Quantifying responses of ecological communities to bioclimatic gradients

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

Doctor of Philosophy

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Contents

Abstract

The biotic change along environmental gradients has been the subject of study for well over a century, forming one of the first tools to understand how environment shapes the species and ecosystems that occur. However, gradient studies have historically relied on limited observations on a single transect, limiting their inductive power. Here, I investigate how this limitation can be addressed. I present case studies to illustrate how next-generation transect studies can integrate observations from a wider range of observations of phenotypes, species and communities; together with observations from multiple taxa and gradients. Leaf carbon isotope data from bioclimatic gradients in China, South Australia and Western Australia are integrated to demonstrate a variety of species- and community-level responses to water availability, providing evidence against the previously asserted claim of a simple and universal response. Vegetation data from the same gradient is surveyed with two separate survey methodologies are co-analysed to demonstrate climate is the primary regional determinant of vegetation structure and composition in South Australia, while topographic and edaphic variables are important at a local scale. I find no evidence of ecological disjunctions that may indicate a threshold of vegetation change associated with climate shifts. Comparison of plant and ant species turnover on a spatial gradient suggested that ant communities are ca. 7.5 times more sensitive than plant assemblages to spatial change, providing evidence that future climate change may force community reorganisation and a decoupling of these two taxa, potentially disrupting important interactions and ecosystem function. Well-designed transect studies have the potential to help resolve long-standing questions around the modes of species adaptation to change, as well as improving our understanding of how climate change will shape ecosystems in to the future.

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.

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Finally, if there are any errors in this thesis, as surely there must be, they are probably the fault of Andy Lowe, as he was my primary supervisor and really should have trained me better.

I dedicate this thesis to my father, Bill Retalic, who died shortly before I submitted this thesis. His mastery of logic and rhetoric, and his strong sense of social justice continue to be a source of inspiration and fond memories.

Chapter 1: Introduction

Climate change is impacting global biodiversity through changing selection pressures, species migration, and local extirpation, compounding other anthropogenic stressors including habitat fragmentation and pollution (Aitken et al. 2008; Parmesan and Yohe 2003). These anthropogenic forces are increasing and are likely to lead to widespread ecosystem transformation and mass extinction (Urban 2015). Consequently, there is a strong incentive to accurately predict how species and ecosystems will respond to climate change (Sutherland 2006; Urban et al. 2016). Substantial attention has been devoted to this issue over the last two decades, resulting in a profusion of research outputs focused on modelling predicted biotic changes with climate and detailing the changes that have already occurred (Pecl et al. 2017; Urban et al. 2016). Given the poor prognosis for many of the world's species and ecosystems due to the threats of climate change (Burrows et al. 2011; Hoegh-Guldberg and Bruno 2010; Urban 2015), there is a need to move beyond describing biodiversity decline. In this thesis, I address the pressing issue of how we can mobilise knowledge of climate change impacts gained from measuring biotic change on spatial environmental gradients to improve the ongoing monitoring and management of susceptible species and ecosystems. Linking biotic change to spatial environmental gradients is achieved through the establishment of a linear network of reference sites aligned with a known gradient (a "transect"). I provide case studies of trait and species/community level change on the same environmental gradient in South Australia to describe how the biota respond to this gradient, make predictions about how these systems may change in the future, and make recommendations regarding future work (Figure 1).

Ecological forecasting is an iterative process and requires making biodiversity observations to create a baseline, against which predictions of the direction and magnitude of change can be made. This process relies on ensuring the baseline is appropriate, as well as possessing an understanding of the environmental drivers and mechanisms of biodiversity change (Luo et al. 2011; Oliver and Roy 2015). While we may have a concept of ecosystems as being temporally stable, this is largely a matter of time scale – ecosystems are dynamic and change over time, particularly in response to large-magnitude extrinsic change (e.g. large climate events; invasions, etc.) and potentially even in relatively stable environmental conditions. Indeed, the lack of temporal stability in ecosystems means that ecosystem change in response to a new stressor is not likely to occur from a "standing start", but rather build upon the instability brought about by previous ecological perturbation leading to ecological change of a greater magnitude (Perry et al. 2014). The temporal instability of ecosystems somewhat undermines the credibility of a "pre-disturbance baseline", but in practice, we are only able to pick a reference point in time and measure change against it (Foley et al. 2017).

Once a baseline has been established, we can then monitor to measure the magnitude and nature of biotic change. Models of ecological change with environmental change can be established, and ongoing monitoring used to validate those predictions. Ideally, the accuracy of predictions will be increased over time through continual evaluation, improvement of mechanistic models and iterative input of data (Dietze et al. 2018; Urban et al. 2016).

Change in ecosystems driven by environmental change can occur at three levels of biological organisation: a) phenotypic change within species (adaptation, plasticity or behaviour), b) species and community change (migration or extirpation of individual species; altered assemblages); and c) ecological transformation (substantial alteration of physical structure or the replacement of one ecosystem type with another) (Walther et al. 2002). Environmental variables are likely to have some impact at each of these scales. For example, a small temperature increase within an ecosystem could result in a phenotypic change in one species and the emigration or extirpation of a second species. If the second species was a dominant or keystone species (e.g. a canopy tree or key herbivore), the loss of that species could result in ecological transformation. In the following paragraphs, I outline in turn the motivation for considering phenotypic, and species and community change, and how they interact to lead to functional and/or transformative change in ecosystems.

Intra-specific or phenotypic change

The ability of species to adapt to environmental change through their inherent capacity for genetic and phenotypic change is a fundamental facet of evolutionary processes (Jump et al. 2009; Pauls et al. 2013). Aside from shifting to a more favourable environment (migration), there are several mechanisms through which new phenotypes can arise to cope with new conditions (Christmas et al. 2015). For plants, the primary mechanisms are: a) adaptation, through which natural selection improves the fitness of a population by providing a selection pressure for the best-suited genotype for an environment (Hoffmann and Sgrò 2011); b) phenotypic plasticity, under which organisms change phenotype without genetic modification (typically through epigenetic means) (Nicotra et al. 2010); and c) phenological change, through which plants modify the timing of key life cycle phases (e.g. flowering time) (Körner and Basler 2010). Fauna may also be able to respond through behavioural change (e.g. a shift from diurnal to nocturnal activity, or a change from a declining prey species to one with greater abundance). Change within species may result in a more advantageous phenotype, neutral variation, or deleterious effects (e.g. inbreeding depression) (Chown et al. 2010).

Changes in plant physical traits including leaf morphology (Hopkins et al. 2008), wood density (Swenson and Enquist 2007), and reproductive strategy (Pellissier et al. 2010) have been assumed to be environmentally driven, but demonstrating a clear mechanistic link has been difficult. Similarly, genetic clines paralleling environmental gradients have been detected in several taxa, including plants (Christmas et al. 2017), insects (Hoffmann and

Weeks 2007), fish (Chlaida et al. 2009) and mammals (Mullen and Hoekstra 2008), which are assumed to be adaptive. However, genetic clines alone are limited in their capacity to explain environmentally driven change because changes in allele frequency may be related to geographic rather than environmental isolation (Warren et al. 2014), and if they are environmentally driven, may involve multiple genes acting in concert to confer a phenotypic advantage. For these reasons, where a clear causative mechanism for trait change is not known, it is important to combine genetic studies with studies of phenotypic trait change in order to demonstrate that a trait is environmentally sensitive.

A popular trait in investigating plant adaptation to environment is the leaf carbon isotope ratio ($\delta^{13}C$), because it represents an integrated measure of water use efficiency in C_3 plants and is readily measured (Cernusak et al. 2013; Farquhar et al. 1989). Leaf $\delta^{13}C$ can be measured in any plant with leaves, making it possible to compare values across many species and growth forms. In Chapter 4, I investigate the utility of this trait in understanding biotic response to environment on three spatial gradients. The environmental drivers of leaf $\delta^{13}C$ are well understood (Cernusak et al. 2013; Farquhar et al. 1989), and it is possible to make a priori predictions on how changed environmental conditions will affect leaf $\delta^{13}C$. The expectation of this study is that leaf $\delta^{13}C$ will become more positive with increasing aridity (Kohn 2010), but testing multiple species, growth forms and gradients allows the ubiquity of this relationship to be tested. A similar response across all tested species irrespective of growth forms and location would imply a simple carbon discrimination response to aridity irrespective of individual species traits. Conversely, disparate responses may indicate that other environmental variables and morphological traits have a strong impact on carbon isotope discrimination. Resolving this question would help resolve whether all plants display similar phenotypic responses to aridity, or whether there are multiple successful response pathways.

Species and community level change

The assembly of species into ecological communities is central to understanding the spatial patterns of diversity across landscapes, and how they may change over time. The pressing issue of adapting to global environmental change requires a detailed understanding of how the environment shapes the current diversity within ecological communities. In doing so, it is important to consider the type of change, which taxa to focus on if not everything can be surveyed, and how taxonomically different assemblages (such as ants and plants) may respond to the common environmental pressures across space and time. I will outline each of these issue in turn and how they will be tackled in this study.

By monitoring the presence, absence and/or abundance of species, it is possible to detect the decline or migration of species in response to climate change. Changes in species abundances are of interest in their own right, but species each form a part of a network, so declines in “keystone” species that provide important services or regulate other species are of particular concern (Gilman et al. 2010; Griffith et al. 2017; Mills et al. 1993). Vegetation is probably the most common (and easiest) group on which to monitor compositional change, as vegetation provides food and habitat for many other taxa, and for dominant species. The ready and increasing availability of high resolution aerial imagery also provides opportunities for expensive and labour intensive on-ground monitoring to be at least partly replaced with cheaper remote sensing. In Chapters 5 and 6, I use abundance data for vascular plants to represent a biotic gradient, which I correlate to an environmental gradient.

Community composition of indicator groups that are thought to be proxies for broader ecological function, are also common monitoring targets, including birds (Furness and Greenwood 2013), invertebrates (particularly ants) (Andersen et al. 2004), and other taxa

(Lindenmayer et al. 2012a). The utility of indicator taxa as representative of broader ecosystem composition and/or function has been criticised, often on the basis of quantitative evidence that one group is unlikely to be always representative of another (Carignan and Villard 2002). Nevertheless, of the many taxa that have been put forward as representative indicators, the case for ants is particularly strong. Ants are cosmopolitan, intimately connected to both the soil and vegetation, and phenotypically diverse, making their composition and abundance sensitive to disturbance and environmental change (Andersen and Majer 2004; Majer et al. 2007). In Chapter 6, I combine vegetation and ant responses to test the comparative sensitivity of these two commonly-investigated groups, as well as project their likely congruence under future climate change.

Methodologies for ecological forecasting

Knowledge of how ecosystems were structured and functioned in the past compared with the current ecosystems is an obvious starting point to understand how ecosystems are likely to change in the future (Williams and Jackson 2007). However, while historic data sets can be informative, long-term data of sufficient resolution is not available for most regions (Lindenmayer et al. 2012b). Perhaps more importantly, a steady increase in anthropogenic impacts (Ellis et al. 2010), particularly through modification of the global climate (Williams et al. 2007), means that the past may not be a good model for the future behaviour of many ecosystems (Fordham et al. 2016; Veloz et al. 2012; Williams and Jackson 2007). In order to be able to responsibly utilise past environmental and biodiversity data as an indication of the future, a clear mechanistic model must be presented (Warren et al. 2014).

Contemporary environmental gradients, in which biodiversity changes in tandem with one or more environmental variables, is an efficient method to associate abiotic change with biotic response (Blois et al. 2013a; Parker et al. 2011). This approach has grown from the gradsect survey technique, in which surveys are conducted along one or more environmental gradients in order to maximise species detection while minimising survey effort (Austin and Heyligers 1991). Demonstrating a correlative link between environment and biotic response is reasonably straightforward, but replication or complimentary experimentation is required to establish a causative link (Caddy-Retalic et al. 2017; Warren et al. 2014).

Understanding how ecological change occurs across spatial gradients can give a good insight into both the individual and combined environmental drivers of biodiversity change, as well as the magnitude of response that may be expected by a change in one or more drivers (Blois et al. 2013a; Jennings and Harris 2017). This approach of using space as a proxy for time has the benefit of being based on direct observations, rather than modelled data, and allows intraspecific through to biome-level observations to be collected. Space-time substitutions are further discussed in Chapter 7, in which the sensitivity of plant and ant groups on the TREND are assessed for sensitivity to spatial environmental change, and potential future climate change, is assessed.

Ecological thresholds

Ecological thresholds have been defined in many ways (see Huggett (2005) and Bestelmeyer (2006)), but can essentially be regarded as points or regions on environmental gradients at which the rate of change in a biotic variable (e.g. trait change, species turnover, physical structure, etc.) is markedly greater than for the gradient as a whole (Figure 2). Examples include the estuarine zone separating marine and freshwater ecosystems; and the alpine “tree line” on mountains which delineates forested slopes and barren peaks.

Ecological thresholds are an attractive concept for ecologists and ecosystem managers because they provide an explanation for rapid transitions in ecosystems, as well as information to support the understanding of biodiversity to changing environmental conditions. Together, this improved knowledge base facilitates the development of a management framework to conserve or promote desirable ecosystem attributes (e.g. temperature must be limited below a value of x in order to avoid large scale coral deaths).

Thesis aims

In this thesis, I aim to investigate the utility of spatial bioclimatic gradients in understanding the environmental drivers of within-species, species and community level biotic change. Each chapter has its own specific aims:

- Demonstrate how single bioclimatic gradient studies can be augmented to improve their power and utility in creating generalizable biodiversity change models (Chapter 2);
- Determine the variability in leaf carbon isotope response to aridity and test for generalizable patterns between species and growth forms on three replicated bioclimatic gradients (Chapter 4);
- Describe the change in vegetation composition and structure on a mediterranean-arid zone gradient, test for the presence of ecological thresholds at which rapid change occurs, and determine the degree to which survey methodology impacts on our ability to describe vegetation change for this region (Chapter 5); and
- Quantify the relative sensitivity of plant and ant assemblages to environmental change and determine the likely ecological responses of plant and ant assemblages to future climate change (Chapter 6).

Over the entire thesis, attempt to I address the pressing issue of how we can improve our knowledge of climate change impacts gained through spatial gradient studies to gain insight in to the likely trajectories of species and ecological communities under climate change, and how this knowledge can be mobilised to promote the ongoing monitoring and management of species and ecosystems.

Thesis structure

This thesis comprises seven chapters (Figure 1).

In Chapter 1 (this chapter), I outline the structure of the thesis and give a brief overview of the context of gradient research in ecology. Additional conceptual material has been integrated in to Chapter 2.

In Chapter 2, I present an overview of how bioclimatic gradients have traditionally been used. I undertake a critical evaluation of the power and limitations of bioclimatic transects as platforms for elucidating the drivers and mechanisms of biodiversity change. I explain how studies based on environmental gradients can be structured to overcome potential design weaknesses, particularly through addressing biodiversity change at multiple scales (intra-species, species, and community level change), as well as replication of transects and study taxa. I also provide further information on the Australian Transect Network, whose infrastructure has been used in these studies. Chapter 2 also includes a glossary which defines many of the main terms and concepts used throughout the thesis.

In Chapter 3, I introduce the TREND, an 800-kilometre aridity gradient spanning the transition from the Mediterranean to arid zones of South Australia, in order to provide an overview of the gradient that I use in this thesis. I provide a background of the development of the TREND through its establishment to its adoption as a part of the Australian Transect

Network, a group of analogous subcontinental-scale bioclimatic transects used as infrastructure on which to explore biodiversity change with climate.

Chapter 4 investigates intra-species change using analogous gradients to test whether the magnitude of trait-change is similar for different species and the gradients as a whole. I present a case study of change in leaf carbon isotope ratio (an integrated measure of water use efficiency) measured on three bioclimatic transects in Australia and a further transect in China to explore differences in trait variation in species on replicated transects. I also test the "universal scaling hypothesis", a theory posited following a previous gradient study in China suggesting that carbon isotope responses to aridity would function identically at both the species and community scale.

In Chapter 5, I investigate species and structural change in a comparative study of vegetation structure and species change on the TREND. This analysis shows that the vegetation on the Adelaide Geosyncline changes monotonically, and finds little support for any disjunctions of higher than expected species or structural change.

Having explored within-species change (Chapter 4) and species level change (Chapter 5), in Chapter 6, I compare community level responses in plants and ants on the same transect. I further predict the disruptive pressure of future climate change on ant and plant communities as an example of investigating multiple taxa on the same gradient. I find that plant and ant species composition is likely to be affected by future climate change, with ants likely to be particularly sensitive, but these species are unlikely to migrate in tandem. The asymmetrical responses of vegetation and ants (in both trajectory and magnitude) implies that these communities will become somewhat decoupled in the future, potentially leading to a loss of important ecological relationships and function.

Finally, Chapter 7 (Discussion and Conclusions) critically evaluates each chapter and provides a cross-cutting synthesis of the work presented here as a whole. I also provide suggestions for future research, with particular emphasis on future work that could be supported by the Australian Transect Network.

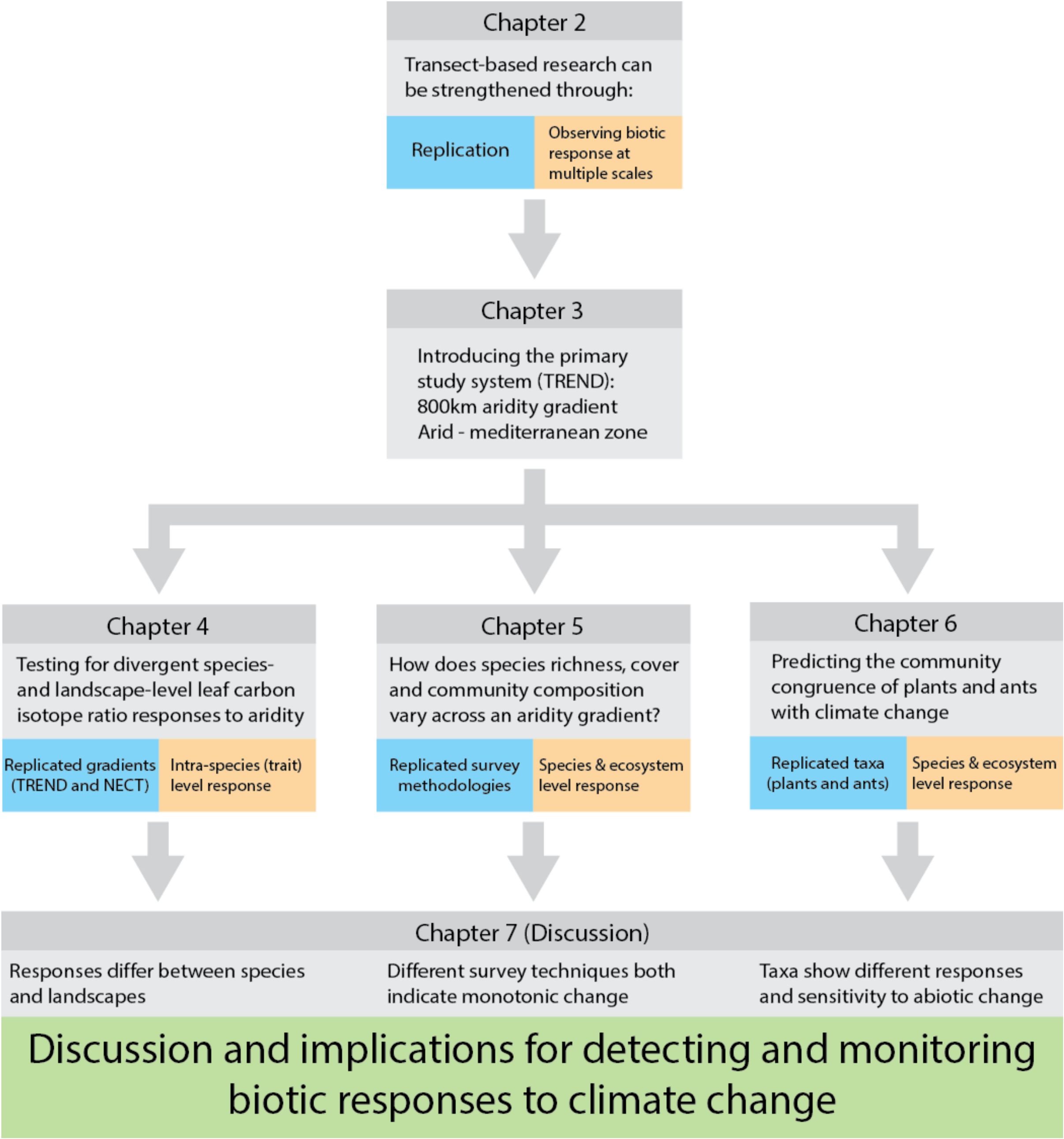

Figure 1: Chapter structure for this thesis. TREND = TRansect for ENvironmental monitoring and Decision making (the primary study platform used in each of the following chapters), NECT = North East China Transect.

