern mesic region (Byrne *et al.*, 2008b). In addition, the eastern mesic region has a longer latitudinal gradient with more diverse topography and greater elevation range, which would allow species to either move south, or move higher in altitude, in response to Pleistocene climatic oscillations. Both these historical biogeographic factors are likely to have reduced the intensity of bottlenecks in the eastern biome compared to the western and tropical biomes.

Population genetic differentiation

Our expectations for the effect of range disjunctions and species abundance on population differentiation were confirmed. Species with distributions that include range disjunctions where gene flow is expected to be limited showed a higher level of differentiation than species with non-disjunct distributions. The effect of range disjunction on population differentiation was consistent for both allozyme and microsatellite data, indicating that this strong effect is readily detected. While some of these species may have disjunct ranges due to recent widespread habitat fragmentation in southern Australia (Bradshaw, 2012; Guerin *et al.*, 2016), it is more likely that the high levels of divergence reflect genetic processes associated with historical ecogeographic barriers to gene flow over significant time frames (Byrne *et al.*, 2008b, 2011b). Abundance was also found to influence population differentiation as predicted, due to increased mean differentiation in patchily distributed species, although this was only observed for microsatellite studies.

We were surprised to observe deviations from our expectations for genetic differentiation (measured with allozymes), and plant growth form as the greater genetic differentiation in Australian trees compared to herbs was the opposite of the trends observed in global analyses. The low genetic differentiation observed for Australian herbs (0.11) was more similar to that observed for long-lived perennial woody plants (0.08) reported in earlier global reviews than for

herbaceous species (0.23; Hamrick & Godt, 1989). This is surprising because 93% of herbs included in our study were classified as insect-pollinated, which is a pollination syndrome that should increase the strength of population differentiation due to limited capacity for gene flow compared with pollination by large animals or wind (Rossetto *et al.*, 2007, 2009). This unexpected result may also be due to the dominance of terrestrial orchids in our dataset (28% of studies) as these species have readily dispersed dust-like seed (Jersáková & Malinová, 2007) whose widespread dispersal should reduce population differentiation compared to many other herbaceous species. Other herbaceous species in our dataset are likely to be primarily outcrossing as few Australian herbs are obligate selfers [e.g. *Drosera* (Stace *et al.*, 1997); *Ranunculus* (Pickering, 1997); *Stylidium* (Coates, 1982)]. Therefore, these Australian herbs are unlikely to show the high genetic differentiation typical of selfing species that have been observed in other floras (Hamrick & Godt, 1989). Observations of weak genetic differentiation in Australian herbs suggest that these species may have broader geographic scales of pollen and seed dispersal than has been observed elsewhere. Alternatively, our findings may reflect the contraction of these species from larger and more continuous populations in the more recent past. This may be a productive area of further research because we may be underestimating the pollen dispersal capacity of insects in Australian systems as high dispersal has been observed in some studies on trees and shrubs (e.g. Byrne *et al.*, 2008a; Millar *et al.*, 2011, 2014).

Our results also showed high levels of genetic differentiation for allozyme studies of Australian trees (0.18), which were on average double the estimates for trees in global studies (0.08; Hamrick & Godt, 1996). This result confirms previous observations comparing Australian trees to conifers and Northern Hemisphere wind-pollinated temperate/boreal angiosperms (Moran & Hopper, 1987; Moran, 1992). This may be due to the high prevalence of animal and particularly insect pollination in Australian trees compared to the dominance of wind pollination in temperate/boreal Northern Hemisphere trees. We did note one extreme outlier in the tree dataset, the highly localized *Eucalyptus caesia* $G_{ST} = 0.60$, that may have a strong effect on our mean value for trees. However, even when this outlier was removed, the level of differentiation was still high (mean for trees with *E. caesia* = 0.18; mean for trees without *E. caesia* = 0.16). Moran & Hopper (1987) noted the same trend when just widespread Australian trees are compared with Northern Hemisphere trees and suggest that in addition to pollination syndrome, this difference could also be due to a more patchy distribution of widespread Australian trees due to their greater edaphic specialization.