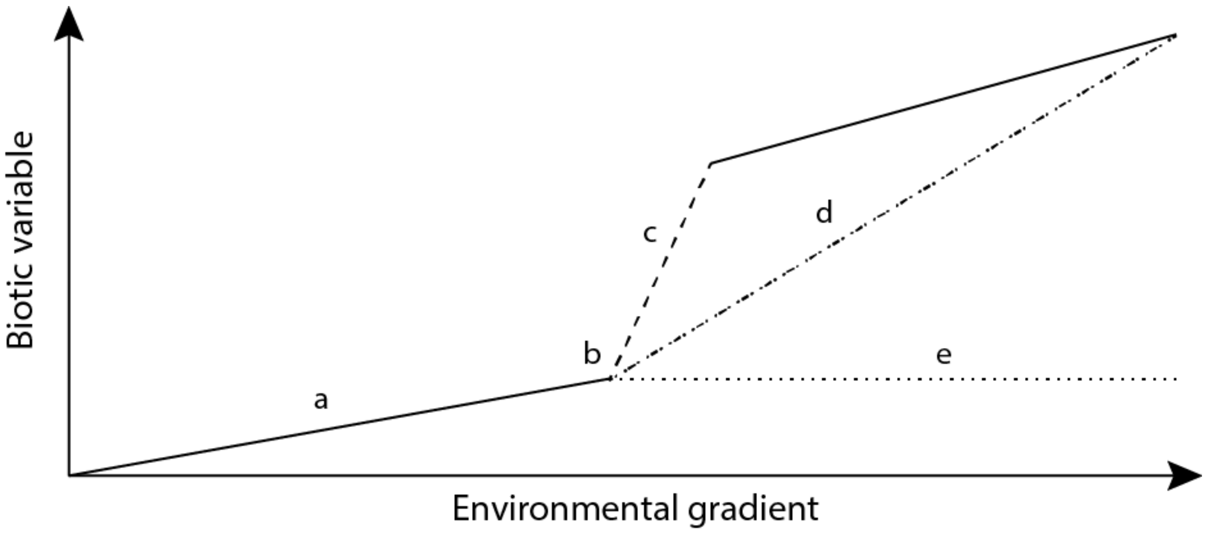

Figure 2: Linear biotic change in response to an environmental gradient (a) continues until a threshold point (b) or region (c) is reached. Once the threshold point is exceeded, the change becomes non-linear and may increase in magnitude (d) or reach saturation (e), with no further biotic response. Similar responses may occur following a threshold region, as well as a return to the previous rate of change (solid line). Biota on a gradient may display several thresholds of varying magnitudes.

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Chapter 2: Bioclimatic transect networks: Powerful observatories of ecological change

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Overall percentage (%)	70		
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Bioclimatic transect networks: Powerful observatories of ecological change

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Abstract

Transects that traverse substantial climate gradients are important tools for climate change research and allow questions on the extent to which phenotypic variation associates with climate, the link between climate and species distributions, and variation in sensitivity to climate change among biomes to be addressed. However, the potential limitations of individual transect studies have recently been highlighted. Here, we argue that replicating and networking transects, along with the introduction of experimental treatments, addresses these concerns. Transect networks provide cost-effective and robust insights into ecological and evolutionary adaptation and improve forecasting of ecosystem change. We draw on the experience and research facilitated by the Australian Transect Network to demonstrate our case, with examples, to clarify how population- and community-level studies can be integrated with observations from multiple transects, manipulative experiments, genomics, and ecological modeling to gain novel insights into how species and systems respond to climate change. This integration can provide a spatiotemporal understanding of past and future climate-induced changes, which will inform effective management actions for promoting biodiversity resilience.

KEYWORDS
change detection, community turnover, ecological forecasting, environmental gradients, spatial analogues, transect replication

1 | BIOCLIMATIC TRANSECTS

Understanding the adaptive potential of species and resilience of communities is vital for effective conservation management in the face of climate change. A particular challenge is scaling up knowledge from detailed local studies to understand ecological dynamics at regional scales. Large-scale transects that traverse major climate gradients have been recently highlighted as useful platforms for climate change research (de Frenne et al., 2013; Parker, Schile, Vasey, & Callaway, 2011).

Bioclimatic transects are a long-standing method for studying ecological change. By the early 20th century, it was understood that vegetation across Europe and North America responded to a longitudinal rainfall gradient and a latitudinal temperature gradient (Turner, Gardner, & O'Neill, 2001). Whittaker's (1956) classic study of vegetation change in the Smoky Mountains of the United States led to increased interest in environmentally driven biotic change, with a proliferation of large-scale transect studies since the late 1960s (Figure 1). Two decades later, a global series of subcontinental scale transects was established under the International Geosphere-Biosphere Program (IGBP)

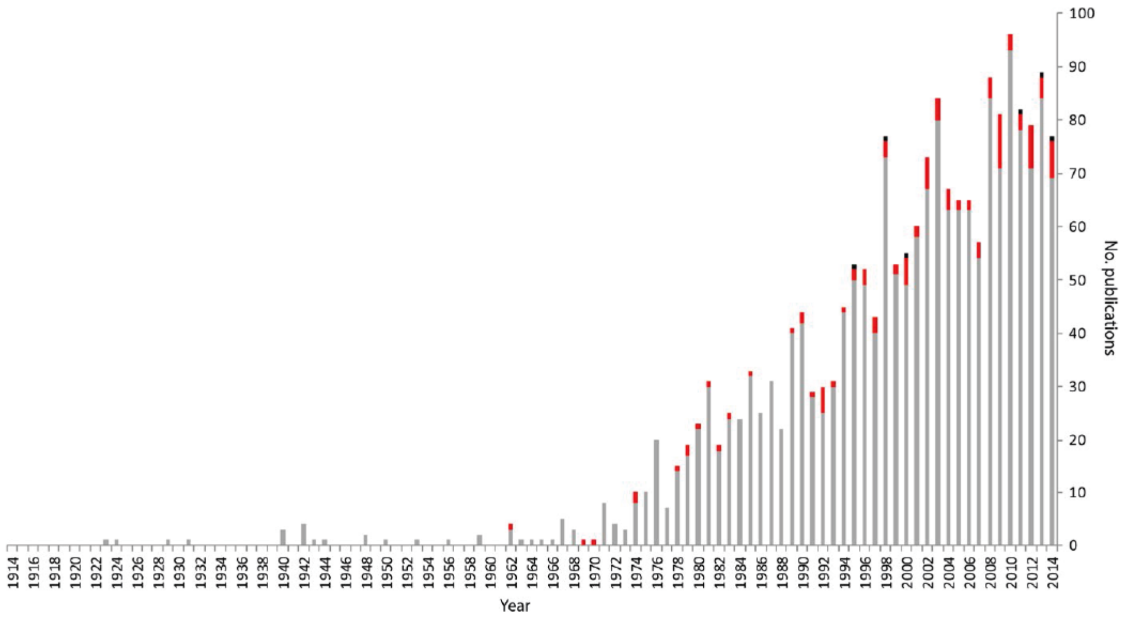

FIGURE 1 Results from a Web of Science search for peer-reviewed papers published between 1914 and 2014 containing "transect" in the title in the fields of "environmental science" and "ecology." Search was undertaken on 12 March 2016. Most studies used single large-scale transects (e.g., altitudinal or coastal gradients) or several small-scale transects (e.g., grids for counting birds) (gray bars). A small subset of studies used multiple or replicated transects (e.g., paired altitudinal transects) (red bars). Investigations that included manipulations (e.g., common gardens or translocations) were very rare (black bars)

to investigate how climate and land use drive change in ecosystems (Austin & Heyligers, 1991; Koch, Vitousek, Steffen, & Walker, 1995).

Since the IGBP was established, interest in exploring the impacts of climate change on species and ecosystems has resulted in many independent studies using spatial bioclimatic change as a proxy for temporal climate change (Blois, Williams, Fitzpatrick, Jackson, & Ferrier, 2013; Parker et al., 2011). Transects are attractive research platforms because they help stratify environmental variation, reducing the sampling (and therefore resources) required to describe variability (de Frenne et al., 2013), and minimizing confounding factors. They therefore represent a cost-effective approach for linking biodiversity patterns to environmental drivers in ecosystems (Box 1).

Transects can be used to examine variation at multiple biological scales, from functional traits and genes within species, to ecosystem turnover, thus providing insights into the relationships between abiotic variables and the adaptive limits of species and communities. Such studies clarify patterns and processes of micro- and macro-evolution, as well as processes that facilitate species persistence and ecosystem resilience, particularly in relation to climate change. Consequently bioclimatic transect research addresses the following fundamental questions:

1. To what extent is phenotypic variation linked to climate, and how much is variation determined by genetics vs. plasticity?
2. What climatic thresholds limit the distribution of species and communities?
3. How do responses to climate change vary among biomes?

Although bioclimatic transects allow for efficient sampling of species and community change across environmental variation, they also have significant limitations (Metz & Tielbörger, 2016; Warren, Cardillo, Rosauer, & Bolnick, 2014). Many environmental variables (e.g., temperature and rainfall) may covary along single transects and so the true driver of biotic change may be difficult to discern (Meirmans, 2015). In addition, species distributions are likely to be strongly influenced by historical factors and not determined solely by contemporary environmental conditions, so current distribution can sometimes be a poor basis for predicting future change (Warren et al., 2014). Results from experiments can be strikingly different from those based on observations over environmental gradients (Metz & Tielbörger, 2016). Thus, caution is required when making predictions based only on contemporary spatial patterning.

Building networks of replicated transects with embedded experiments can address these limitations and help underpin the development of generalized models of how climate affects biodiversity at gene, species, community, and ecosystem levels. In this study, we draw on research facilitated by the Australian Transect Network (ATN; Figure 2; Box 2), a facility of Australia's Terrestrial Ecosystem Research Network, to describe how a network of transect-based research, augmented by embedded experiments, can overcome the weaknesses of individual transect studies to provide cost-effective insights into ecological and evolutionary adaptation associated with climate change at the continental scale. Akin to other global networks (e.g., the Pacific-Asia Biodiversity Transect Network (Mueller-Dombois & Daehler, 2005)), the ATN has developed a network of bioclimatic transects that cover Australia's major biomes. The ATN straddles most of Australia's climate space and captures the diversity of biomes across the continent. Thus, developing an Australian transect network provides insights that are directly relevant to understanding climate change impacts at multiple scales and provides a framework which could be replicated by other

Box 1 Defining Transects

The term "transect" is used in a broad sense to mean a path (usually linear) through an area along which data are collected. Data collection could include species presence and abundance (e.g., for biodiversity surveys), phenotypic traits, tissue for genetic analysis (e.g., for assessing population genetic structure), and environmental variables. Transects can be used at varying scales: Transects spanning just meters are used as a survey method for measuring vegetation structure within a plot (e.g., White et al., 2012); transects spanning profound environmental change, and potentially along hundreds of kilometers, are more commonly used to assess community composition and adaptive changes along environmental gradients on a large scale (Figure 3, and the focus of this article).

Gillison and Brewer (1985) proposed that positioning a transect to follow a significant environmental gradient was the most efficient method to capture habitat heterogeneity and maximize species detection in biodiversity surveys. This approach differed from traditional survey methods based on random, systematic, or simple stratified sampling (Smartt & Grainger, 1974). Systematic sampling is resource intensive, and Gillison and Brewer criticized randomized sampling as potentially counterproductive, as species' distributions are rarely random. Instead, they proposed that greatest biodiversity would be found in line with the most significant environmental gradient or gradients within a study area, in a nonrandom distribution. They termed these gradient-orientated transects "gradsects," which have remained a popular survey methodology (e.g., Austin & Heyligers, 1991; Parker et al., 2011).

Large-scale (subcontinental) transects follow some gradsect principles. They are placed along a major environmental (often climatic) gradient, site selection is based on logistical considerations (e.g., accessibility), and they follow sound experimental design with opportunities for replication and randomization within a transect. However, where gradsects were designed as a biodiversity survey tool, the goals of bioclimatic gradient studies are typically to assess biotic response to environmental change, and to interpret these results in the context of the gradient.

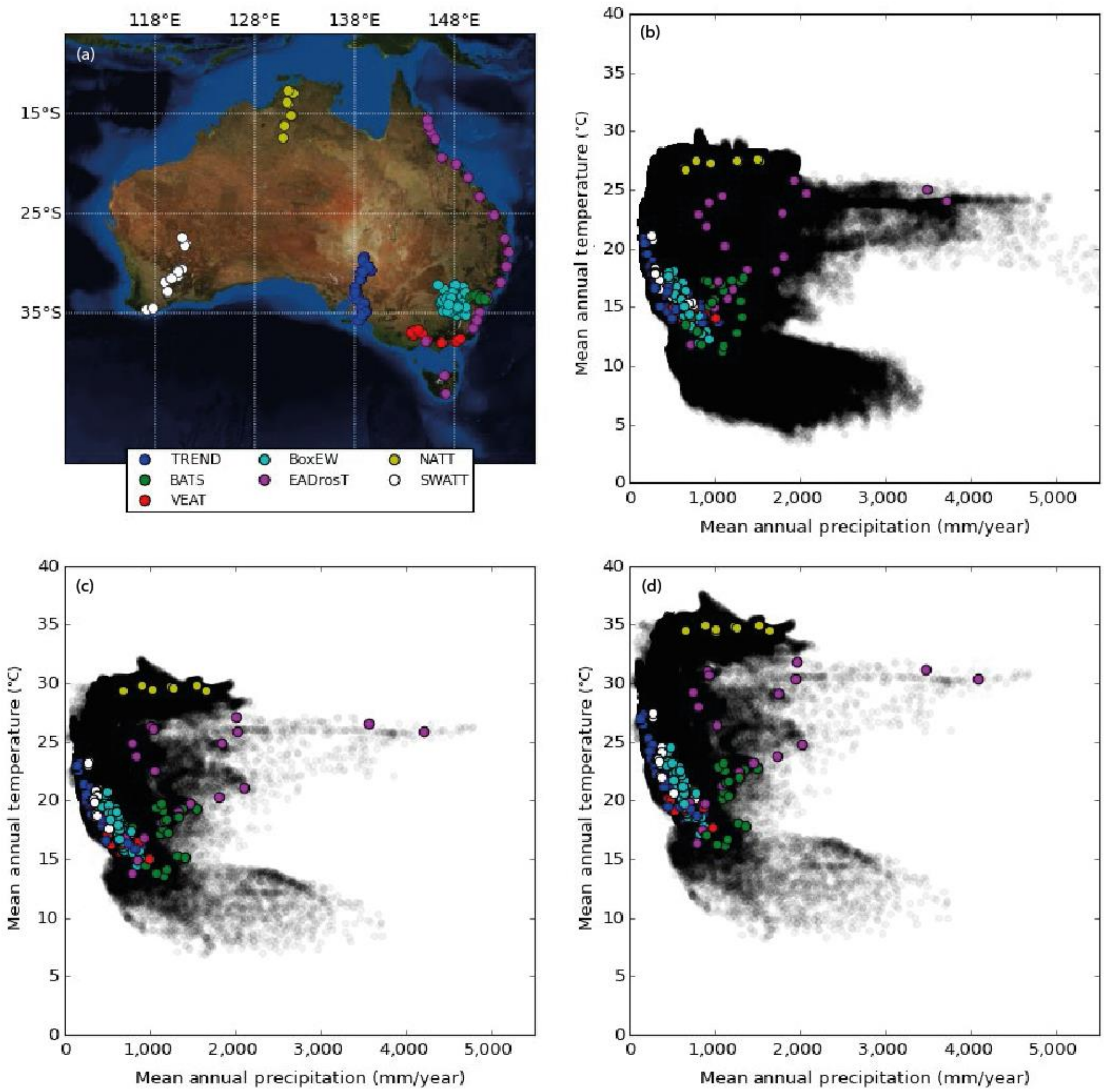

FIGURE 2 Spatial (a) and bioclimatic (b–d) context of Australian Transect Network sites against recent (1970–2005) and projected (2006–2050) climate space. (b) Recent (1970–2005) ANUClimate v 1.0, 0.01 degree climate data (Hutchinson, Kesteven, & Xu, 2014) mean annual temperature and mean annual precipitation for each site, and all of Australia (gray circles). (c) 2006–2050 ensemble mean of seven global climate models for the RCP4.5 scenario (stabilization of ~650 ppm atmospheric CO_2 equivalent (Thomson et al., 2011)). (d) 2006–2050 ensemble mean of seven global climate models for the RCP8.5 scenario (comparatively high greenhouse emissions (Riahi et al., 2011)). Models selected to be consistent with current Australian Government climate modeling (CSIRO and Bureau of Meteorology, 2015). Refer to Appendix 1 for details of climate models

countries wishing to understand the responses of species to changing climates.

Taking globally derived principles, demonstrated using specific case studies from the ATN, we highlight in the study the important insights that can be derived from transect research at both intraspecies (i.e., phenotype and genetic variation) and interspecies (i.e., community) levels, and the importance of combining these two levels. We also summarize key aspects of transect design to mitigate shortcomings of transect methods and highlight the future opportunities provided by such approaches through the application of genomics and modeling approaches. Finally, the continental scope of the ATN provides a model for the establishment of a globally informative network, incorporating variation across the world's major climate zones.

Box 2 Background of the Australian Transect Network (ATN)

The ATN was established as a facility within Australia's Terrestrial Ecosystem Research Network (TERN) with the aim of improving understanding of the climatic drivers and likely future of Australia's biodiversity. The ATN was formed through the development of new and existing transects across several of the major climate transitions in Australia (Figure 2).

ATN Transects with attributes

		Common metrics			
Transect	Gradient	Floristics	Focal species	Soil attributes	Indicator species
BATS [a]	170-km distance 634–1,330 mm MAR [h] 11.3–17.5°C MAT [i]	Yes	Yes	Yes	Yes
BoxEW [b]	290-km distance 451–930 mm MAR [h] 11.8–18.1°C MAT [i]	Yes	Yes	No	No
EADrosT [c]	3,500-km distance 724–3,719 mm MAR [h] 11.8–25.8°C MAT [i]	No	Yes	No	Yes
NATT [d]	800-km distance 640–1,535 mm MAR [h] 27.0–28.0°C MAT [i]	Yes	No	Yes	Yes
SWATT [e]	900-km distance 261–746 mm MAR [h] 15.2–21.2°C MAT [i]	Yes	Yes	Yes	No
TREND [f]	800-km distance 175–1,049 mm MAR [h] 13.5–20.9°C MAT [i]	Yes	Yes	Yes	Yes
VEAT [g]	500-km distance 491–1,018 mm MAR [h] 13.9–14.9°C MAT [i]	Yes	Yes	Yes	No

[a] Biodiversity and Adaptation Transect Sydney; [b]Box-gum transect East–West; [c]East Australian Drosophila Transect; [d]Northern Australian Tropical Transect; [e]Southwest Australian Transitional Transect; [f]TRansect for ENvironmental monitoring and Decision making; [g]Victorian Eucalyptus Adaptation Transect; [h]Mean Annual Rainfall (accumulated mm per year); [i]Mean Annual Temperature (min + max/2).

It is the vision of the ATN to standardize data collection across all transects to improve multi-transect analysis. The methodology published by White et al., (2012) is used to ensure data collection and analysis of soils, floristics and indicator species is standardized; with the intention of developing consistent genetic approaches for focal taxa in the future.

Testing how ecosystems respond to changing conditions is a classic example of transdisciplinary research, which involves researchers and the users of that research collaborating to improve on-ground conservation outcomes (Campbell et al., 2015). This approach is exemplified by the TREND (Figure 3), which was developed in partnership with the South Australian government's environment agency, and research was tailored to address management driven questions such as "what shifts in distribution, species composition and ecological characteristics can we expect?" (Caddy-Retalic, Guerin, Sweeney, & Lowe, 2014).

The ATN continues this approach, in part, to provide a platform through which the data and samples collected across several transects can support the ongoing science needs of environmental managers. High-level questions have been developed to shape the projects supported by the ATN:

1. To what extent can biodiversity be predicted on the basis of environmental variables?
2. Can thresholds be identified where there are abrupt changes in biodiversity?
3. How will ecosystems change in the fact of expected climatic shifts?

Given the dual theoretical and applied interest in answering these questions and potential of transect-based studies to address them, the development of a continental scale transect network is a powerful approach to understanding and predicting biodiversity change.

2 | INSIGHTS FROM TRANSECT STUDIES

As highlighted above, transect networks provide the opportunity to understand responses to climate variation on multiple scales. Here, we detail how the ATN has provided information at a variety of scales using case studies that illustrate ecological principles and research findings.

2.1 | Studying Climate Change Within Species

Discounting migration, populations have three main modes of climate change response: (1) plasticity, involving environmental phenotype alteration to increase fitness (Anderson & Gezon, 2015); (2) epigenetics, which improves fitness through the activation and/or deactivation of genes through generations (Heard & Martienssen, 2014); and (3) genetic adaptation, whereby phenotypes adapt over generations through shifts in allele frequencies resulting in improved fitness (Pauls, Nowak, Bálint, & Pfenninger, 2013). Distinguishing between the mechanism(s) underlying apparent responses to climate change (e.g., plastic vs. heritable changes) is often difficult, but is critical for predicting biotic responses to future climate change (Warren et al., 2014).

Identifying causal relationships requires mechanisms to explain relationships between environmental and phenotypic variation (e.g., variation in specific genes, gene expression changes, alteration of chemical pathways, etc.; Savolainen, Lascoux, & Merilä, 2013). Future climatic conditions will probably represent a novel combination of environmental variables; hence, a clear understanding of how changes in climate affect phenotypes is required in order to make predictions of biotic response to future change (Warren et al., 2014).

Phenotypic plasticity and adaptation are often observed as clines in traits that can be related to environmental gradients. For example, potential climatic control over leaf traits has been investigated in the sticky hop bush, Dodonaea viscosa (hereafter Dodonaea). Dodonaea exhibited clinal variation in leaf area, narrowing with increasing temperature and decreasing rainfall along the along the TRansect for ENvironmental monitoring and Decision making (TREND; Figures 2, 3) in South Australia (Guerin, Wen, & Lowe, 2012). A probable mechanism for this process has been proposed: Leaf narrowing in plants reduces surface area (reducing transpiration and limiting radiation loads), potentially increasing fitness under arid conditions (Guerin et al., 2012). A subsequent analysis of historical herbarium specimens revealed a similar temporal trend: a 40% decrease in leaf width over the last 127 years, with most change occurring since 1950 (Guerin & Lowe, 2013). Whether the phenotypic cline observed in Dodonaea is the result of plasticity or genetic adaptation has yet to be determined. However, genomic analysis of this species on the TREND identified 55 genetic variants that strongly associated with temperature and water availability, along with a further 38 genetic variants associated with the elevation of populations (Christmas, Biffin, Breed, & Lowe, 2016a). Many of the variable genes related to environmental stressor responses, such as drought response (Christmas et al., 2016a). These findings suggest that climate is a clear agent of selection pressure on Dodonaea populations along TREND and has resulted in local genetic adaptation.

On the same transect, analysis of flowering times of the wallflower orchid, Diuris orientis, from herbarium records over the last 100 years identified a shift toward earlier flowering, likely an avoidance response to increasingly arid summers associated with recent climate shifts across its natural range (Macgillivray, Hudson, & Lowe, 2010). A similar phenological change was observed along an altitudinal transect, indicating that ongoing phenological shifts are expected for this species (Macgillivray et al., 2010). These results are consistent with an adaptive response to climate change. The detection of the same trends in both spatial and

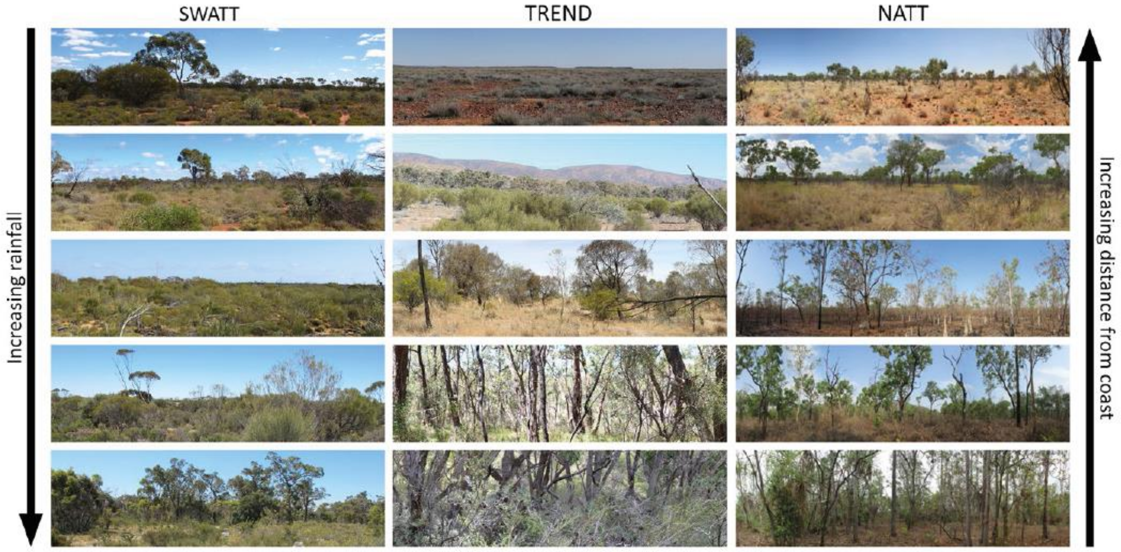

FIGURE 3 Environmental change across three subcontinental transects

temporal transects validates the relationship and provides a sound basis on which to seek confirmation through manipulative field and genomic studies.

Rates of adaptation, and thus adaptive potential with climate, are primarily driven be micro-evolution (i.e., changes in gene frequency) (Visser, 2008). Advances in observing micro-evolutionary processes of climate adaptation have been made through studying fruit flies (Drosophila) along the East Australian Drosophila Transect (EADrosT; Figure 2) (Hoffmann & Weeks, 2007; Rane, Rako, Kapun, Lee, & Hoffmann, 2015). Genetic differentiation among populations has been demonstrated in numerous traits by culturing flies under controlled conditions for multiple generations. Clear differentiation has also been demonstrated in chromosome inversions, specific genes, transposable elements, and maternally inherited bacteria (Hoffmann & Weeks, 2007; Levine, Eckert, & Begun, 2011; Rane et al., 2015). Many of these genetic changes have been shown to be adaptive. For example, cold temperatures led to selection on body size and winter egg retention, and geographic patterns in genetic changes were associated with climate adaptation. Indeed, shifts in gene and inversion clines through time have provided some of the first evidence of adaptive evolution under contemporary climate change (Umina, Weeks, Kearney, McKechnie, & Hoffmann, 2005).

2.2 | Studying climate responses within ecological communities

When species are pushed beyond their adaptive capacity, some species will be lost and others will shift in space, leading to localized changes in species composition (Figures 4, 5). Measures of species turnover along bioclimatic gradients can provide important insights into how

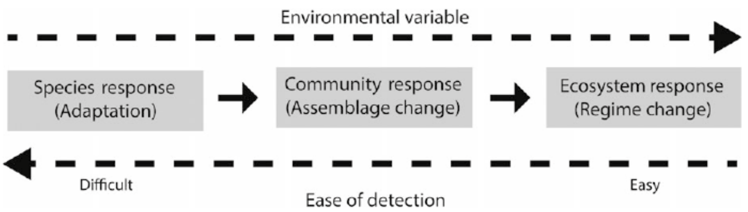

FIGURE 4 Schematic representation of the hierarchy of ecological change along an environmental gradient. Change progresses from sensitive (but difficult to detect) intraspecific changes in genes or traits (i.e., adaptation), through changes in species assemblage, generally requiring intensive field surveys, to profound (but more readily detectable) biome-level responses that can be detected using rapid surveys or remote sensing

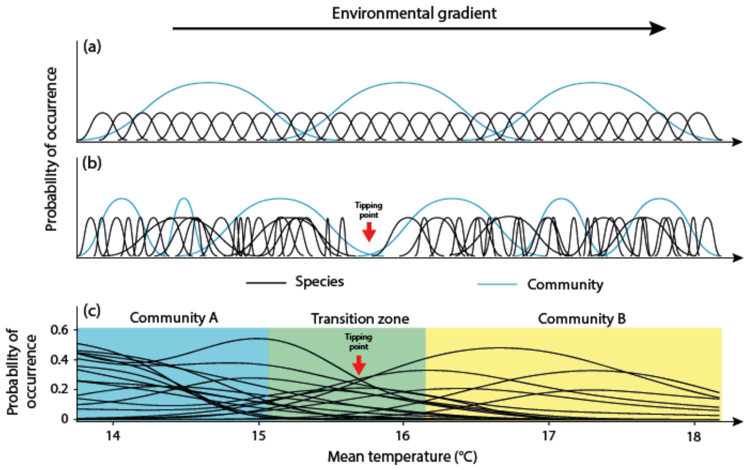

FIGURE 5 Turnover in species and communities on a hypothetical bioclimatic transect (a, b) and occurrence data from the TRansect for ENvironmental monitoring and Decision making (TREND) in South Australia (c). Regular species turnover would be expected if all species and communities had the same niche width and sensitivity along an even gradient (a). However, landscapes are likely to have a mix of generalist and specialist species with differing tolerances, genetic variation or niche widths, potentially displaying an uneven response between taxonomic and functional groups (b). Red arrows indicate a nonlinear ecological disjunction or "tipping point." Nonparametric distribution models for 19 common species on the TREND based on surveys of 3,567 field plots by the Biological Survey of South Australia (c). TREND data are provided by the South Australian Department of Environment, Water and Natural Resources, accessed 20 August 2010 (Guerin et al., 2013). Conceptual diagrams after Austin (1985)

different communities might respond to future climate change. For example, analysis of woody plants along the Northern Australian Tropical Transect (NATT; Figures 2, 3) revealed a systematic decline in species richness with declining rainfall (Bowman, 1996). In contrast, ant species richness was resilient to changes in rainfall, remaining uniformly high across the NATT (Andersen, del Toro, & Parr, 2015). Plant species richness on the South-West Australian Transitional Transect (SWATT) was positively correlated with rainfall, but beta diversity (spatial turnover) was consistently high at local and regional scales (Gibson, Prober, Meissner, & van Leeuwen, 2017), suggesting species turnover is at least partially driven by neutral processes such as dispersal limitation. Systematic plant community turnover has been observed along the TREND (Figure 5), with families characteristic of mesic ecosystems (e.g., Cyperaceae and Xanthorrhoeaceae) dominating at the temperate end, giving way to a greater prevalence of arid-adapted families (e.g., Amaranthaceae and Solanaceae) at the drier end (Guerin, Biffin, & Lowe, 2013). Plant community turnover on the SWATT was high and occurred through species replacement (rather than nestedness) across the transect at a local scale, irrespective of environmental factors (Gibson et al., 2017).

Bioclimatic transects are particularly useful for identifying climate-sensitive zones, where rapid, nonlinear ecological change occurs (Kreyling, Jentsch, & Beier, 2014) (Figure 5). For example, ant species composition along the NATT showed marked discontinuities between the arid and monsoonal zones in the south and between the semi-arid and mesic zones in the north (Andersen et al., 2015). Along the TREND, particularly rapid species turnover occurred in the range of 15–16 °C in mean annual temperature and mean annual rainfall of 400–600 mm (Guerin et al., 2013). A similarly abrupt transition from mesic eucalypt woodlands to arid Acacia woodlands was detected on the SWATT (Butt, Horwitz, & Mann, 1977). The identification of such climate sensitive zones and biomes is particularly important for conservation planning and prioritization. The transects used in all of these studies has facilitated the stratified collection of biotic and abiotic variables and therefore revealed not only spatiotemporal ecosystem changes, but also the mechanisms responsible for these changes.

Land use (grazing, cropping, reserves, etc.) and intensity can have a major impact on local ecology and can interact with climate to form synergistic effects, particularly if land use changes as a result of climate (Brodie, 2016; Sirami et al., 2016). Transect studies are also useful for investigating community responses to interacting climatic and land use variables. For example, plants at intermittently livestock-grazed sites across the Box-gum East-West Transect (BoxEW; Figure 2) were compositionally more similar to the dry end of the gradient than to ungrazed sites. Characteristic taxa from drier woodlands (e.g., grasses, annual forbs, succulents) become more prominent in grazed mesic woodlands. Conversely, mesic grasses and some perennial forbs that occurred along the whole gradient in ungrazed sites were rare in drier grazed woodlands (Prober & Thiele, 2004). The interaction between community composition and land use history demonstrates the potential for rapid and extensive shifts in plant composition associated with grazing (Prober, Stol, Piper, Gupta, & Cunningham, 2014).

3 | STRENGTHENING TRANSECT RESEARCH

3.1 | Replication

Deriving causation from analyses of single transects can be problematic. Covariation of many variables (e.g., temperature, rainfall, soil, land use) with geographic distance (Meirmans, 2015) makes it difficult to interpret patterns across single transects, even when manipulative studies are undertaken. Additionally, confounding impacts (such as fire or grazing) occurring on a single transect could be mistaken for a climate signal. Replicating studies along two or more similar gradients helps ameliorate these limitations and filter out confounding factors and enables disassociation of individual drivers, facilitating comparisons of occurrence and variation in genes, and traits between species and communities.

Interpretations of patterns of adaptive change would be strengthened by replicating studies along analogous environmental gradients. Such replicated studies can, for example, help identify whether many genes with small effect, or a few genes with larger effect, provide the basis of adaptive evolution. If the same genes are associated with adaptation across species (and transects), this suggests that there are only a few genetic solutions available to cope with climate change (Bell & Aguirre, 2013; Yeaman et al., 2016). Conversely, if many genes or combinations of genes are adaptive across replicated gradients, there could be substantial flexibility in genetic responses. Studies in three eucalypt species across the SWATT and Victorian Eucalyptus Adaptation Transect (VEAT) have demonstrated that adaptation to climate is a genome-wide phenomenon involving multiple genes and gene pathways in different species (Steane et al., 2017). While there has been extensive discussion around theoretical expectations of the predictability of evolution (e.g., Rockman, 2012), well-designed transect studies will help resolve this question. Similar investigations of community attributes (such as species diversity) are likely to improve our understanding of community-level change.

Studies on single transects might identify a correlation between environment and some biotic response, but they are also potentially affected by evolutionary and ecological processes that are disconnected from adaptive processes. For example, habitat fragmentation might limit gene flow and therefore the spread of adaptive genes across a landscape (Breed, Ottewell, Gardner, & Lowe, 2011). Differences between populations might then be interpreted as representing adaptation, whereas they could simply reflect neutral divergence that happens to match an abiotic gradient in a continuous manner (Warren et al., 2014). This problem can be reduced through integrating multiple gradients, such as the elevational and latitudinal sampling approach undertaken on the TREND (Guerin et al., 2012) and EADrosT (Klepsatel, Gáliková, Huber, & Flatt, 2014) (Figure 2). Establishing multiple transects improves characterization of environmental variability, by potentially including multiple gradients running in different directions. In this situation, a single transect would inadequately capture the environmental driver of interest (Travis, Brooker, Clark, & Dytham, 2006). Analyzing data from multiple transects can also disentangle the relative contribution of neutral (e.g., migration—isolation by distance) and adaptive (e.g., selection—isolation by environment) processes to avoid interpreting divergence due to isolation as adaptation (Sexton, Hangartner, & Hoffmann, 2014; Steane et al., 2017).

3.2 | Embedding experiments

If observations of phenotypic change are repeatedly linked to a climate driver, manipulative experiments (such as reciprocal transplants) and further investigations to identify underlying mechanisms are justified. Transect networks are ideal for such experiments, as independent taxa can be used to determine the generality of biotic responses to climatic drivers. Predictions can then be made as to whether a relationship is likely to persist or change under novel conditions.

Transects provide a robust, cost-effective platform for investigating phenotypic change through reciprocal transplant experiments, allowing differentiation of plastic and genetic adaptive changes (e.g., Grady et al., 2013; McLean et al., 2014). Indeed, a major focus of many transect research programs is combining growth experiments with genetic data collected along gradients to reveal associations between phenotypic and genetic variation with climate.

This approach has been used to study the red ironbark, Eucalyptus tricarpa, and New South Wales waratah, Telopea speciosissima, along the VEAT (Figure 2) and Biodiversity and Adaptation Transect Sydney (BATS; Figure 2), respectively (McLean et al., 2014; Rossetto, Thurlby, Offord, Allen, & Weston, 2011; Steane, Potts, McLean, Prober et al., 2014). Local adaptation in functional traits was demonstrated for E. tricarpa using common gardens at each end of the VEAT aridity gradient (McLean et al., 2014; Steane, Potts, McLean, Prober et al., 2014). Some traits displayed complex combinations of plasticity and genetic divergence, and several traits showed clinal genetic variation in plasticity itself (McLean et al., 2014).

A combination of genetic adaptation and phenotypic plasticity was also observed in studies of york gum, Eucalyptus loxophleba, and gimlet, Eucalyptus salubris, on the SWATT (Figures 2, 3) (Prober et al., 2015; Steane, Potts, McLean, Collins 2014). Similarly, studies of T. speciosissima along the BATS revealed genetic differentiation of coastal and upland genotypes, with substantial mixing at mid-elevations (Rossetto et al., 2011). Germination trials showed significant interactions between genotype and germination temperature in growth cabinets and field conditions, where coastal and upland genotypes showed highest germination rates at 30 and 10°C, respectively, suggesting differential selection by optimal germination temperatures in these ecotypes (Rossetto et al., 2011).

3.3 | Transect network development

The approach of the ATN, IGBP, and other networks in coordinating experiments run by local institutions in ecosystems across continents or globally has become increasingly popular (Fraser et al., 2013). The benefits of coordinated networks are clear: By combining resources and expertise, a consortium can build more than individual researchers, and more reliable results can be obtained by comparing the results from many complementary investigations run simultaneously (Suresh, 2012). Coordinated networks are also able to better target future investment. For example, the ATN is currently focused on ensuring a set of common variables are collected for all transects and embedding experiments on some transects. A major challenge in ensuring the longevity of networks such as the ATN is the availability of centralized funding. If centralized funding is insufficient to support individual researchers and institutions to undertake the work needed to support the network, the function and therefore persistence of the network is quickly jeopardized. Improved long-term priority setting and funding security for science funding agencies can alleviate this problem.

4 | NEW AVENUES FOR TRANSECT RESEARCH

Having transect networks available as a research infrastructure resource creates opportunities to apply novel and developing methods to understand species responses to climate change, particularly in the rapidly developing field of genomics and modeling.

4.1 | Genomics and transectomics

Recent applications of new genomic tools on ATN transects include exploring variation in genome-wide single nucleotide polymorphisms to understand neutral and adaptive processes in plants (Christmas, Biffin, Breed, & Lowe, 2016b; Steane, Potts, McLean, Prober et al., 2014; Steane et al., 2017) and the nature of genetic changes within chromosomal inversions in Drosophila (Rane et al., 2015). Genomic and transcriptomic approaches can test the importance of epigenetics and other modes of gene regulation in natural systems under climate change, which are still not yet well understood (Franks & Hoffmann, 2012), but are likely to be significant (Palumbi, Barshis, Traylor-Knowles, & Bay, 2014). For example, epigenetic changes have been implicated in drought responses in plants (Rico, Ogaya, Barbeta, & Penuelas, 2014). Transcriptomic studies also indicate that gene regulation is expected to influence phenotypic plasticity and therefore is a likely target of selection (Chen et al., 2012). Experiments to establish causal relationships between molecular changes and trait variation along transects would entail rearing organisms across multiple generations under common conditions to identify epigenetic effects and reciprocal transplants or controlled manipulation experiments to isolate environmental effects. This understanding could facilitate screening for genotypes more resilient to future climates, and assessing benefits of assisted migration for key species (e.g., seed sourcing for restoration programs (Steane, Potts, McLean, Prober et al., 2014; Breed, Stead, Ottewell, Gardner, & Lowe, 2013; Prober et al., 2015)).

4.2 | Next generation ecological models

Recent advances in forecasting range dynamics and distributions of species have focused on integrating physiological tolerance, adaptive potential, dispersal, metapopulation dynamics, and species interactions (Fordham, Akçakaya, Brook et al., 2013; Fordham, Akçakaya, Araújo et al., 2013; Kearney, Porter, Williams, Ritchie, & Hoffmann, 2009). Transect sampling remains the most efficient way to capture environmentally driven variation across ranges of species and communities (Gillison & Brewer, 1985). Transect networks with wide spatial coverage of bioclimatic space and temporal replication can therefore provide the detailed life-history data required to parameterize, validate, and refine increasingly realistic ecological models. Physiological and genetic data collected across transect networks can further strengthen model predictions (Fordham, Brook, Moritz, & Nogués-Bravo, 2014; Wisz et al., 2013; Figure 4). For example, information on physiological adaptation and acclimation to climate variability can be used to modify vital rates in climate-biodiversity models, improving the reliability of ecological predictions and understanding of eco-evolutionary dynamics (Thuiller et al., 2013). Resampling transect networks provides opportunities to quantify how species occurrence, abundance and demographic traits vary temporally as well as spatially. Integrating this information into ecological models is important because modeled range dynamics are sensitive to assumptions regarding inter-annual climate variability (Bateman, Vanderwal, & Johnson, 2012). Building ecological models using transect network data is therefore likely to result in models that more accurately and explicitly reflect species' ecology and responses to changing conditions in both space and time.

5 | CONCLUDING REMARKS

By re-examining the strengths and limitations of bioclimatic transects for conducting climate change adaptation research, we conclude that a network of bioclimatic transects is a powerful and effective platform to answer the most pressing questions in climate adaptation research. Further understanding of the processes underpinning biotic response to climate change requires manipulative studies that exploit the gradients of change along transects. The case studies illustrate how genetic and phenotypic variation can be linked to improve species distribution models and to forecast changes in biodiversity and ecosystem function. By integrating these approaches into a unified framework, we can improve our understanding of contemporary biodiversity responses to changing climate that will inform effective management actions to promote biodiversity resilience.

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CONFLICT OF INTEREST

None declared.

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APPENDIX 1

Global Climate Models used in Figure 3

Model	Developer
ACCESS1.0	Bureau of Meteorology, Australia
CESM1- CAMS	National Center for Atmospheric Research, USA
CNRM- CM5	Météo- France & Centre Européen de Recherche et de Formation Avancée en Calcul Scientifique, France
GFDL- ESM2M	National Oceanic and Atmospheric Administration, USA
HadGEM2- CC	Met Office, UK
CanESM2	Canadian Centre for Climate Modelling and Analysis, Canada
MIROC5	International Centre for Earth Simulation, Switzerland
NorESM1- M	Norwegian Climate Centre, Norway

The World Climate Research Programme's Working Group on Coupled Modelling is responsible for the Coupled Model Intercomparison Project, and the climate modeling groups developed the models used in Figure 2. For CMIP the U.S. Department of Energy's Program for Climate Model Diagnosis and Intercomparison provided coordinating support and led development of software infrastructure in partnership with the Global Organization for Earth System Science Portals.

APPENDIX 2

Glossary

Adaptation: A heritable change in genotype and/or gene expression in response to environmental change that improves a population's mean fitness.