CONCLUSIONS

We show that aggregating population genetic data across many studies can provide important insights into the associations between species attributes, using an extremely broad and diverse sample of the Australian flora, and the level and structuring of population genetic diversity in these species. The plant attributes that had the greatest influence on genetic diversity across this sample of the Australian flora were range size, growth form, species abundance and biome. The best predictors of population genetic differentiation were range disjunctions and abundance. Most of these findings were consistent with global observations, based largely on Northern Hemisphere or Neotropical floras, providing further evidence for the robustness of our understanding of genetic diversity and differentiation in plant species. However, we found some notable differences with global trends, which highlights that caution is needed when extrapolating trends from global analysis to regional floras. The unexpected lower levels of genetic diversity in Australian shrubs compared to trees and herbs appear to be a result of confounding effects of distribution that would need to be considered in general application of broad predictions. We also noted an unexpected difference in the levels of genetic diversity in eastern Australian species compared to those from western and tropical biomes that appears associated with effects of species distribution in these plants, and demonstrates the strength of the influences of variables despite different environments. For genetic differentiation, we identified a notable impact of range disjunction. This relationship has rarely been evaluated in previous studies of this kind, and our analysis suggests range disjunction merits more attention as a possible driver of differentiation in global studies.

Our study has identified general associations between the attributes of Australian plant species and the level and structuring of genetic diversity, affirming the observations of previous studies of different regions. This is important because these associations provide simple and cost-effective surrogates for predicting population genetic diversity and differentiation, although not necessarily adaptive variation, where this information is not readily available. Such predictions assist in the inclusion of a genetic component into decision-making approaches and will assist in the development of rapid and cost-effective frameworks for the conservation and management of the Australian flora.

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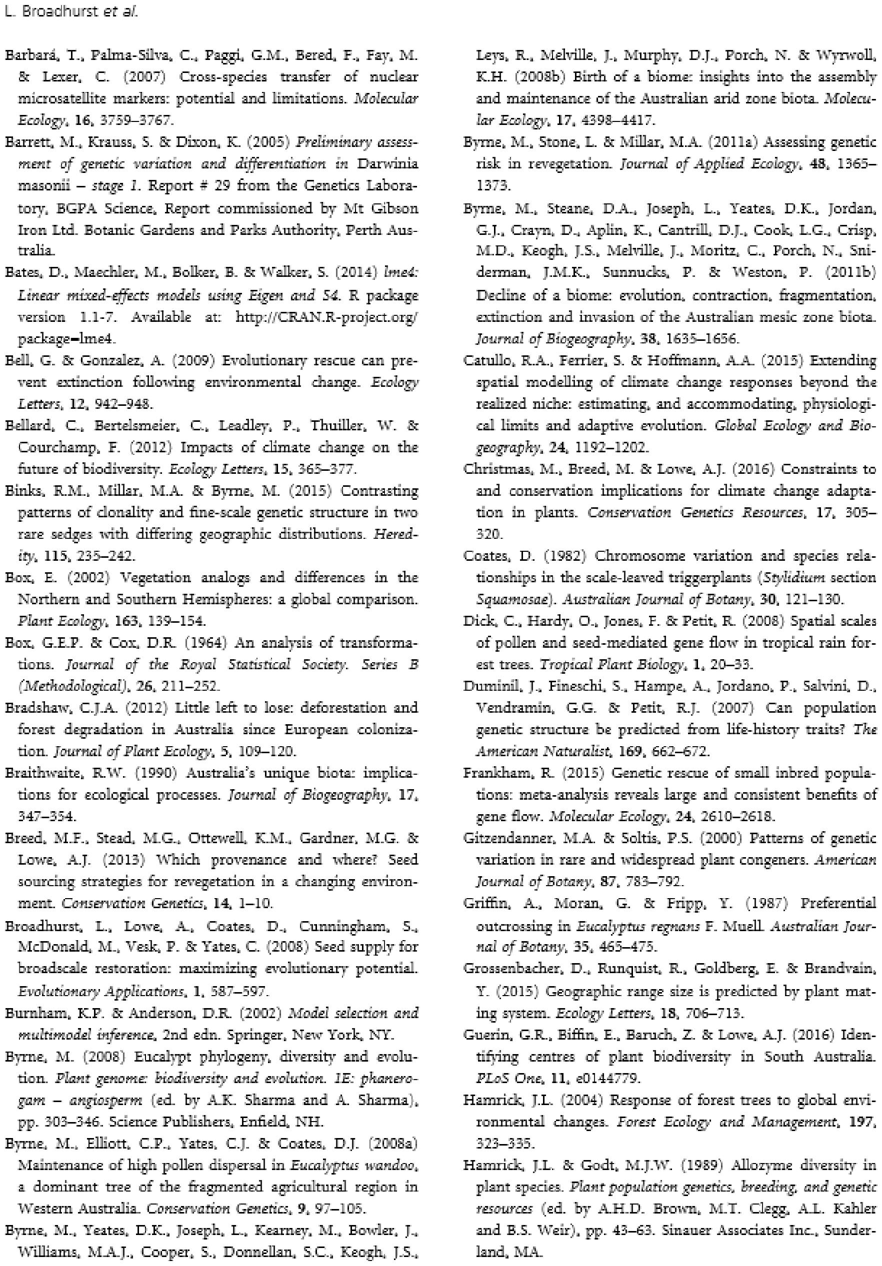