Adaptive potential: The capacity of a population, species, community, or other biological system to undergo adaptation. Adaptive potential is both facilitated and limited by the levels of standing genetic variation, gene flow, de novo mutation, and the inherent plasticity associated with a genotype.

Bioclimatic gradient: A continuous change in one or more climatic variable(s) with associated change in biodiversity. For example: a mesic woodland transitioning to an arid grassland.

Biome: A category of large-scale ecosystem determined by the structure of the dominant vegetation, such as savanna or tundra. Biomes could comprise a number of constituent ecological communities.

(Ecological) community: An assemblage of organisms that co-occur and interact in a steady state.

Ecological space: An n-dimensional hypervolume, where n represents every variable required for a species' persistence (e.g., sunlight, winter rainfall, food availability).

Epigenetic change: Gene expression moderated by one or more factors external to the gene—such as DNA methylation—that does not alter the gene sequence.

Facilitation: A relationship between two or more organisms conferring an advantage on at least one party. For example, the presence of shading vegetation could create a microhabitat in which smaller plants are able to persist in an otherwise hostile environment.

Functional group: A collection of organisms with shared traits, for example, growth form or climatic requirements.

Functional trait: A trait that is indicative of an organism's interaction with its environment. Functional traits are often governed by balancing fitness trade-offs in biochemistry and/or physiology. For example, wood-density is a functional trait of trees that balances growth rate with durability.

Niche: The ecological space in which a species can persist. Generalist species occupy wide niches and are capable of persisting across most (or all) of a climate gradient and might, therefore, display greater

adaptive potential. Specialist species occupy narrow niches and could be less likely to persist if environmental conditions change.

Nonlinear change: Change occurring on a gradient associated with one or more tipping points. Nonlinear change could be difficult to model or predict and potentially lead to transformative change within ecosystems.

Phenotypic plasticity: The potential of a genotype to produce variation in phenotype. Variation involves changes in one or more functional trait(s) without changes in gene frequency. Plastic responses can be temporary or permanent for an organism's life. Genotypes vary in their plasticity, and evolution and plastic responses can occur in tandem. Examples include learning or nonheritable changes in gene expression. The mechanisms underlying phenotypic plasticity are not well understood but are likely to involve changes in gene expression in many cases.

Replicated transects: Statistically independent transects traversing similar environmental gradients. Replicated transects can occupy different spatial scales (e.g., a short-scale altitudinal transect and continental-scale gradient) but should be otherwise analogous.

Tipping point: The point (in geographic or climate space) at which continuous change in a single environmental factor, or coalescence of multiple factors, reaches a threshold prompting a major ecological disjunction (e.g., a transition from one biome to another).

Tolerance: The ability of an individual, genotype, species, community, or biome to persist in the face of extrinsic change.

Transect network: An arrangement of transects placed across separate environmental gradients on which the same or analogous variables can be measured to develop generalized models of change. Transect networks could include replicated transects as well as transects across different gradients (e.g., aridity, salinity, anthropogenic impact, etc.).

Chapter 3: Overview of the TREND

The TRansect for ENvironmental monitoring and Decision making (TREND) was established in 2012 with the support of the South Australian government. The TREND program was designed to explore the medium to long-term sustainability of South Australia's landscapes in four thematic areas: terrestrial ecosystems, marine ecosystems, agricultural landscapes and regional towns (Caddy-Retalic et al., 2014).

Under the terrestrial ecosystems theme, policy drivers and questions were identified through discussions with government and management stakeholders (Guerin et al., 2016). The research that was subsequently undertaken was designed to determine the drivers of species composition in South Australia and how it might be affected by climate change, as well as identify strategies that could improve the resilience of key species and communities (Guerin et al., 2016).

As a part of this program, a linear network of 85 permanent monitoring plots (hereafter referred to as the TREND-Guerin transect) was established at 17 locations on the Adelaide Geosyncline between Deep Creek on the tip of the Fleurieu Peninsula, and Mount Hack in the northern Flinders Ranges in 2012 (Guerin et al., 2014). The arrangement of these plots was designed to exploit a strong gradient from the mesic Fleurieu Peninsula through to the arid Flinders Ranges. In addition to a climatic gradient, an obvious vegetation change was also evident, with a transition from mesic closed woodlands in the south to more open, arid-adapted shrubland and woodland communities in the north.

Following the formation of the Australian Transect Network as a facility of the Terrestrial Ecosystems Research Network (Thurgate et al., 2017), the TREND was resurveyed using the AusPlots Rangelands methodology (White et al., 2012). In addition to all TREND-Guerin locations being surveyed, additional plots were situated in gaps in the transect, and a number of plots were added at the northern end, extending further into the arid zone. This expanded TREND-AusPlots transect (Figure 1), which forms the core sampling effort of the present study, encompasses a total of 42 one-hectare sites, and the transect forms a key component of the Australian Transect Network, which has established similar transects across several major biogeographic transitions in Australia (Caddy-Retalic et al., 2017).

The TREND-AusPlots transect traverses 6.7° C in mean annual temperature (difference between northernmost and southernmost sites), with sites becoming warmer moving north. There was a greater difference in maximum temperature of the warmest quarter (13.3° C) than coolest temperature of the coldest quarter (2.4° C) (Figure 1). Precipitation was negatively related to temperature, increasing from north to south, with 673.6 mm difference in mean annual precipitation (i.e. monthly average of 56.1 mm), 24 mm difference for the wettest month and 3.8 mm difference for the driest month (Figure 1). Moisture Index showed a similar pattern to temperature. There was no consistent elevational pattern from north to south, with sites located at high and low elevation across the spine of the Mt Lofty, Flinders and Gammon Ranges until reaching the low-altitude Stony Plains at the northernmost extent. A soil pH gradient of ~4.7 pH units was also present (Figure 1), potentially related to ion accumulation due to high evaporation in the arid north, and soil leaching effects in the high rainfall (southern) sites.

For consistency, throughout this thesis, each AusPlot is referred to by a four letter code (e.g. BLCK for Black Hill). Corresponding TREND-Guerin plots are referred to with an additional letter (e.g. BLCK-A, BLCK-B, etc). A full list of site codes can be found in Chapter 5. The following pages provide a brief overview of the 42 locations at which the permanent survey plots investigated in the following pages were established, ordered from north to south. I include a brief description of the landform features and vegetation community, including dominant species and a photographic panorama for each AusPlot.

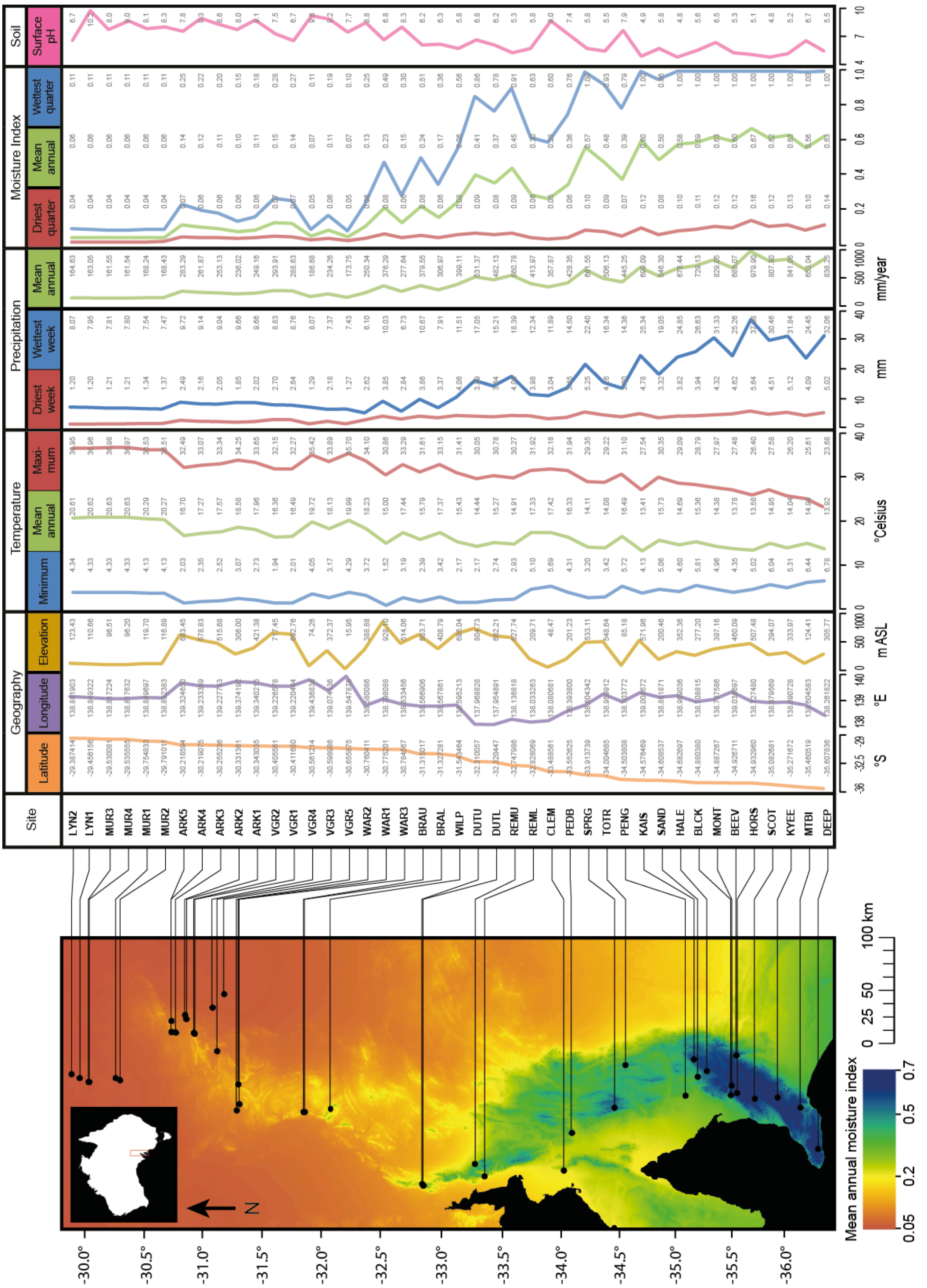

Figure 1: The location of the 42 locations at which AusPlot surveys were undertaken, with key environmental variables. AusPlots are colocated with TREND-Guerin plots at 17 locations (WAR1, BRAU, BRAL, WILP, DUTU, DUTL, REMU, REML, SPRG, TOTR, KAIS, SAND, HALE, BLCK, MONT, HORS and DEEP).

Transect panoramas

LYN2 (SATSTP0008) – Murnpeowie Station (1/42)

Low, sparse grassland dominated by *Astrebla pectinata*, with scattered *Plantago drummondii* and *Tripogon loliiformis.* Little relief, heavy gibber strew. Some grazing pressure, no evidence of fire. Surveyed 18/8/2013. Coordinates: -29.38786°, 138.88138°.

LYN1 (SATSTP0005) – Murnpeowie Station (2/42)

Dune system with moderate slopes to the west and steep slopes to east. Dunes run parallel NE-SW. Mid-sparse shrubland dominated by *Dodonaea viscosa* and *Acacia ligulata*. Ground stratum comprised of *Zygochloa paradoxa*, *Polycalymma stuartii* and *Enchylaena tomentosa* with scattered *Aristida holathera*. Surveyed 17/8/2013. Coordinates: -29.45660°, 138.84880°.

MUR3 (SATSTP0006) – Murnpeowie Station (3/42)

Low, open woodland dominated by *Eucalyptus coolabah* with mid-stratum of *Duma coccoloboides* and juvenile *E. coolabah.* A sparse, mixed ground stratum dominated by *Calotis hispidula*, *Tetragonia moorei*, scattered *Enchylaena tomentosa* and *Eragrostis setifolia*. On the edge of ephemeral swamp with evidence of historic cattle grazing. Surveyed 17/8/2013. Coordinates: -29.53052°, 138.81670°.

MUR4 (SATSTP0007) – Murnpeowie Station (4/42)

Low, open woodland dominated by *Eucalyptus coolabah* with sparse mid-stratum of *E. coolabah* and isolated *Duma coccoloboides* and a sparse ground stratum of *Trigonella suavissima* and *Verbena officinalis*. Situated in an ephemeral swamp with evidence of heavy grazing though no cattle present at time of site visit. Surveyed 18/8/2013. Coordinates: -29.53606°, 138.81711°.

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MUR1 (SATSTP0003) – Mount Lyndhurst Station (5/42)

Gibber plain with bare soil, gravel, scattered grasses and vegetated gilgais. Low, sparse tussock grassland dominated by *Eragrostis setifolia*, *Calotis hispidula* and scattered *Astrebla pectinata*. Some impact from cattle grazing. Surveyed 15/8/2013. Coordinates: -29.75529°, 138.84916°.

MUR2 (SATSTP0004) – Mount Lyndhurst Station (6/42)

Low, open shrubland dominated by *Senna artemisioides*, *Eremophila maculata*, *Acacia victoriae* and scattered *Santalum lanceolatum*. A ground stratum of *Eragrostis setifolia*, *Zygophyllum apiculatum* and *Sida intricata*. Minor grazing by cattle. Surveyed 15/8/2013. Coordinates: -29.79147°, 138.83186°.

ARK5 (SATFLB0018) – Arkaroola Wilderness Sanctuary (7/42)

Steep, north facing slope. Low, hummock grassland dominated by *Triodia irritans* with emergent *Codonocarpus* and scattered *Eucalyptus intertexta* and *E. flindersii.* Long unburnt with some evidence of recent grazing by macropods and historic grazing by goats. Surveyed 8/8/2013. Coordinates: -30.21906°, 139.32413°.

ARK4 (SATFLB0019) – Arkaroola Wilderness Sanctuary (8/42)

Low, open hummock grassland dominated by *Triodia irritans* with emergent *Eucalyptus intertexta* and mid-stratum of *Rhagodia parabolica* and *Maireana triptera.* NW facing slope with extensive igneous surface strew and larger boulders. Long unburnt with little obvious grazing impact. Surveyed 9/8/2013. Coordinates: -30.21953°, 139.23286°.

ARK3 (SATFLB0020) – Arkaroola Wilderness Sanctuary (9/42)

Low, mallee woodland dominated by *Eucalyptus socialis* with *Myoporum platycarpum* mid layer. *Amyema miquelii* on most trees. Sparse ground stratum of *Triodia scariosa.* Long unburnt with little obvious grazing impact. Surveyed 20/8/2013. Coordinates: -30.25567°, 139.22722°.

ARK2 (SATFLB0017) – Arkaroola Wilderness Sanctuary (10/42)

Tall, open shrubland dominated by *Acacia aptaneura* and *A. tetragonophylla* with sparse understratum of forbs dominated by *Sida fibulifera*. Possibly burnt within last 10 years, with moderate (presumably macropod) grazing impact. Surveyed 7/8/2013. Coordinates: -30.33181°, 139.37367°.

ARK1 (SATFLB0016) – Arkaroola Wilderness Sanctuary (11/42)

Scattered shrubland dominated by *Eucalyptus gillii*, with scattered *Melaleuca lanceolata* and *Acacia araneosa*. Ground stratum dominated by *Enneapogon cylindricus* with scattered *Euphorbia drummondii* and *Salsola australis*. Site long unburnt, with some grazing impact from goats and rabbits, as well as rabbit diggings. Surveyed 6/8/2013. Coordinates: -30.34347°, 139.33969°.

VGR2 (SATFLB0022) – Vulkathunha-Gammon Ranges National Park (12/42)

Low woodland of *Eucalyptus intertexta, E. dumosa* and scattered *Callitris glaucophylla*, with a ground stratum of *Setaria constrita, Trioida scariosa* and *Ptilotus obovatus.* Extensive cobbles and boulders with heavy lichen. Long unburnt with no obvious grazing impact. Surveyed 14/8/2013. Coordinates: -30.40605°, 139.22606°.

VGR1 (SATFLB0021) – Vulkathunha-Gammon Ranges National Park (13/42)

Open woodland dominated by *Eucalyptus intertexta* and *E. flindersii* with *Callitris glaucophylla*. Mid stratum dominated by *Melaleuca uncinata*, *Senna artemisioides* and several *Eremophila* spp. Ground stratum of *Triodia scariosa*. Extensive cobbles and boulders with lichen. Long unburnt with no obvious grazing impact. Surveyed 14/8/2013. Coordinates: -30.41119°, 139.22097°.

VGR4 (SATSTP0001) – Vulkathunha-Gammon Ranges National Park (14/42)

Low, open tussock grassland dominated by *Astrebla pectinata* with scattered *Enneapogon avenaceus. Malvastrum americanum* and *Sclerolaena* spp. Flat site on alluvial plain, with mixed surface lithology. No evidence of recent burning or substantial grazing. Surveyed 10/8/2013. Coordinates: -30.56167°, 139.26964°.

VGR3 (SATFLB0023) – Vulkathunha-Gammon Ranges National Park (15/42)

Low, sparse shrubland dominated by *Senna artemisioides*. Ground stratum dominated by *Carrichtera annua* with scattered *Zygophyllum iodocarpum* and *Enneapogon cylindricus*. South-facing slope to base of low range of hills. Mixed dolomite and limestone lithology. Moderate grazing impact from goats and rabbits, and moderate weed impact (largely weedy understory). No evidence of recent fire. Surveyed 15/8/2013. Coordinates: -30.59944°, 139.07361°.

VGR5 (SATSTP0002) – Vulkathunha-Gammon Ranges National Park (16/42)

Tall, sparse shrubland dominated by *Acacia ligulata* with *Enchylaena tomentosa* and scattered *Santalum lanceolatum*. Some isolated *Maireana pyramidata* in mid stratum. Scattered understory dominated by tussock grasses, mainly *Enneapogon* spp. and *Sida fibulifera*. Low grazing impact, with some evidence of cattle, macropods and rabbits. Low weed impact, although isolated *Sonchus oleraceus* and clumps of *Cenchrus ciliaris.* Site long unburnt. Surveyed 12/8/2013. Coordinates: -30.65633°, 139.54736°.

WAR2 (SATFLB0024) – Warraweena (17/42)

Sparse chenopod shrubland dominated by *Maireana pyramidata*. Understory dominated *Carrichtera annua*. Very gently north sloping site with dolomite and mixed strew wash from further up slope. Site long unburnt with moderate to high weed impact with nearly entirely weedy understory. Moderate grazing impact from cattle, goats and macropods. Surveyed 16/8/2013. Coordinates: -30.76086°, 138.57956°.

WAR1 (SATFLB0006) – Warraweena Mount Hack (18/42)

Low woodland dominated by *Eucalyptus polybractea* and *E. flindersii* with *Casuarina pauper*, *Acacia havilandiorum* and *Grevillea aspera*. Sparse ground stratum dominated by *Chrysocephalum semipapposum*. Site on escarpment, facing south. Evidence of heavy goat grazing. Long unburnt. Heavy surface strew and some outcrop. Surveyed 21/8/2013. Coordinates: -30.77565°, 138.79757°.

WAR3 (SATFLB0025) – Warraweena (19/42)

Low woodland dominated by *Casuarina pauper* in upper and mid stratum. Mid stratum also included *Chenopodium desertorum* and *Maireana* spp. Ground stratum of *Atriplex vesicaria* and *Carrichtera annua*. SE slope, becoming steeper to the north. Moderate to high weed impact, with patches of *C. annua* and other annual forbs. Low grazing impact, though evidence of goats. Long unburnt. Heavy surface strew, with patches of outcrop with lichen cover. Surveyed 17/8/2013. Coordinates: -30.78503°, 138.63294°.

BRAU (SATFLB0005) – Flinders Ranges National Park Brachina Upper (20/42)

Low woodland dominated by *Eucalyptus flindersii* with emergent *Callitris glaucophylla*. Diverse mid stratum dominated by *Dodonaea viscosa* with *Olearia decurrens*, *Cassinia laevis* and *Calytrix tetragona*. Ground stratum dominated by *Chrysocephalum semipapposum* with *Goodenia vernicosa*. Site straddles the top of the range above Brachina Gorge. Extensive boulders, cryptogam cover and little bare ground. Long unburnt with some grazing impact from goats. Minimal weed impact. Surveyed 20/8/2012. Coordinates: -31.31546°, 138.56636°.

BRAL (SATFLB0004) – Flinders Ranges National Park Brachina Lower (21/42)

Low woodland dominated by *Callitris glaucophylla* and *Eucalyptus intertexta*. Mid-stratum dominated by *Rhagodia paradoxa* and *Hakea leucoptera* with scattered *Cassinia laevis* and *Dodonaea viscosa*. Sparse ground stratum dominated by *Ptilotus obovatus*. High grazing impact from goats, macropods and rabbits. Uneven slope with drainage channels in gully. Rocky surface with cryptogam and some outcrop. Long unburnt. Surveyed 18/8/2012. Coordinates: -31.32774°, 138.56733°.

WILP (SATFLB0007) – Wilpena Pound (22/42)

Open shrubland dominated by *Allocasuarina muelleriana*, *Casuarina pauper* and scattered *Eucalyptus flindersii*. Ground stratum sparse but dominated by *Chrysocephalum apiculatum*. Site located on lower side slopes of Mt Ohlssen. Very rocky, with large boulders and outcrop. Recently burnt, with eucalypts regenerating. Evidence of some goat grazing. Surveyed 22/8/2012. Coordinates: -31.54388°, 138.59458°.

DUTU (SATFLB0009) – Dutchman's Stern Conservation Park Upper (23/42)

Mixed woodland dominated by *Eucalyptus cladocalyx*. Dense mid stratum dominated by *Cassinia laevis*, *Xanthorrhoea quadrangulata* and *Casuarina pauper*. Ground stratum dominated by *Wurmbea biglandulosa* and *Cheilanthes sieberi*. Site located on NW slope near top of Dutchman's Stern ridge. Low weed and grazing impact, with evidence of eucalypts recovering from drought. Surveyed 24/8/2012. Coordinates: -32.31052°, 137.96827°.

DUTL (SATFLB0008) – Dutchman's Stern Conservation Park Lower (24/42)

Mixed shrubland dominated by *Cassinia laevis*, *Casuarina pauper* and *Xanthorrhoea quadrangulata*. Dense ground stratum of *Triodia scariosa* with scattered *Gonocarpus elatus*. Steep slope facing east with slight drainage line running to SE. Low grazing impact with no recent burning. Surveyed 23/8/2012. Coordinates: -32.320959°, 137.95439°.

REMU (SATFLB0011) – Mt Remarkable Conservation Park Upper (25/42)

Low, open woodland dominated by *Eucalyptus cladocalyx* and *E. goniocalyx*. Diverse, dense mid stratum dominated by *Xanthorrhoea quadrangulata* and *Hibbertia* spp. Ground stratum dominated by *Astroloma humifusum*, *Dianella revoluta* and *Lomandra densiflora*. Grazing and weed impact minimal. Site slopes to the SW, with extensive surface strew. Long unburnt. Surveyed 26/8/2012. Coordinates: -32.74842°, 138.13627°.

REML (SATFLB0010) – Mt Remarkable Conservation Park Lower (26/42)

Open mallee woodland dominated by *Eucalyptus odorata*. Mixed mid stratum dominated by *Rhagodia paradoxa*, *Enchylaena tomentosa*, *Olearia decurrens* and scattered *Pimelea microcephala*. Extensive surface strew with cryptogam and little understory. Long unburnt. Moderate weed impact from *Carrichtera annua* and *Lycium ferocissimum*. Surveyed 25/8/2012. Coordinates: -32.82851°, 138.03267°.

CLEM (SATEYB0002) – Clement's Gap Conservation Park (27/42)

Mallee woodland dominated by *Eucalyptus socialis* and *E. gracilis*. Mid layer dominated by Geijera, *Senna artemisioides*, Myoporum and Alectryon. Ground layer dominated by Olearia, Zygophyllum, *Carrichtera annua* and grasses. Extensive bare ground and cryptogam. Site is a sand plain, with moderate weed impact. No evidence of grazing or recent fire. Surveyed 30/10/2014. Coordinates: -33.48902°, 138.08010°.

PEDL (SATFLB0028) – Pedler's Block (28/42)

Low mallee woodland dominated by *Eucalyptus odorata* and *Allocasuarina verticillata* with mid stratum of *Acacia pycnantha*, *Bursaria spinosa* and *Senna artemisioides*. Lower stratum dominated by *Eutaxia microphylla*, *Lomandra multiflora* and *Avena barbata* with weedy annual grasses. Long unburnt. Evidence of rabbits but well-fenced and no other grazing impact evident. Surveyed 6/11/2014. Coordinates: -33.55318°, 138.39322°.

SPRG (SATFLB0013) – Spring Gully Conservation Park (29/42)

Low, open woodland dominated by *Eucalyptus macrorhyncha*. Mid stratum dominated by *Xanthorrhoea quadrangulata*, with *Hibbertia exutiacies* and *Bursaria spinosa*. Ground stratum dominated *Astroloma conostephioides*. Gently sloping, close to the top of the Spring Gully ridge. Unburnt for at least ten years. Moderate weed impact, with *Lavandula stoechas*, *Rosa rubiginosa*, *Prunus domestica* and some tussock grasses. Low grazing impact. Surveyed 19/11/2012. Coordinates: -33.91420°, 138.60381°.

TOTR (SATFLB0014) – Tothill Range Private Reserve (30/42)

Open mallee woodland dominated by *Eucalyptus odorata* with some *E. goniocalyx*. Dense, mixed mid-stratum dominated by *Xanthorrhoea quadrangulata, Allocasuarina verticillata* and *Exocarpos cupressiformis*. Very sparse ground stratum. Site slopes to the west near the top of the range, with little exposed ground. Unburnt for more than 100 years. Low weed impact, though some weedy grasses at the lower end of the site. 21 years since stock removed, no current grazing impact. Surveyed 20/11/2012. Coordinates: -34.00514°, 138.95937°.

PENG (SATEYB0001) – Pengilly Scrub Reserve (31/42)

Mallee woodland dominated by *Eucalyptus socialis*, with scattered *Callitris gracilis,* and *E. dumosa*. Mid stratum of *Rhagodia parabolica* and *Alyxia buxifolia*. Ground stratum dominated by mixed tussock grasses. Site is on dune crest with slope to northeast, east and southwest. No obvious fire or grazing impact except abandoned rabbit warrens. Weeds in ground layer, especially *Asparagus asparagoides* (control program currently underway). Surveyed 28/10/2014. Coordinates: -34.50429°, 138.70317°.

KAIS (SATFLB0003) – Kaiserstuhl Conservation Park (32/42)

Low, open *Eucalyptus baxteri* woodland. Mid stratum dominated by *Xanthorrhoea semiplana* and *Acacia pycnantha*. Ground stratum dominated by *Lepidosperma semiteres*, *Spyridium parvifolium* and *Lomandra fibrata*. Long unburnt. Weed impact low. No grazing. Gently sloping shallow gully from north to south. Several large rock outcrops with little strew. Surveyed 15/8/2012. Coordinates: -34.57693°, 139.00610°.

SAND (SATFLB0001) – Sandy Creek Conservation Park (33/42)

Low, open forest dominated by *Callitris gracilis, Eucalyptus fasciculosa* and *Allocasuarina verticillata*. Mid stratum of *Astroloma conostephioides* and *Babingtonia behrii*. Sparse ground stratum, with *Drosera auriculata* prominent as well as some mixed grass cover. Gentle slope across low dune, with vegetation becoming slightly more sparse on the top of the dune. Very long unburnt. Grazing impact low but heavy in neighbouring paddock. No surface strew. Surveyed 13/8/2012. Coordinates: -34.60901°, 138.86135°.

HALE (SATFLB0002) – Hale Conservation Park (34/42)

Low mallee woodland dominated by *Eucalyptus obliqua*. Dense mid stratum dominated by *Xanthorrhoea semiplana*, *Acacia paradoxa* and *Astroloma conostephioides*. Ground stratum dominated by *Chamaescilla corymbosa*. SE facing slope with some outcrop and surface strew. Low intensity fire within last five years. Weed impact low, though some *Asparagus asparagoides*. Grazing impact low. Surveyed 14/8/2012. Coordinates: -34.68310°, 138.90860°.

BLCK (SATFLB0012) – Black Hill Conservation Park (35/42)

Dense mixed shrubland dominated by *Allocasuarina muelleriana* with emergent *Eucalyptus fasciculosa*. Ground stratum dominated by *Gonocarpus elatus* and *Neurachne alopecuroidea*. NW facing slope, curving around hill to the north. Long unburnt and minimal evidence of grazing. Weed impact low, though some weedy grasses including *Vulpia muralis*. Surveyed 16/11/2012. Coordinates: -34.88077°, 138.70826°.

MONT (SATFLB0026) – Montacute Conservation Park (36/42)

Open woodland dominated by *Eucalyptus obliqua*. Mid stratum dominated by *Acacia pycnantha* on upslope sections, with *Xanthorrhoea semiplana* scattered throughout the site. Ground stratum dominated by *Hibbertia exutiacies* and mixed tussock grasses. Steep, (35-45°) site with some outcrop and boulders. Long unburnt. Moderate weed impact, including from *Senecio pterophorus*, *Genista monspessulana, Briza maxima* and *Rubus* sp. Some grazing impact from goats and deer. Surveyed 29/10/2014. Coordinates: -34.88750°, 138.78739°.

BEEV (SATKAN0004) – Mount Beevor Private Reserve (37/42)

Low woodland dominated by *Eucalyptus fasciculosa* with a mixed mid stratum of *Eutaxia microphylla*, *Acacia paradoxa*, juvenile *E. fasciculosa* and *Bursaria spinosa*. Very diverse ground stratum. Site is a shallow gully, sloping 12° NE. Site was burnt 10 years ago but has recovered well. Low grazing impact from macropods. Moderate weed impact, with introduced grasses and *Senecio quadridentatus* evident. Surveyed 5/11/2014. Coordinates: -34.92714°, 139.03812°.

HORS (SATFLB0015) – Horsnell Gully Conservation Park (38/42)

Closed forest dominated by *Eucalyptus obliqua* and *E. baxteri*. Mid stratum dominated by *Xanthorrhoea semiplana*, *Acacia pycnantha* and *Hakea rostrata*. Ground stratum dominated by *Lepidosperma semiteres* and Hibbertia crinita. Fire in last 10 years, with scarring on trees, but shrub layer has regenerated. Weed impact high, including *Rubus anglocandicans*, *Genista monspessulana* and *Watsonia* sp. Site slopes 7° south in centre, falling 15° to the NW and NE on the northern edge. Moderate grazing impact from kangaroos. Extensive echidna diggings. Surveyed 22/11/2012. Coordinates: -34.93343°, 138.72695°.

SCOT (SATFLB0027) – Scott Creek Conservation Park (39/42)

Open woodland dominated by *Eucalyptus leucoxylon* with isolated *E. fasciculosa*. Mid layer of *Acacia pycnantha*, *E. cosmophylla* and other scattered shrubs. Sparse ground layer dominated by *Hibbertia exutiacies* and *Cheilanthes austrotenuifolia*. Steep site on hill slope facing NE, average of 20° but up to 30° at the SE and NE corners. Minimal grazing impact, mainly from macropods. No evidence of recent fire. Site probably cleared in the past, as some very old cut stumps are evident. Moderate weed impact, with several weed species present, including *Chysanthemoides monilifera* and *Olea europaea,* but overall cover is low. Surveyed 05/11/2014. Coordinates: -35.08277°, 138.67890°.

KYEE (SATKAN0002) – Kyeema Conservation Park (40/42)

Open woodland dominated by *Eucalyptus obliqua*. Dense mid-stratum dominated by *Hakea rostrata* with *Pultenaea involucrata* and ground stratum dominated by *Lepidosperma semiteres*. Gently NW sloping site. Shallow ephemeral creek in NW corner. Burnt within last 5 years. Minimal grazing and weed impact. Surveyed 22/8/2012. Coordinates: -35.27211°, 138.69017°.

MTBI (SATKAN0003) – Mount Billy Conservation Park (41/42)

Open woodland dominated by *Eucalyptus leucoxylon*, with mid stratum of *Acacia pycnantha*, *Dodonaea viscosa* and *Acacia paradoxa*. Ground stratum sparse, with *Vulpia* sp. and *Hibbertia riparia*. Substrate dominated by litter and cryptogam and extensive coarse woody debris. Little evidence of tree recruitment. East facing hill slope. No evidence of grazing. Tree stumps indicate some past logging but no recent activity. Surveyed 3/11/2014. Coordinates: -35.46097°, 138.60405°.

DEEP (SATKAN0001) – Deep Creek Conservation Park (42/42)

Low, open woodland dominated by *Eucalyptus obliqua* with a dense, mixed mid-stratum dominated by *Pultenaea involucrata*, *Xanthorrhoea semiplana* and *Banksia marginata*. Ground stratum dominated by *Lepidosperma semiteres*, *Lomandra fibrata* and mixed tussock grasses. Very long unburnt. Moderate weed impact, with *Ulex europaeus*, and weedy annual grasses and forbs evident. Steep slope down towards valley in the NE. Surveyed 21/8/2012. Coordinates: -35.60827°, 138.26127°.

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Chapter 4: Empirical testing refutes the existence of a universal scaling relationship for leaf carbon isotope response on aridity gradients

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Contribution to the Paper	Designed the study, led the field work in South Australia, conducted data analysis and wrote the manuscript.		
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Signature		Date	11 Oct 2017

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Title: Empirical testing refutes the existence of a universal scaling relationship for leaf carbon isotope response on aridity gradients

Running headline: Plant carbon isotope responses on gradients

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Abstract

Leaf carbon isotope ratio ($\delta^{13}C$) is reflective of aridity during leaf formation, with higher values in drier environments. A postulated "universal scaling relationship", where all C_3 species display a single isotopic response to aridity gradients has never been empirically tested in multiple locations. We compared plants growing along aridity gradients in South Australia, Western Australia, and northeast China (160-980 mm, 220-745 mm and 145-710 mm mean annual precipitation, respectively) to test for universal scaling. We found $\delta^{13}C$ responses to aridity at the species-level and landscape level differed widely, providing strong evidence against a universal scaling phenomenon. The trade-off between maintaining CO_2 uptake and limiting water loss in arid environments does result in plants responding to aridity in a generally consistent way, but our data suggests that the magnitude of this response is highly variable and probably related to location and individual species traits. We suggest the magnitude of both species- and landscape-level $\delta^{13}C$ responses to aridity may be useful in classifying the sensitivity of species and regional floras to aridification under climate change.

Key words

Aridity response, biotic homeostasis, climate change, $\delta^{13}C$, gradient, NECT, SWATT, TREND

Introduction

Climate change is rapidly altering the composition and distribution of the world's vegetation. Migration of many species has already been detected (Chen et al. 2011; Kelly and Goulden 2008; Parmesan 2006) but it has been suggested that some species will be unable to disperse fast enough to keep up with climate shifts, particularly where habitat is fragmented (Jump and Peñuelas 2005; Loarie et al. 2009). Determining the ecophysiological responses of species *in situ* to environmental variability across their range has been suggested as a method to gauge sensitivity and likely persistence of those species under future changed climates and can inform projections of future vegetation distribution (Moritz and Agudo 2013).

Temperature rise and increased rainfall variability, and their interactions with rising atmospheric CO_2 concentration, are the major changes expected to alter the plant physiology and vegetation composition of temperate ecosystems under projected future climates (Kertész and Mika 1999; Murphy and Timbal 2008). Measuring variation in traits of species occurring along environmental gradients (e.g. increasing aridity through increased temperature and reduced rainfall) is an established methodology for mapping phenotypic diversity and possible biotic responses to environmental change (Blois et al. 2013). To be most useful in an ecological context, selected traits should reflect responses to the environment, and the physiological mechanisms that control trait expression should be understood (Caddy-Retalic et al. 2017). Stable carbon isotope ratios are indicative of water use efficiency and water stress, and are therefore useful traits for predicting the capacity of temperate species to cope with projected climate change. By comparing the isotopic response of individual species to the aggregated mean response of numerous species across a landscape, we can infer the relative sensitivity of those species and the emergent sensitivity of aggregated communities.

The carbon isotope ratio ($\delta^{13}C$) of leaves is indicative of the environmental conditions during leaf formation, constrained by species physiology (Cernusak et al. 2013; Farquhar et al. 1989). Leaf $\delta^{13}C$ reflects the $\delta^{13}C$ of atmospheric CO_2, modified by the isotopic discrimination (Δ) that occurs during photosynthesis, calculated as

$$\Delta = \frac{\delta_a - \delta_p}{1 + \delta_p} \tag{1}$$

where δ_a is atmospheric CO_2 $\delta^{13}C$ and δ_p is leaf $\delta^{13}C$ (Cernusak et al. 2013; Farquhar and Richards 1984).

There are many factors that can affect C_3 carbon isotope discrimination (Δ_3), including stomatal conductance, enzyme bias, mesophyll conductance (Flexas et al. 2008), respiration, light and nutrient availability (Cernusak et al. 2013) and tissue composition (Cernusak et al. 2009). While each of these factors can vary between species and environmental conditions, the majority require complex instrumentation to quantify and cannot be measured for dead leaves. Moreover, mesophyll and stomatal conductance are thought to generally respond to environmental variables in a similar way (Flexas et al. 2008). For these reasons, the simplest model of carbon isotope discrimination in C_3 plants can be appropriate for landscape-scale studies:

$$\Delta_3 = a + (b - a)\frac{c_i}{c_a} \tag{2}$$

where a is the fractionation caused by diffusion of CO_2 molecules through the stomata; b is the fractionation which occurs during carboxylation through the discrimination against $^{13}CO_2$ by the RuBisCO enzyme; c_i is the CO_2 concentration of the intercellular air spaces within the leaf; and c_a is the CO_2 concentration of the atmosphere (Farquhar et al. 1989). Given that a and b are relatively fixed, the ratio of c_i to c_a is regarded as the major determinant of change in Δ_3, and therefore, leaf $\delta^{13}C$ for plants growing in natural environments (Cernusak et al. 2013; Farquhar et al. 1989).

Factors c_a and δ_a can vary in space and time. Burning fossil fuels is steadily increasing c_a, which has risen from ~280ppm to >400ppm since industrialisation (Pachauri et al. 2014). Combustion of ^{13}C-depleted hydrocarbons has led to a concurrent lowering of δ_a with a ~1.5‰ decrease in $\delta^{13}C$ of atmospheric CO_2 since 1800 (Francey et al. 1999). Air spaces which have restricted mixing with the atmosphere, such as dense forest canopies, may also display large diurnal changes in c_a (due to the shift between photosynthesis and respiration of trees) and δ_a (due to the "canopy effect" in which air trapped by forest canopies is progressively ^{13}C enriched when photosynthesis predominates and ^{13}C depleted when respiration predominates) (Hymus et al. 2005)). Despite these effects, short term c_a and δ_a can be considered to be relatively constant, especially in ecosystems that are not isolated from the open atmosphere by dense canopies (Cernusak et al. 2013). The c_i is controlled by the movement of CO_2 into the leaf (stomatal conductance) and the photosynthetic demand of the leaf. Photosynthetic demand is determined by a range of drivers, including nutrient and light availability and prevailing climatic conditions (Cernusak et al. 2013).

Water availability is an important control of plant growth. Vapour pressure deficit (VPD) and soil moisture largely determine a plant's water budget and are usually negatively correlated (Zhou et al. 2014). In order to limit water loss, plants in arid conditions must limit stomatal conductance, which also decreases the diffusion of CO_2 to the intercellular air spaces, thus decreasing c_i. The resultant reduction in c_i/c_a decreases the discrimination of C_3 plants and shifts leaf δ^{13}C values closer to that of atmospheric δ^{13}C. Therefore, leaf δ^{13}C (or Δ) provides a time-integrated proxy for c_i/c_a in C_3 plants (Cernusak et al. 2013), and in turn reflects the level of aridity.

In wet conditions, C_3 plants have more negative δ^{13}C values (i.e. -31.5‰), whereas in very arid conditions, these can rise to above -23‰ (Diefendorf et al. 2010; Kohn 2010). A positive relationship between aridity and leaf δ^{13}C has been demonstrated in regional and global analyses; however the sensitivity of isotopic ratios to aridity has been reported to vary among locations and species (Kohn 2010; Ma et al. 2012). The slope of the regression of δ^{13}C versus moisture availability for a C_3 species (m_s) occurring along an aridity gradient can be used to gain insight into the change in stomatal regulation as water becomes increasingly limited. We suggest that species' m_s values can be compared to infer relative climate sensitivity, and compared to a “common slope” of $\delta^{13}C$/moisture availability for the community mean of many species along a gradient (m_c). Measuring m_c for multiple regions would enable the comparative climate-vegetation sensitivity to be assessed for entire bioregions.

Zonation is a biogeographic phenomenon in which individual species occupy overlapping ranges along an environmental gradient, with turnover referring to the rate of species change relative to abiotic (e.g. climate) change (Figure 1a). Noting that all plants are limited by water loss during photosynthesis, and discounting life history traits that could impact water use efficiency, we might expect all species on a water availability transect to have similar m_s, which would also approximate m_c (Figure 1b). This pattern was observed for several species occurring along the Northeast China Transect (NECT; Figure 2) where a consistent relationship was found between $\delta^{13}C$ and moisture index (MI) and mean annual precipitation (MAP), both within and between species (i.e. $m_s \approx m_c$) (Prentice et al. 2011). The authors described this relationship as “universal scaling” (Prentice et al. 2011). In contrast, trees (*Eucalyptus* spp.) on the North Australian Tropical Transect (NATT) had a steeper m_s than m_c. This meant that individual species displayed an initially lower leaf $\delta^{13}C$ value than the species it replaced along the gradient of increasing aridity, i.e. $m_s << m_c$ (Figure 1c) (Schulze et al. 1998). Prentice *et al.* (2011) referred to this pattern as “biotic homeostasis”, but did not consider it viable in the absence of a clear causative mechanism. Re-analysis of NATT *Eucalyptus* data reported that “the proposed saw-tooth pattern of species was not observed” (Miller et al. 2001), casting further doubt on the existence of a biotic homeostasis pattern on natural gradients. The idea that all C_3 plants respond similarly on gradients (i.e. conform to universal scaling) is intriguing, but the claimed “universality” of this scaling warrants further investigation to test the consistency of carbon isotope ratio patterns on different climate gradients. Additionally, there is value in comparing m_s and m_c between and within transects to explore relative sensitivity of entire species assemblages and their constituent species to aridification.

We sought to explore the $\delta^{13}C$ response of a range of flora on two new transects, the TRansect for ENvironmental monitoring and Decision making (TREND; Figure 2) in South Australia the South West Australian Transitional Transect (SWATT; Figure 2) in Western Australia. We present new data on $\delta^{13}C$ responses for 150 C_3 species on the TREND and 49 C_3 species on the SWATT, which we contrast with a reanalysis of data from 36 species collected on the NECT previously published by Prentice *et al*. (2011) and a global $\delta^{13}C$ data set (Kohn 2010).