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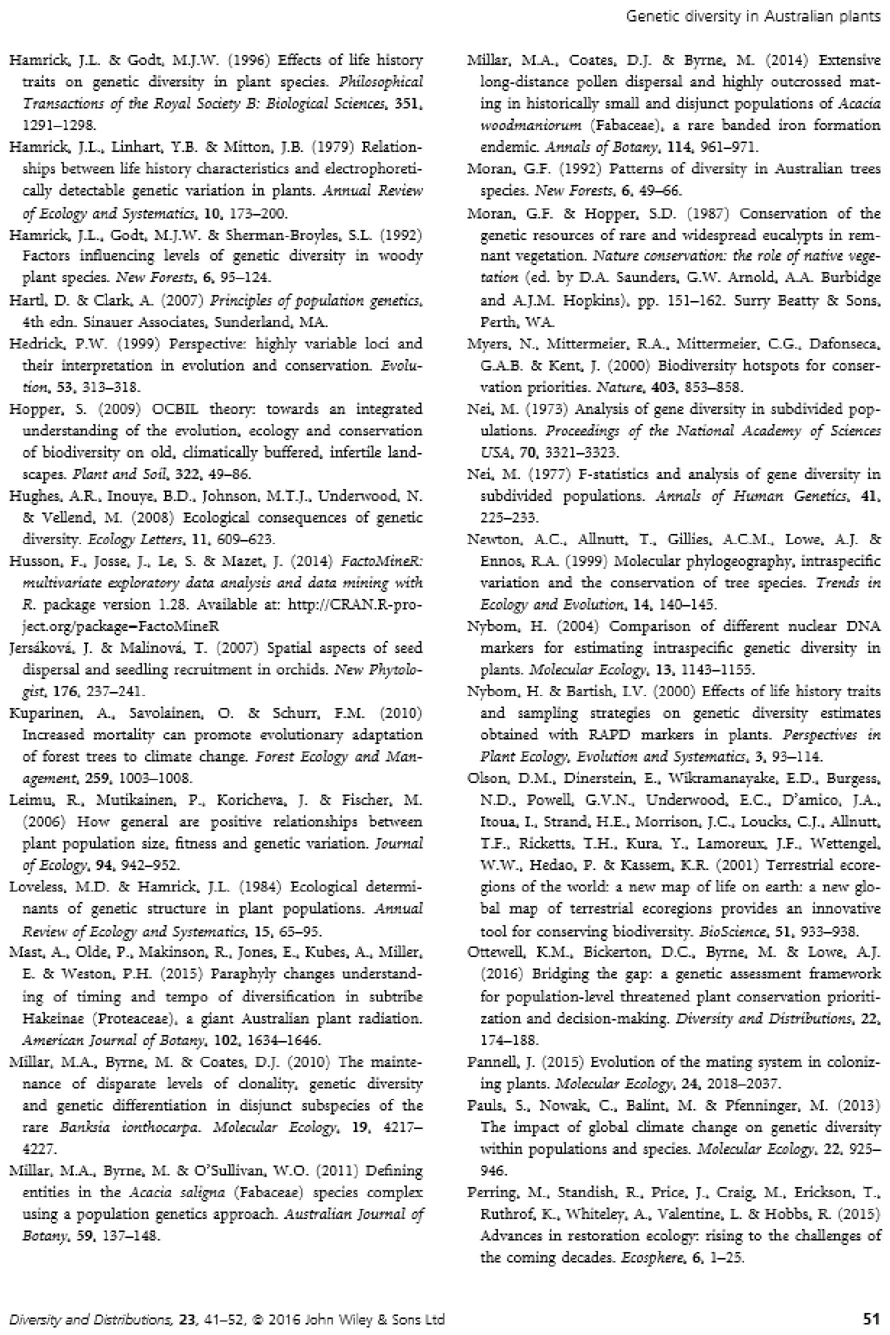