The species we tested represent a broad range of functional types including forbs, grasses, ferns, vines, shrubs, trees and mistletoes. Together, these data are subjected to new statistical analysis to provide a comprehensive test of the universal scaling hypotheses. By combining species distributions with carbon isotope values, we aimed to delineate the ecophysiological strategies employed by plants and test the consistency of these patterns between communities on analogous bioclimatic transects. Finally, we assessed the potential of m_s as a proxy measure to determine species' sensitivity to aridification.

Materials and methods

Study locations and survey methodology

Australia

The TREND consists of 42 sites spanning 6.2° of latitude (~800 km) and 150-980 mm mean annual precipitation (MAP) near Adelaide in South Australia (Figure 2). The SWATT consists of 127 sites spanning 9.5° of latitude (~1,000 km) and 220-745 mm MAP in south-western Australia. Surveys were undertaken from August to November (the austral spring) from 2012 to 2014.

Sites were located in remnant native vegetation on nature reserves. Vouchers of all vascular plant species present at a site were collected and deposited with the State Herbarium of South Australia (TREND) and the Western Australian Herbarium (SWATT). Identifications were undertaken by the Herbarium botanists. South-Western Australia is an area of exceptionally high species diversity, and analysis of species distributions on SWATT revealed very high species turnover (Gibson et al. 2017). Most species had distributions restricted to a small portion of the gradient, but species distributions were greater in the more arid section of the transect (Coolgardie bioregion) so most species occurring in this area were selected, resulting in a narrower rainfall range (220-400 mm MAP) for samples than for the NECT or TREND.

Leaf $\delta^{13}C$ was measured in samples of species that occurred at ≥4 sites (TREND) and ≥6 sites (SWATT; Supplementary Table S1). For most plants, leaf tissue samples were collected in the field and dried in synthetic gauze bags on silica gel; otherwise samples were taken from herbarium vouchers. Leaf samples were ground, homogenised using a ball mill (Retsch MM400 fitted with a Qiagen TissueLyser 24 adapter set), and 2-2.5 mg sub-samples were weighed to six significant figures using a Simultaneous Thermal Analyser (PerkinElmer STA 6000). Technical replicates were run for 13% of samples. Samples were combusted at 1000°C in an Elemental Analyser (EuroVector EuroEA 3000) in line with a continuous flow isotope ratio mass spectrometer (Nu Instruments Nu Horizon, University of Adelaide). Pure glycine, glutamic acid and triphenylamine (all calibrated to international C & N standards) were used as standards to correct for drift. A standard error of 0.07‰ was achieved. Two-point drift and size corrections based on glycine and glutamic acid standards were undertaken.

Functional habits were attributed to species based on the growth forms described in the National Vegetation Attribute Manual (ESCAVI 2003) derived from Specht (1970), including ‘grass trees’ (members of the endemic Australian family Xanthorrhoeaceae), and with grasses and forbs further divided into annual and perennial groups. For TREND sites only, surface soil samples were suspended in demineralised water and portable probes were used to measure pH and electroconductivity in the field. Climate data were extracted from BIOCLIM layers modelled at 9 second resolution extracted from ANUCLIM 6.1 (Xu and Hutchinson 2013). Soil pH and electroconductivity were measured for the surface layer at all plots.

China

Data analysed here from the Northeast China Transect (NECT) were published previously by Prentice *et al*. (2011). The NECT consists of 33 sites spanning 19.2° longitude (~1,500 km) and 145-710 mm MAP (Figure 2). Full survey methodology, sample treatment and original analysis of data are described in Prentice *et al*. (2011). Briefly, sites appearing to have little anthropogenic or grazing disturbance were selected and the dominant species at each site were collected for isotopic analysis.

Global data set

In order to provide a point of comparison for our transect data, a global baseline was established using a global compilation of site-averaged leaf $\delta^{13}C$ values, primarily representing vegetation in Australia, southern Africa, Europe, North America and Asia published by Kohn (2010). The dataset was trimmed to include only sites from 145-980 mm MAP (the range of MAP for on the NECT, TREND and SWATT) to provide a meaningful comparison with the data from our transects.

Statistical analysis

The previous analysis of NECT data used Principal Component Analysis (PCA) and Redundancy Analysis to determine the primary environmental drivers of isotopic response, and the homogeneity of species responses was tested using standardised major axis regression (Prentice et al. 2011; Warton et al. 2012). Here, we repeat the PCA analysis for all three transects and the global data set and introduce new analyses.

The slope of the regression of $\delta^{13}C$ versus moisture availability for each C_3 species (m_s) was calculated as the slope of a linear least squares regression between leaf $\delta^{13}C$ and MAP for that species. Both raw p values and Bonferroni adjusted p values, which account for multiple tests of significance, were calculated. The common slope (m_c) of each transect was calculated for the regression of all species on the transect combined. Global slope (m_g) was calculated as a linear regression between leaf $\delta^{13}C$ and MAP for site-averaged data published by Kohn (2010) (n=392). For the NECT, m_c was calculated using data from 154 species (n=333), including 36 species found at ≥3 locations (n=186). For the TREND, the m_c regression was calculated for 150 species, each sampled at ≥4 locations (n=996). For the SWATT, the m_c regression was calculated for 47 species, each sampled at ≥7 locations, except for one species from 6 locations (*Grevillea hookeriana*), and three singletons (*Eremophila ionantha*, *Grevillea haplantha*, *Hibbertia rostellata*) that were measured by mistake but included as plants found on the transect and used to develop the common slope (n=552). The number of species occurrences was increased for the SWATT to improve the likelihood of obtaining statistically significant m_s regressions. To test the consistency of the $\delta^{13}C$ response to MAP at a landscape level, each transect m_c regression was compared against m_g and the other transect m_c regressions using Chow tests (Chow 1960).

All data analysis was performed using R (R Core Team 2017). To test the degree to which climatic and soil variables explained leaf δ^{13}C, a Principal Component Analysis (PCA) was conducted using FactoMineR (Lê et al. 2008). Linear regressions were also calculated between each environmental variable and leaf δ^{13}C for all plants on each transect and compared against m_g to test for transect-level differences. Ordinary least squares regressions and a PCA were also used by (Prentice et al. 2011), but were repeated here for all three transects.

In order to test for the presence of multiple species responses consistent with those hypothesised (Figure 1), we used Finite Mixtures of Regression (FMR) models to search for 'species archetypes' (Grun and Leisch 2008). For the purposes of defining the nature of responses, we defined 'biotic homeostasis' as $m_s < 1.5(m_c)$, 'universal scaling' as $m_s = m_c \pm 0.5(m_c)$, 'insensitivity', as $m_s = 0 \pm 0.5(m_c)$, and 'contrary', as $m_s > -0.5(m_c)$ (Figure 1). Models of 1-4 species archetypes for each transect were assessed using Akaike Information Criterion (AIC), with models differing <4 from the model with the lowest AIC for a given transect regarded as having strong support, <7 from the lowest AIC regarded as having moderate support, and an AIC >10 from the lowest AIC as having little to no support (Burnham and Anderson 2004).

To determine whether species response was affected by growth form, we ran linear mixed effect models using a maximum likelihood approach in the lme4 package (Bates et al. 2015). The first model used species as a random effect; and the second model used species as a random effect nested within growth form as a random effect. Model outputs were compared using an ANOVA, with model explanatory power assessed by comparing the correlation between the fitted and the observed values.

Results

Species distributions

For the TREND, a total of 150 species from 44 families were recorded at four or more sites (Table 1). There were 15 alien (non-native) species, including annual forbs (7), perennial forbs (2), annual grasses (5) and a vine on the TREND. We recorded 47 species from 21 families at six or more sites on the SWATT, all of which were native. On the NECT, a total of 42 species from 21 families were recorded at three or more sites, but native/alien status was not available for these species. Refer to Supplementary Table S1 for full species details.

Vegetation on all transects showed strong structural partitioning. On the NECT; forest communities dominated by trees, ferns, vines and sedges were found on the eastern (wet) end of the gradient, transitioning to meadows comprised of grasses, forbs and chenopods in the arid west (Figure 2; Figure S1; Ni and Zhang 2000). On the TREND, sedges, grass trees and vines were located predominantly at the southern (wet) end of the gradient, while chenopods and annual forbs dominated more northern (dry) sites (Figure 2; Figure S2; Chapter 5). The SWATT displayed a similar transition to the TREND, with trees and perennial forbs most common in the southern (mesic) sites, transitioning to chenopods, perennial grasses and a vine species in the more arid northern sites (Figure 2, Figure S3; Gibson et al. 2017). Vegetation complexity also changed with aridity, with wetter sites displaying complex multi-strata woodland communities, eventually transitioning to sparse chenopod shrublands and grasslands in the more arid north (TREND and SWATT) and east (NECT). The transition in growth forms with aridity was most graded on the TREND and SWATT, with most growth forms being found across much of those transects, whereas on the NECT, growth forms were more spatially partitioned.

Carbon isotope responses

Leaf δ^{13}C values varied by 9-11‰ across all transects, regardless of MAP range (NECT =-34.2‰ to -22.6‰, TREND=-34.0‰ to -22.8‰, SWATT=-31.7‰ to -20.5‰). Principal component analyses revealed Component 1 (the primary aridity gradient) explained the majority of observed leaf δ^{13}C variance (67.16%, 75.88% and 65.19% for the NECT, TREND and SWATT, respectively; Figure 3; Table 2). The length of the δ^{13}C response vector (in red) indicates the strength of the relationship with predictor variables. Vectors for predictor variables on a similar angle to the δ^{13}C response were positively correlated. Plant growth forms exhibited some differences in leaf δ^{13}C (Figure S4) but these were likely a result of differences in growth environment, rather than growth form (e.g. chenopods are restricted to drier environments whereas sedges occupy wetter environments).

Linear regressions revealed highly significant correlations of foliar leaf δ^{13}C values with several environmental variables (Table 3). For the NECT, the strongest environmental correlates were mean moisture index (precipitation divided by potential evapotranspiration) of the wettest quarter (MaxMI), MAP and mean annual moisture index (MAMI) (R^2=0.721, 0.715 and 0.714 respectively). For the TREND and SWATT, correlation between leaf δ^{13}C and environmental variables were much weaker. The strongest correlates for the TREND were MAP, mean moisture index of the driest quarter (MinMI) and precipitation of the wettest week (MaxP) (R^2=0.143, 0.139 and 0.133 respectively). On the SWATT, the strongest environmental correlates were MaxP, longitude and MAP (R^2=0.066, 0.025 and 0.013 respectively) (Table 3).

Because MAP has been determined to be the strongest global driver of leaf δ^{13}C (Diefendorf et al. 2010) and was in the top three strongest environmental correlates with leaf δ^{13}C across all of our transects, MAP was used as the predictor variable for subsequent analyses. The slope of the regressions between MAP and leaf $\delta^{13}C$ varied for all three transects (Figure 4; Table 4) and Chow tests confirmed that they were statistically distinguishable from both the global regression (m_g) and each other (Table 5). The NECT regression was much steeper than that for the global dataset published by Kohn (2010), lacking strongly negative $\delta^{13}C$ values for the desert steppe (MAP < 250 mm), and more positive $\delta^{13}C$ values at the forested end (MAP > 500mm) (Figure 4b). By contrast, the TREND regression was similar to the global dataset in slope and the spread of data, but the intercept was offset by -2.11‰ (Figure 4c). The SWATT regression was limited to a smaller MAP range (220-745 mm, with only three values >400mm) and was driven by these few more mesic data points. Nevertheless, the SWATT m_c appeared similar to the TREND and global regressions in terms of spread, slope and intercept (Figure 4d).

There were 28 species that showed statistically significant m_s ($p<0.05$) (10 on the NECT; 13 on the TREND and 5 on the SWATT; Table 6). Following Bonferroni correction, only *Asparagus dauricus* (Asparagaceae; NECT), *Dianella revoluta* (Xanthorrhoeaceae; TREND and SWATT) and *Platysace trachymenioides* (Apiaceae; SWATT) returned a statistically significant m_s (Table 6). The m_s for *D. revoluta* and *P. trachymenioides* was much steeper than the m_c of their transects, while *A. dauricus* had m_s values similar to the NECT m_c (Figure 5; Table 6).

Frequency histograms of m_s values revealed TREND plants were approximately normally distributed around the TREND m_c. The lower number of species meant that normality was difficult to assess for the NECT or SWATT (Supplementary Figure S5). m_s values varied considerably, with no apparent consistency between growth form or transect (Supplementary Figure S1-S3, Table S1). Just over a quarter (25.9%) of species displayed responses close to m_c, consistent with the universal scaling hypothesis presented by Prentice *et al*. (2011). 43.1% displayed steeper slopes than the common slope, consistent with biotic homeostasis, while 14.6% had slopes near 0 (insensitive). A fourth subset (16.4%) displayed a positive relationship between $\delta^{13}C$ and MAP (contrary) (Supplementary Figure S1-S3, Table S1).

Finite Mixture of Regression (FMR) models were used to further probe data sets for evidence of the existence of two, three and four distinct responses (Figure 6). For the NECT, the most supported model (based on AIC value) included three components, but all archetype responses were aligned with a universal scaling response, consistent with the findings of Prentice et al. (2011) for that transect. For the TREND, the most supported model was for two archetypes (universal scaling and insensitive), but the three archetype model (universal scaling, insensitive and biotic homeostasis) was also supported (i.e. had an AIC <7 higher than the two-archetype model; Figure 6). For the SWATT, a single response (i.e. universal scaling) was the strongest model, but two archetype (biotic homeostasis and contrary) and three archetype (biotic homeostasis and insensitive) models were also highly supported (i.e. had an AIC <4 higher than the single archetype model; Figure 6).

We used a linear mixed-effects model to test whether growth form significantly affected leaf $\delta^{13}C$. Due to the comparatively low sampling of NECT species (median observations per species $n = 4$), analysis was confined to the TREND and SWATT (median of six and 11 observations per species respectively). Growth form did not increase the explanatory power of the model (correlation between the fitted and the observed values of model without growth form as a fixed effect was stronger for both the TREND and the SWATT). Growth form was found to significantly affect leaf $\delta^{13}C$ for the TREND ($p<0.001$) but not the SWATT ($p>0.05$).

Discussion

The variety of m_c and m_s responses observed in species sampled from three subcontinental-scale transects is strong evidence that a universal scaling relationship between aridity and leaf $\delta^{13}C$ does not hold. Lack of a consistent relationship among regions could be due to a range of factors influencing both spatial variability between transects in different locations, and variability among species on the same transect due to differing sensitivity to aridity.

Variability between transects

The large difference observed for m_c between the NECT (-13.5 ‰ m^{-1}), TREND (-3.0 ‰ m^{-1}) and SWATT (-5.6 ‰ m^{-1}) is somewhat surprising, given that the transects traverse similar MAP gradients. One of the most obvious differences among transects is that the NECT is longitudinal, the TREND is latitudinal and the SWATT traverses both latitude and longitude (Figure 2). Latitude and longitude were less correlated with leaf $\delta^{13}C$ than climate variation across the gradients (Table 3), reinforcing previous findings that latitude does not have a strong effect once its impacts on climate are accounted for (Diefendorf et al. 2010). On the other hand, elevation is likely to limit discrimination, meaning plants at altitude will experience more positive $\delta^{13}C$ values (Körner et al. 1991). The NECT and TREND experience similar elevation change (1173 m and 913 m respectively) (Prentice et al. 2011; Chapter 3), but on the NECT, altitude changes consistently across the transect (highest at the desert steppe end), potentially exaggerating the effect of MAP on leaf $\delta^{13}C$. By contrast, elevation change on the TREND is not monotonic (Chapter 3), and the SWATT is confined to lower-elevation sand plains (Gibson et al. 2017).

Rainfall extremes may also play a role in influencing transect level leaf $\delta^{13}C$ responses. The difference in correlation strength between leaf $\delta^{13}C$ and MAP (strongest for the NECT and

TREND), versus leaf $\delta^{13}C$ and maximum precipitation (strongest for the SWATT) suggests that rainfall variability may be responsible for differences in m_c between the transects. However, the SWATT m_c is the least robust due to the narrow sampling range, and if the data point at ~750 mm MAP (*Dianella revoluta*) is removed, the regression is no longer significant.

The vegetation present on the gradients is also likely to have influenced transect-level $\delta^{13}C$ responses. Vegetation on the TREND and NECT is largely sclerophyllous and evergreen. However, the NECT traverses a change between sclerophyllous steppe vegetation and deciduous forests. Deciduous plant forms have different nutrient use strategies, and as a whole, deciduous communities are able to display higher carbon isotope discrimination than similar evergreen or xeric communities (Diefendorf et al. 2010). The presence of deciduous communities could therefore drive more negative leaf $\delta^{13}C$ values at the wetter end of the NECT, which, compounded with the effects of altitude increasing leaf $\delta^{13}C$ values at the drier end, creates a steeper overall slope than would be expected for a global compilation or gradient where deciduousness and altitude are not negatively associated.

The difference in observed m_c between transects suggests limitations in the concept of universal scaling as a global explanatory mode, as different regions have different m_c against which m_s are to be compared. Using a global dataset synthesised for a meta-analysis of plant $\delta^{13}C$ values (Kohn 2010), we calculated a global common slope (m_g) for the MAP range sampled on the TREND and NECT (145-980 mm) of -2.8 ‰ m^{-1}. Given that the m_g was similar to the m_c values calculated for the TREND and SWATT, the flora of these regions may be more representative of global carbon isotope responses than the NECT, which displayed an m_c more than three times steeper than the global common slope (Figure 4). While Kohn (2010) appears to be the most comprehensive global dataset published to date, it includes only 392 site-averaged data points within the MAP range of our study (compared to the 1881 individual species observations we present here).

Variability between species

We found a diverse range of species responses across all three transects, providing strong impetus to examine whether all species in an area display a similar isotopic response to aridity. However, the low number of species returning significant linear regressions (following Bonferroni correction; Table 6; Table S1) means some caution is required. For this reason, the Finite Mixture of Regressions approach was used to ‘borrow strength’ across many poorly-sampled species and evaluated using AIC values (Brewer et al. 2016; Burnham and Anderson 2004). The presence of only the universal scaling archetype in the NECT FMR models provides support for the conclusions of Prentice et al. (2011) that universal scaling was the only response mode present on the NECT. However, the small AIC difference in 2-3 archetype models on the TREND and SWATT indicates additional spread present in these larger data sets. Nevertheless, the strong performance of models showing insensitive (i.e. $m_s = 0 \pm 0.5(m_c)$), and biotic homeostasis (i.e. $m_s < 1.5(m_c)$) responses and detection of three species populations (*Dianella revoluta* on both the TREND and SWATT and *Platysace trachymenioides* on the SWATT) displaying a response consistent with biotic homeostasis provides support for the existence of non-universal scaling responses. We were not able to unambiguously confirm the existence of the four theoretical modes that we postulated. Nevertheless, to the degree that we are able to relate leaf $\delta^{13}C$ to MAP and stomatal regulation, our data imply a more complex relationship between leaf $\delta^{13}C$ and MAP than presented by Prentice *et al.* (2011).

Universal scaling ($m_s \approx m_c$) and biotic homeostasis ($m_s << m_c$) modes show a negative relationship between leaf $\delta^{13}C$ and rainfall, conforming to the expectations of more positive carbon isotope ratios with greater aridity due to reduced stomatal conductance and reduced c_i/c_a (Farquhar et al. 1989). Biotic homeostasis, rather than universal scaling, could occur in species that display the capacity to strongly adjust stomatal conductance, leading to higher discrimination in mesic environments, and/or the ability to maintain photosynthesis at low values of c_i in environments too arid for other plants to persist. Universal scaling, which requires a more muted m_s than biotic homeostasis, might then be more likely to occur in species which also adjust other traits in response to aridity, such as belowground carbon allocation and hydraulic architecture, in addition to stomatal conductance (Givnish et al. 2014).

The remaining modes, insensitive ($m_s \approx 0$) and contrary ($m_s >> 0$) are counter to the simplest interpretation of the interaction between water availability and carbon isotope fractionation during C_3 photosynthesis. Some flat or positive slopes may be artefactual, particularly when these occur in poorly sampled species (i.e. three or four occurrences). Yet, given >30% of species were insensitive or contrary, it seems reasonable to regard this phenomenon as genuine. Such responses could be attributed to opportunistic growth in a short window of favourable conditions (Ehleringer 1993), or through interactions with other species that may make climatically adverse conditions more suitable (Metz and Tielbörger 2016). Deep-rooted species may also be able to tap water unavailable to other plants in their region (e.g. groundwater). If plants were opportunistically able to access such water at the drier end of their distribution, it could reasonably override the prevailing climatic signal.

Nevertheless, the regular occurrence of these modes identified on both transects studied here is compelling. Reanalysis of data collected as part of other gradient studies, as we have done with the NECT, as well as examination of other leaf trait data to better constrain the proportion of leaf $\delta^{13}C$ driven by aridity, may provide further support for the existence of four different response modes.

To the extent that variable plant responses can be linked to aridity, m_s could be used to determine the relative sensitivity of C_3 plants to aridification and would be a useful factor to include in models of plant function and persistence under climate change. Understanding the potential mechanisms of each response mode is important in interpreting m_s values in an ecological context and ascribing a sensitivity to those species. Below, we discuss the empirical support for the theoretical modes presented in Figure 1, and some of the possible drivers of such responses.

Universal scaling

The hypothesis of universal scaling postulates that C_3 plants have similar patterns of stomatal adjustment, irrespective of phylogeny and traits (including growth form). Plant attributes are therefore not relevant for the purposes of modelling community and regional ecophysiology for C_3 plants (Prentice et al. 2011). We find that while m_s can approximate m_c, only a minority (25.7%) of the tested species conformed to this model (47.2% on the NECT, 26% on the TREND, 8.5% on the SWATT). A departure from universal scaling may simply represent data scatter, with biotic homeostasis and contrary responses being outlier measurements. However, the heteroscedasticity of species regressions shows a more positive skew than would be expected if universal scaling were the most common response (Supplementary Figure S5). The NECT and SWATT require additional species to be sampled to gain a better sense of m_s distribution. Nevertheless, it is possible that universal scaling may represent a ‘default’ response for plants in ideal conditions.

Biotic homeostasis

Biotic homeostasis was the most common response mode, with 43.3% of tested species showing $m_s > 1.5 \times m_c$ (16.7% on the NECT, 44.3% on the TREND, 63.8% on the SWATT). Plants with a wide capacity for stomatal adjustment and low desiccation tolerance are likely to show the greatest isotopic sensitivity to aridity and could be vulnerable to further drying. On the other hand, plants that are able to maintain photosynthesis in low-water conditions (and thus display more positive leaf δ^{13}C values) through leaf trait adjustment may be the most robust to future aridification. It is also possible that the local conditions of a plant can magnify the steepness of the climate gradient. For example, if individuals at the wetter end of a species' range were collected from unusually wet microhabitats (e.g. within drainage lines) and/or the plants at the drier end were collected from unusually dry microhabitats, this would have the potential to give the effect of a greater climatic gradient, and steeper m_s.

However, happenstance of microhabitat is unlikely to be a primary cause given that response modes are not uniform within groups restricted to a narrow range of microhabitats (e.g. sedges and ferns). It is more likely that high species sensitivity, or current climate stress, is the major driver of this response. If universal scaling were considered to be a 'default' response mode, climatic perturbation may have led to a shift towards biotic homeostasis. This hypothesis could be tested by repeated sampling of species exhibiting a biotic homeostasis response – if climate stress is indeed responsible, steeper m_s (and therefore more species displaying biotic homeostasis) should be recorded with time due to ongoing climate change.

Insensitive response

A minority of species (14.6%) were classified as insensitive (22.2% on the NECT, 15.3% on the TREND, 6.4% on the SWATT). A number of related scenarios could explain why some species had no appreciable isotopic response to changes in aridity. Annual species, and perennial species that limit foliage growth to the wetter parts of the year, are likely to display less leaf δ^{13}C effect from climate pressures because they fix carbon in periods of comparatively high rainfall. Another explanation for an apparently insensitive response is that deep rooted species are able to tap into permanent or ephemeral water resources not available to other plants, compensating for the effect of an arid climate on the plant's water balance and dampening the climate impact on m_s. Similarly, plants inhabiting relatively moist microhabitats (such as under shrubs and in drainage channels) may be able to extract additional moisture from the soil and/or limit water loss through evapotranspiration, which could lead to a shallower m_s than would be expected from the prevailing climatic conditions alone.

Contrary response

There were 16.3% of species that were classified as 'contrary' (13.9% on the NECT, 15.3% on the TREND, 21.3% on the SWATT). It may be tempting to dismiss these values as artefactual or attributable to sampling error, but four contrary species returned significant regressions prior to Bonferroni correction. Occasional positive slopes may be artefactual, particularly when these occur in poorly sampled species (i.e. three or four occurrences). Yet, given the abundance of these contrary species, one of which (*Ptilotus obovatus*) was measured at 13 locations, it seems reasonable to regard this phenomenon as biologically real. Within the stomatal conductance framework, contrary slopes could result from some plants accessing hidden water sources, allowing them to maintain low water use efficiency in otherwise arid conditions, or through a "grow fast" approach, in which plants persist throughout the year but confine most growth to a short growing season. Further investigation is likely to reveal a range of circumstances in which contrary responses can occur.

Strengths of the transect approach

By measuring species *in situ* in relatively intact habitat, the plants we measured are likely to have benefitted from interactions with other plants (e.g. through facilitation (Maestre et al. 2009) and soil biota (Wardle et al. 2004)). It is probable that these interactions would make plants more resilient to aridification, both in space and time. While it was beyond the scope of this study to quantify biotic interactions and their impacts, the approach we have taken allows a realistic inference of plant response to environment to be interpreted with potentially important associations intact.

Many studies of plant carbon isotopes on climatic gradients have focused on fewer than five species (e.g. Bai et al. 2008; Gouveia and Freitas 2009; Lambrecht and Dawson 2007), several closely related species (e.g. eucalypts; Schulze et al. 2006) or only one functional group (e.g. woody vegetation; Bai et al. 2008; Schulze et al. 2006). Functionally similar or phylogenetically related species are often spatially partitioned on environmental gradients, presumably due to optimisation to a particular environmental envelope. Restricting analysis to a subset of plants is helpful in the identification of a pattern within that group, but is likely to limit the range of responses observed. We caution against developing regional m_c values based on restricted collections of only a few species or growth forms, as it is likely to skew broader inference of plant sensitivity for that region. We recommend extensive bioclimatic gradients be used for the development of additional m_s and m_c values in additional biomes, which will enable the development of a more comprehensive m_g than is currently available.

Ideally, such studies would be combined with common garden or greenhouse experiments to help disentangle the different environmental drivers of leaf $\delta^{13}C$ (Caddy-Retalic et al. 2017).

Ecological implications and potential for monitoring

The data presented here indicate that it may be possible to posit the current and likely future sensitivity of plant species to aridification by comparing the derived m_s for individual species across their natural range with a regional m_c or global m_g value. In this study, the most extreme (and variable) m_s values were recorded for species collected at less than six locations, suggesting that collection of seven or more sites is required to provide a systematically reliable classification of aridity response. Indeed, given that only three of 233 C_3 species tested returned statistically significant m_s values following Bonferroni correction, future research should focus on more intensive collection within species.

One weakness of the approach detailed here is that any leaf $\delta^{13}C$ change within or between species is attributed to stomatal conductance. Other factors that have been established to affect carbon isotope discrimination, as detailed in Cernusak *et al* (2013), were not considered. For example, mesophyll conductance can modify leaf $\delta^{13}C$ by 2-4‰ (Flexas et al. 2008) and respiration by >3‰ (Gillon and Griffiths 1997). Given that the mean within-species leaf $\delta^{13}C$ range was 3.4‰ (though >8.5‰ for the vines *Glycine rubiginosa* and *Clematis microphylla*) in this study, unaccounted-for discrimination factors could introduce much of the variability in the data presented here. The impact of other known determinants of leaf $\delta^{13}C$ including shading and nutrient availability could be investigated through the examination of other leaf traits including stomatal density, leaf nutrient content and specific leaf area. Ongoing research will improve our ability to model the degree to which these factors contribute to carbon isotope discrimination for different species and environmental conditions.

Leaf carbon isotope measurement represents a useful tool for ongoing monitoring of climate stress in C_3 plants and is a rapid and cost-effective method requiring minimal fieldwork. The quantitative results yielded by this approach can be used to further refine our understanding of the tolerances and adaptive capacity of vascular plants and improve predictive models for the future. Careful consideration should be given to which species to monitor. A diverse and ecologically representative mix of species should be selected if possible, including dominant species, as their responses may result in localised community transformation.

Directions for future research

Our analyses interpret leaf $\delta^{13}C$ as exclusively a factor of stomatal conductance that, while likely to drive the majority of observed $\delta^{13}C$ change across aridity gradients, is not the only determinant. Future research should focus on relating changes in leaf $\delta^{13}C$ on bioclimatic gradients to a larger range of variables, potentially through the measurement of other leaf traits (e.g. specific leaf area and nutrient content). Controlling other drivers of $\delta^{13}C$ would allow the degree to which stomatal conductance controls leaf $\delta^{13}C$ to be quantified and verify the utility of species-specific regressions as a tool for predicting sensitivity to aridity.

Conclusion

We provide evidence for multiple modes of leaf stable carbon isotope ratio response to moisture availability for C_3 plants. Comparison of data from three subcontinental-scale transects indicates that there is no ‘one size fits all’ approach to predicting aridity sensitivity at a species or regional level. Nevertheless, developing species-specific regressions shows potential as a tool to determine sensitivity to aridity and would be an informative and effective approach to monitoring impacts on plant function and persistence under climate change. Indicative delineation of four modes provides a framework for the interpretation of future carbon isotope studies in an ecological context.

Author contributions

SCR and FAM designed the study. SCR led the field work in South Australia, conducted data analysis and wrote the first draft of the manuscript. FAM and SCR interpreted the results. SJvL and MB contributed samples from Western Australia. All authors reviewed and edited the final manuscript.

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Data accessibility

All data used in this manuscript has been uploaded to publically accessible data repositories. The species presence and isotope data from the NECT, TREND and SWATT have been uploaded to the AEKOS data portal (www.aekos.org.au). The isotope data from the global meta-analysis is available as Supplementary Information Dataset S1 from Kohn (2010).

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Table 1: Number of tested species by growth form for each transect

Growth form	NECT	TREND	SWATT
Annual grasses	-	5	-
Perennial grasses	4	5	3
Sedges	2	2	4
Annual forbs	-	23	-
Perennial forbs	17	41	2
Ferns	1	3	-
Vines	-	5	1
Chenopods	1	10	3
Grass trees	-	2	-
Shrubs	5	40	30
Trees	6	12	4
Mistletoes	-	2	-
Total	**36**	**150**	**47**

Table 2: Component description for Principal Component Analyses of each gradient

	NECT		TREND		SWATT	
	Component 1	Component 2	Component 1	Component 2	Component 1	Component 2
Leaf δ^{13}C	-0.85***		-0.37***			-0.16***
MAT	0.28***	0.96***	-0.92***	0.35***	-0.98***	0.16***
MinT		0.68***	0.48***	0.86***	-0.39***	0.53***
MaxT	-0.57***	0.66***	-0.96***		-0.97***	
MAP	0.99***		0.98***	0.09***		0.41***
MinP	0.90***	-0.29***	0.95***	-0.16***	0.84***	-0.46***
MaxP	0.91***		0.95***	0.20***		0.95***
MAMI	0.99***	-0.12*	0.98***		0.92***	0.36***
MinMI	0.94***	-0.25***	0.96***		0.87***	-0.25***
MaxMI	0.99***		0.96***		0.92***	0.27***
Elevation	-0.79***	-0.56***	0.18***	0.97***	-0.76***	-0.17***
Soil pH	[redacted]	[redacted]	-0.85***	0.12***	[redacted]	[redacted]
Soil EC	[redacted]	[redacted]			[redacted]	[redacted]
Variance explained	67.16%	22.98%	75.88%	17.50%	65.19%	18.91%
Total variance explained	90.14%		93.38%		84.10%	

Mean Annual Temperature (MAT; Bio01); Mean temperature of warmest quarter (MaxT; Bio10); Mean temperature of coldest quarter (MinT; Bio11); Mean Annual Precipitation (MAP; Bio012); Precipitation of wettest week (MaxP; Bio13); Precipitation of driest week (MinP; Bio14); Mean annual moisture index (MAMI; Bio28); Mean moisture index of the driest quarter (MinMI; Bio33) and Mean moisture index of the wettest quarter (MaxMI; Bio32) were extracted from BioClim layers of a 1960-2014 long-term average at 9 second (ca. 30m resolution). *p<0.05, ***p<0.001

Table 3: Linear regression R^2 statistics for leaf $\delta^{13}C$ with environmental variables

Category	Variable	NECT	TREND	SWATT
Temperature (°C)	MAT	0.032***	0.116***	-0.002 ns
	MaxT	0.254***	0.124***	-0.002 ns
	MinT	0.120***	0.017***	-0.002 ns
Precipitation (mm)	MAP	0.715***	0.143***	0.013**
	MaxP	0.568***	0.133***	0.066***
	MinP	0.587***	0.112***	0.004 ns
Moisture Index	MAMI	0.714***	0.128***	0.010*
	MaxMI	0.721***	0.113***	0.006*
	MinMI	0.641***	0.139***	0.003 ns
Soil	Soil pH		0.092***	
	Soil EC		0.002 ns	
Landscape	Elevation (m asl)	0.445***	0.017***	-0.002 ns
	Latitude	0.157***	0.094***	-0.000 ns
	Longitude	0.711***	0.018***	0.025***

Mean Annual Temperature (MAT; Bio01); Mean temperature of warmest quarter (MaxT; Bio10); Mean temperature of coldest quarter (MinT; Bio11); Mean Annual Precipitation (MAP; Bio012); Precipitation of wettest week (MaxP; Bio13); Precipitation of driest week (MinP; Bio14); Mean annual moisture index (MAMI; Bio28); Mean moisture index of the driest quarter (MinMI; Bio33) and Mean moisture index of the wettest quarter (MaxMI; Bio32) were extracted from BioClim layers of a 1960-2014 long-term average at 9 second (ca. 30m resolution). * ($p < 0.05$), **$p<0.01$, ***$p<0.001$, ns (not significant).

Table 4: Descriptive statistics for $\delta^{13}C$ regressions with MAP (m_c) for the TREND, NECT, the subset of Kohn (2010) values within the climatic range of TREND and NECT (m_g); and an updated global compilation of TREND, NECT and Kohn data (m_g).

	Kohn global subset (m_g)	NECT (m_c)	TREND (m_c)	SWATT (m_c)
n species	na	153	150	50
n observations	392	333	996	552
Slope (‰ m^{-1})	-2.8	-13.5	-3.0	-5.6
Intercept	-25.3572	-22.0081	-27.4683	-25.4224
Adjusted R^2	0.171	0.701	0.143	0.013
p value	<0.0001***	<0.0001***	<0.0001***	0.0037**

**p<0.005, ***p<0.001

Table 5: Chow tests comparing each m_c regressions for each transect with the global regression (m_g) and the other transects.

Test	F value	Degrees of freedom (numerator)	Degrees of freedom (denominator)	*p* value
m_g vs NECT m_c	39.020	2	940	<0.001***
m_g vs TREND m_c	251.269	2	1384	<0.001***
m_g vs SWATT m_c	264.778	2	721	<0.001***
NECT m_c vs TREND m_c	175.512	2	1325	<0.001***
TREND m_c vs SWATT m_c	89.184	2	1544	<0.001***
NECT m_c vs SWATT m_c	42.459	2	881	<0.001***

***p<0.001

Table 6: Linear regression statistics for species displaying a statistically significant regression between mean annual precipitation and leaf $\delta^{13}C$

Species	n	Growth form	MAP range	$\delta^{13}C$ range	m_s (‰ m^{-1})	R^2	p	Adj. p	Transect
Acacia pycnantha	12	Tree	565.92	5.6‰	-6.0	0.382	0.0191	2.8588	TREND
Agropyron michnoi	3	Per. Grass	49.98	2.1‰	-43.0	1.000	0.0075	0.2713	NECT
Allium mongolicum	6	Per. Forb	200.32	5.5‰	-22.1	0.751	0.0160	0.5752	NECT
Allium ramosum	11	Per. Forb	239.10	2.8‰	-7.0	0.553	0.0052	0.1887	NECT
Allocasuarina spinosissima	17	Shrub	58.66	3.9‰	-20.8	0.208	0.0377	1.7731	SWATT
Allocasuarina verticillata	9	Tree	565.92	5.3‰	-8.1	0.493	0.0210	3.1517	TREND
Artemisia frigida	12	Per. Forb	173.43	3.2‰	-9.6	0.298	0.0387	1.3916	NECT
Artemisia sacrorum	5	Per. Forb	241.75	3.3‰	-12.4	0.839	0.0185	0.6670	NECT
Asparagus dauricus	7	Per. Forb	437.79	6.3‰	-12.7	0.882	0.0011	0.0385*	NECT
Avena barbata	6	Ann. Grass	449.94	2.8‰	-5.4	0.719	0.0206	3.0911	TREND
Bulbine bulbosa	4	Per. Forb	400.70	3.0‰	-6.6	0.859	0.0482	7.2289	TREND
Cassinia laevis	6	Shrub	253.81	3.9‰	-13.3	0.592	0.0454	6.8114	TREND
Dianella revoluta	23	Per. Forb	491.50	6.98‰	-10.9	0.402	0.0007	0.0324*	SWATT
Dianella revoluta	15	Per. Forb	603.61	4.3‰	-7.4	0.656	0.0002	0.0228*	TREND
Dodonaea viscosa	16	Shrub	644.75	3.7‰	-4.2	0.493	0.0014	0.2175	TREND
Eremophila maculata	4	Shrub	132.37	4.8‰	35.7	0.992	0.0027	0.4102	TREND
Eucalyptus leptopoda	13	Tree	81.98	2.41‰	-19.0	0.281	0.0361	1.6987	SWATT
Goodenia blackiana	7	Per. Forb	334.93	4.9‰	-11.4	0.576	0.0293	4.3988	TREND
Lespedeza davurica	9	Per. Forb	254.93	3.9‰	-11.8	0.721	0.0023	0.0833	NECT
Lysiana exocarpi	6	Mistletoe	372.55	3.2‰	-7.6	0.691	0.0251	3.7676	TREND
Nicotiana simulans	6	Ann. Forb	120.23	5.2‰	32.5	0.623	0.0383	5.7510	TREND
Pittosporum angustifolium	4	Tree	138.28	3.0‰	20.9	0.883	0.0399	5.9793	TREND
Platysace trachymenioides	12	Shrub	58.66	4.0‰	-58.2	0.720	0.0003	0.0140*	SWATT
Potentilla chinensis	7	Per. Forb	358.70	4.1‰	-10.9	0.577	0.0291	1.0461	NECT
Pteridium aquilinum	4	Fern	154.73	3.6‰	-21.9	0.943	0.0191	0.6864	NECT
Ptilotus obovatus	13	Shrub	45.46	5.58‰	82.8	0.473	0.0056	0.2625	SWATT
Rhagodia parabolica	7	Chenopod	283.71	5.8‰	-13.9	0.716	0.0102	1.5229	TREND
Stipa sareptana	10	Per. Grass	198.18	3.3‰	-9.3	0.424	0.0247	0.8888	NECT

n=number of individuals sampled; m_s = Species level slope of the linear regression between MAP and leaf $\delta^{13}C$.

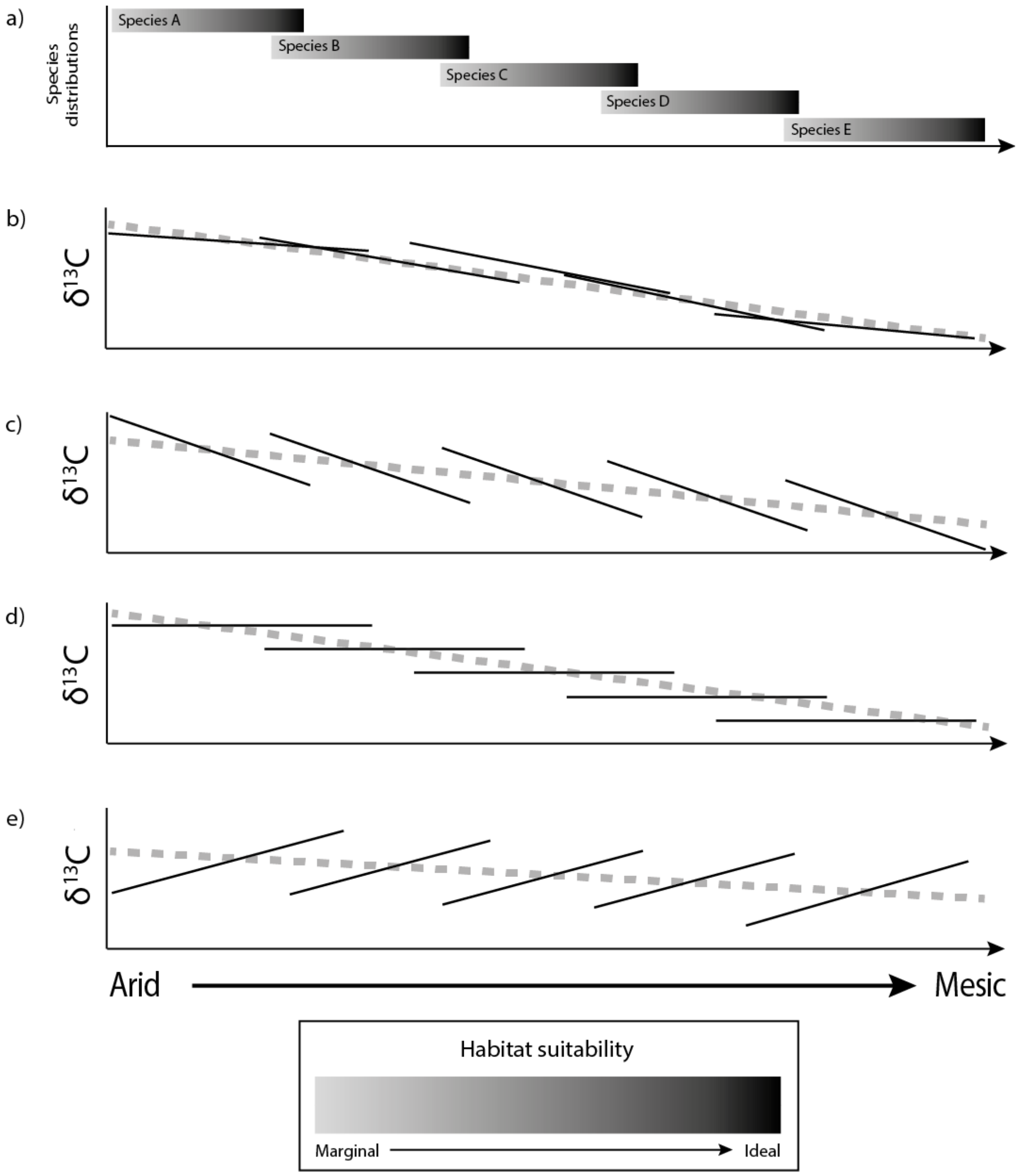

Figure 1: Theoretical framework for potential m_s responses in C_3 plants occupying successive niches on an aridity gradient. Solid lines represent individual species (m_s), dashed line represents all species on a gradient (m_c). a) hypothetical species distribution on a gradient; b) "universal scaling", with response of similar slope both within and between species; c) "biotic homeostasis", with individual species response slopes more than 1.5x the common slope; d) insensitivity within species, but stepped response between species; and e) inverse or "contrary" response (i.e. not consistent with expectations of carbon isotope discrimination increasing with moisture availability).