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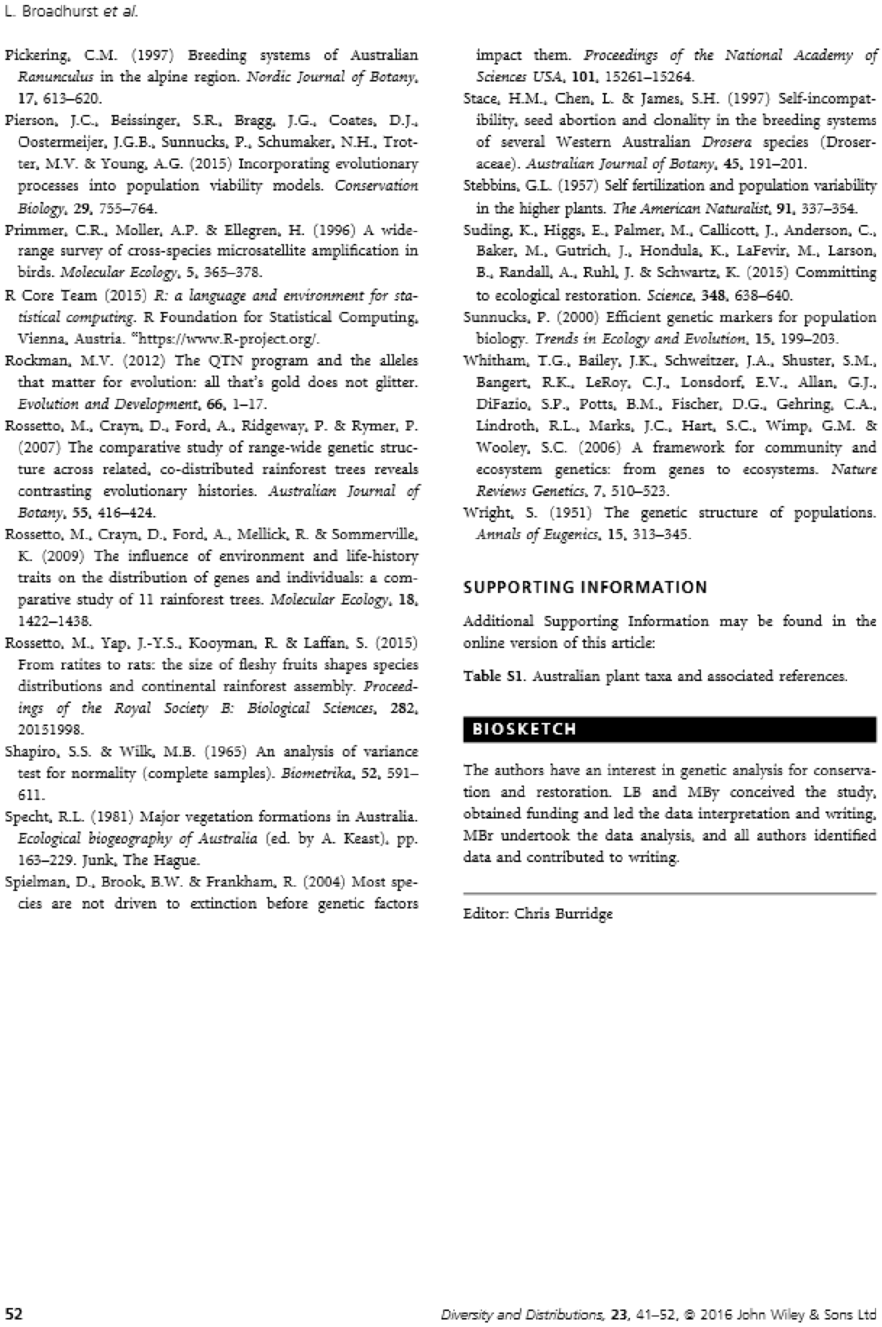

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1. Australian plant taxa and associated references.

BIOSKETCH

The authors have an interest in genetic analysis for conservation and restoration. LB and MBy conceived the study, obtained funding and led the data interpretation and writing, MBr undertook the data analysis, and all authors identified data and contributed to writing.

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