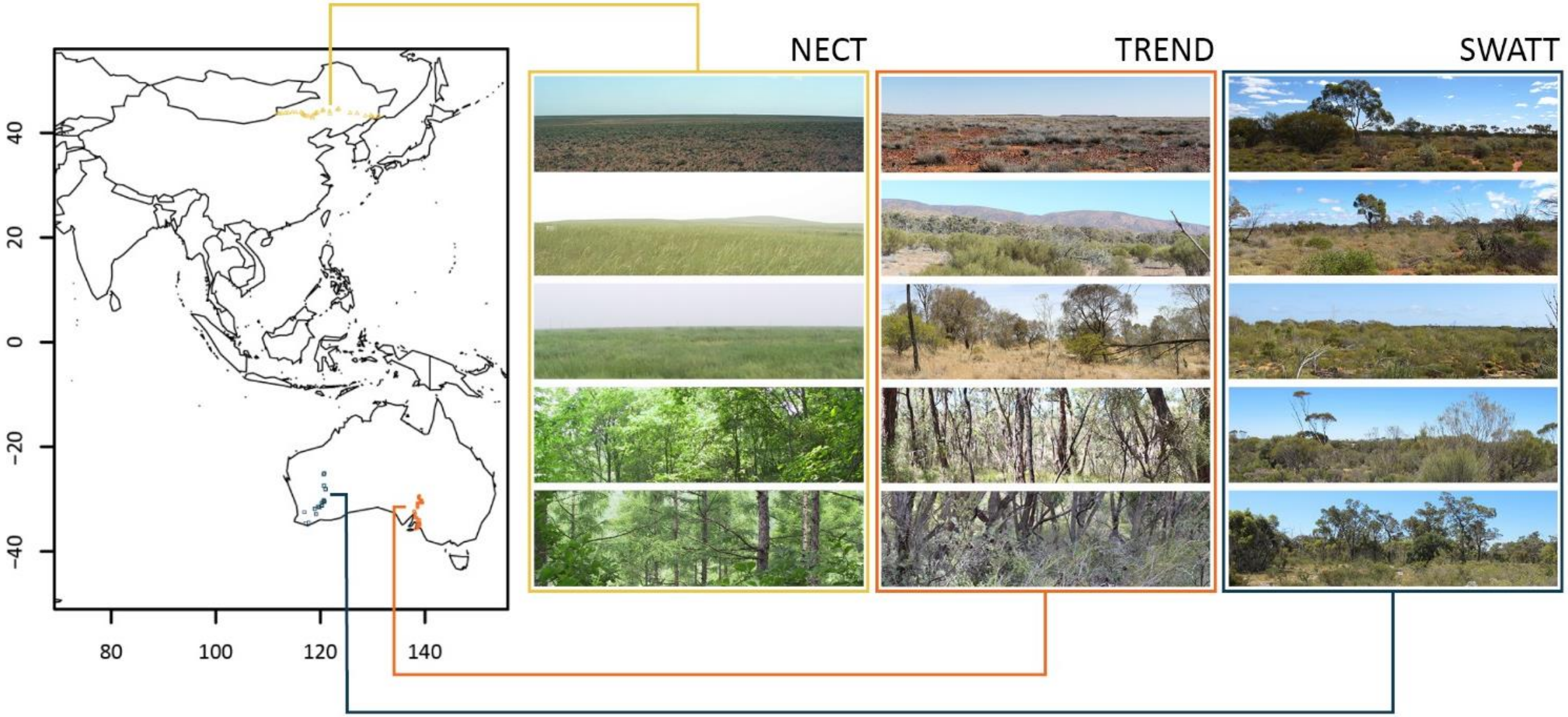

Figure 2: Plot locations for the NECT (China; yellow triangles), TREND (South Australia; orange circles) and SWATT (Western Australia; blue squares) with exemplar photographs to show ecosystem change (note NECT transition is east to west; TREND and SWATT are north to south).

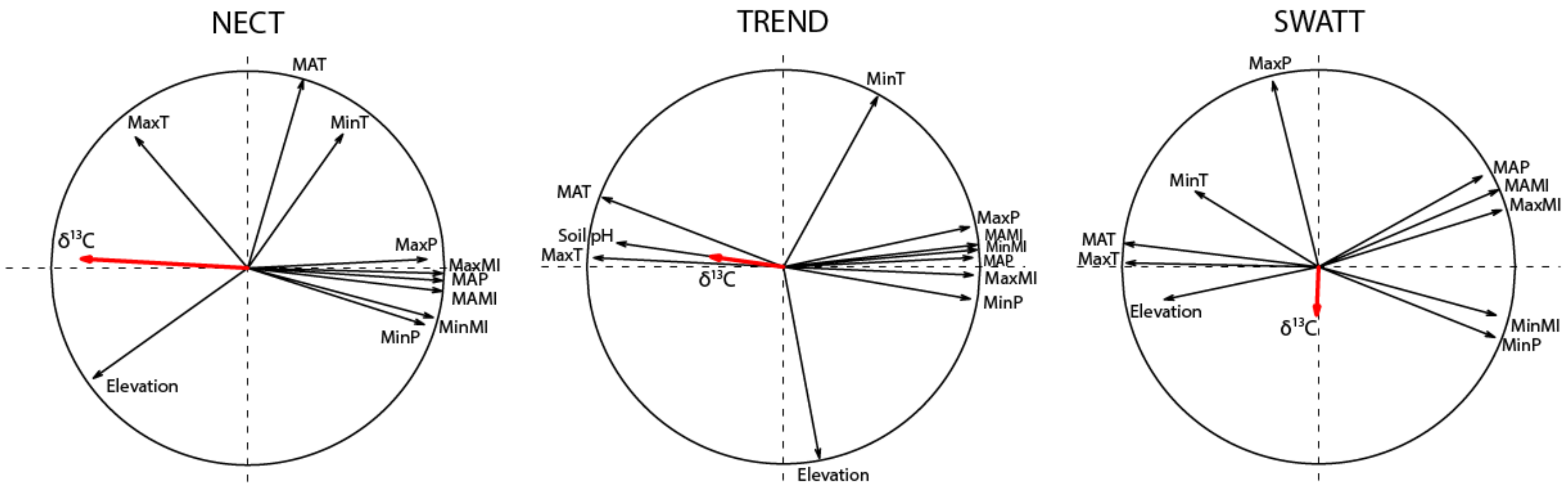

Figure 3: Principal component analysis showing vectors for measured traits and environmental variables. Circle width from -1 to 1. Leaf $\delta^{13}C$ response variable shown in red. NECT and TREND leaf $\delta^{13}C$ most strongly (negatively) correlated with MAP and MAMI; SWATT leaf $\delta^{13}C$ most strongly (negatively) correlated with MaxP. Mean Annual Temperature (MAT; Bio01); Mean temperature of warmest quarter (MaxT; Bio10); Mean temperature of coldest quarter (MinT; Bio11); Mean Annual Precipitation (MAP; Bio012); Precipitation of wettest week (MaxP; Bio13); Precipitation of driest week (MinP; Bio14); Mean annual moisture index (MAMI; Bio28); Mean moisture index of the driest quarter (MinMI; Bio33); Mean moisture index of the wettest quarter (MaxMI; Bio32).

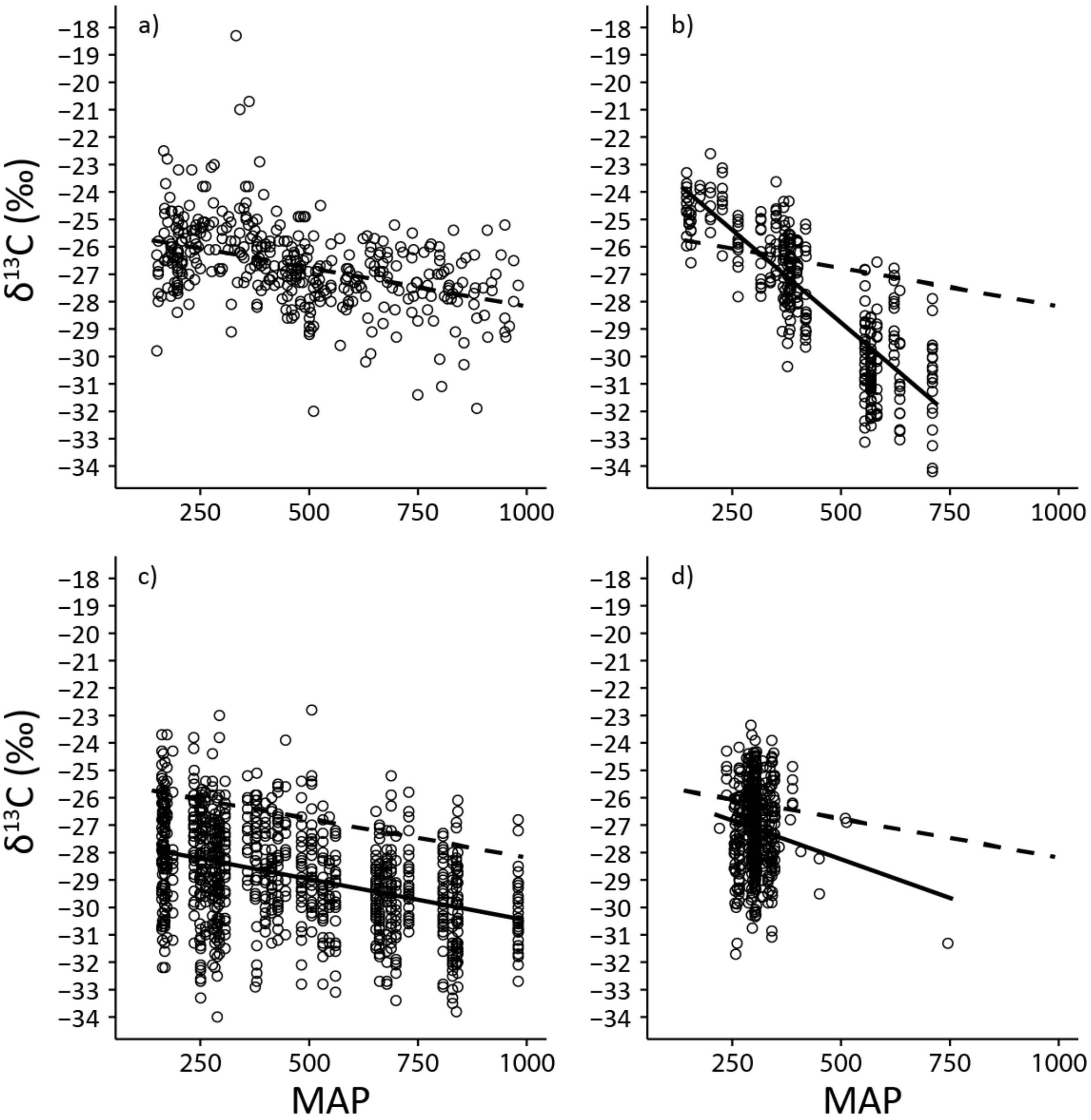

Figure 4: Leaf stable carbon isotope ratio ($\delta^{13}C$) relationship with mean annual precipitation (MAP) for (a) a global meta-analysis of C_3 plants (published by Kohn (2010) and trimmed to 145-980 mm MAP to match the transects presented here), (b) the NECT (*n*=333), (c) the TREND (*n*=996), and (d) the SWATT (*n*=552). Dashed line=m_g (MAP~leaf $\delta^{13}C$ regression) for (a); *n*=392, r^2=0.17, $p<0.001$). Solid lines= m_c (MAP~leaf $\delta^{13}C$ regressions) for the NECT ((b); r^2=0.70, p<0.001), TREND ((c); r^2=0.14, $p<0.001$) and the SWATT ((d); r^2=0.01, $p<0.05$).

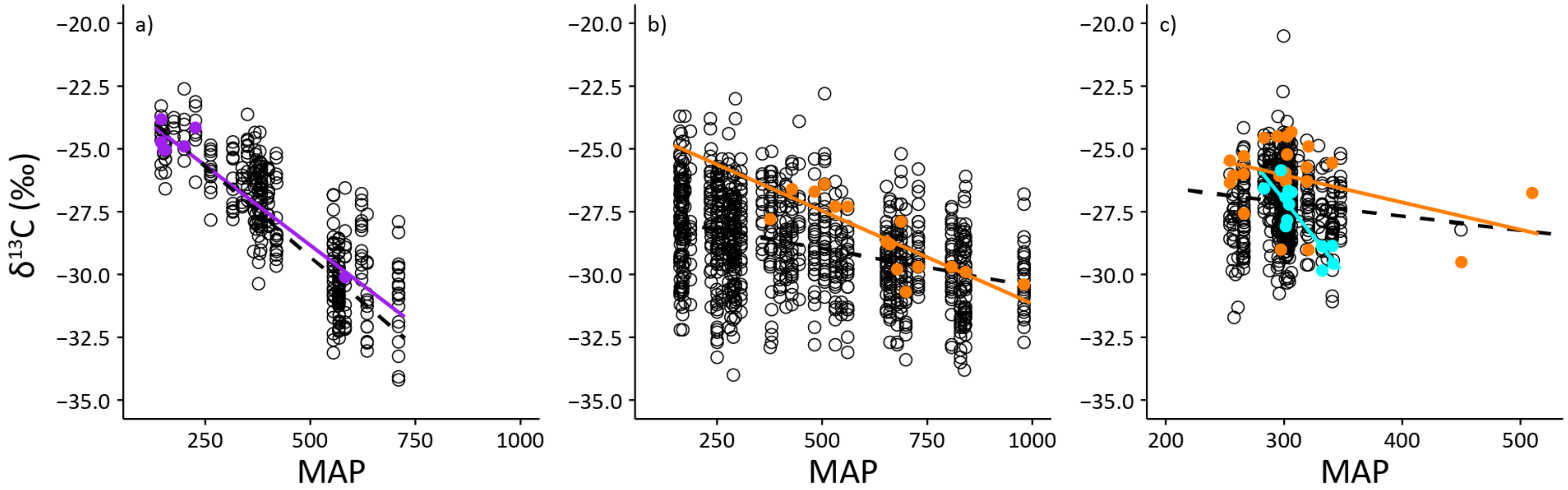

Figure 5: Leaf stable carbon isotope ratio ($\delta^{13}C$) relationship with mean annual precipitation (MAP) for the NECT (a), the TREND (b) and SWATT (c) with species displaying a significant MAP~$\delta^{13}C$ regression (m_s) following Bonferroni correction. Highlighted species are *Asparagus dauricus* (NECT; purple; r^2=0.88*); *Dianella revoluta* (TREND and SWATT; orange; NECT r^2=0.40*; TREND R^2=0.66*) and *Platysace trachymenioides* (SWATT; cyan; r^2=0.72*). Black dashed lines represent transect-level MAP~$\delta^{13}C$ regression (m_c). *=adj. p <0.05.

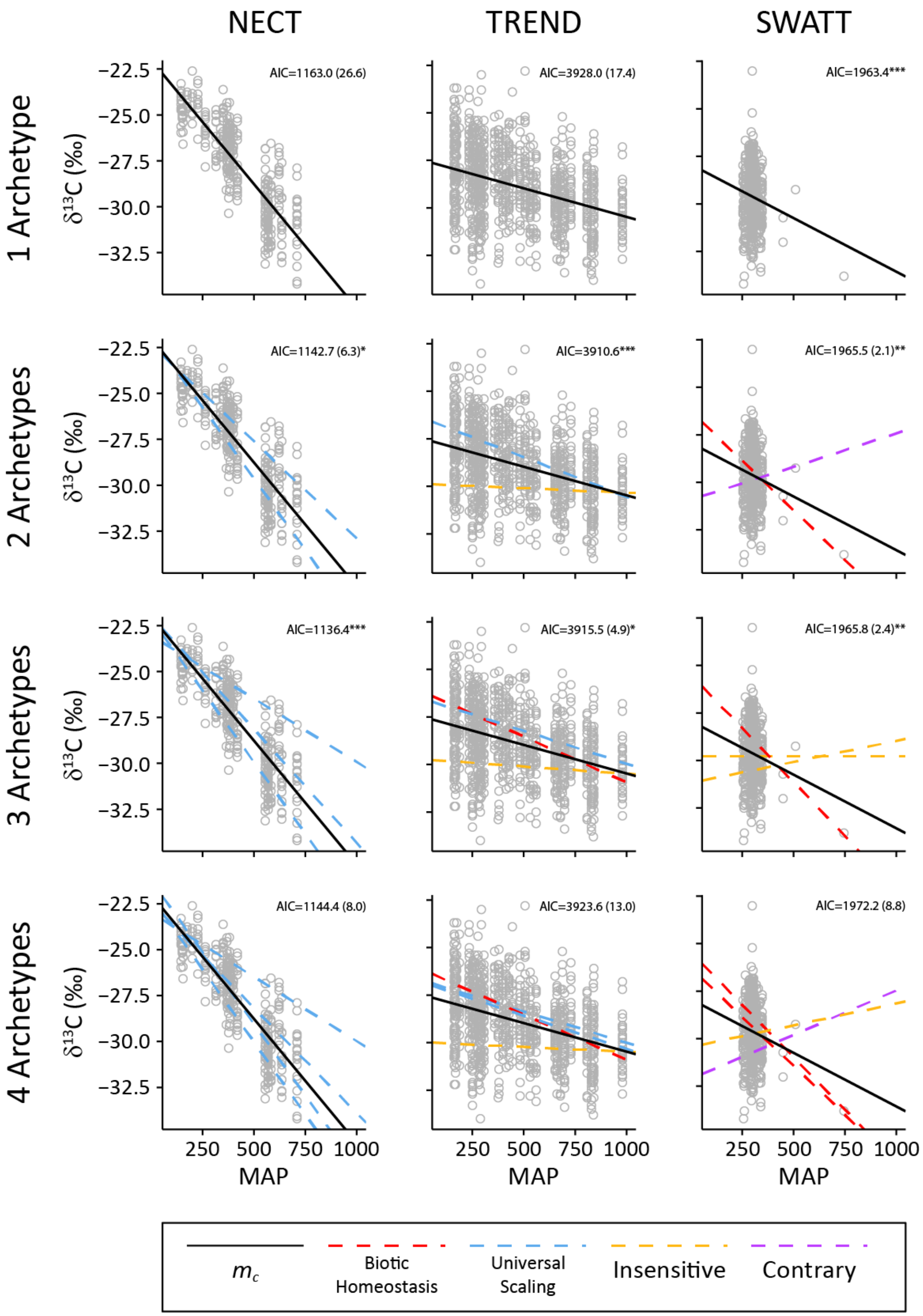
NECT
TREND
SWATT
1 Archetype
2 Archetypes
3 Archetypes
4 Archetypes
δ13C (‰)
−22.5
−25.0
−27.5
−30.0
−32.5
AIC=1163.0 (26.6)
AIC=3928.0 (17.4)
AIC=1963.4***
AIC=1142.7 (6.3)*
AIC=3910.6***
AIC=1965.5 (2.1)**
AIC=1136.4***
AIC=3915.5 (4.9)*
AIC=1965.8 (2.4)**
AIC=1144.4 (8.0)
AIC=3923.6 (13.0)
AIC=1972.2 (8.8)
250
500
750
1000
MAP
m_c
Biotic Homeostasis
Universal Scaling
Insensitive
Contrary

Figure 6: Response archetypes for two, three and four component species archetype models overlaid on C_3 leaf $\delta^{13}C$ data for the NECT, TREND and SWATT including a transect-wide leaf $\delta^{13}C$~MAP response (m_c). Archetype models (dashed lines) are classified based on their conformance to theoretical "modes" of carbon isotope response (Figure 1). Models were assessed based on AIC score (***=most supported model, **highly supported (AIC <4 from most supported model), *moderately supported (AIC <7 from most supported model). The most well-supported-models were three archetypes (all universal scaling) for the NECT, two or three archetypes (universal scaling, insensitive and biotic homeostasis) for the TREND and one to three archetypes (biotic homeostasis, contrary and insensitive) for the SWATT.

Supplemental Information

Table S1: Statistics for all tested species

Species*	*n*	Family	Growth form	Origin	Min MAP	Max MAP	Min δ^{13}C	Max δ^{13}C	Intercept	m_s (‰ m^{-1})	R^2	*p*	Transect	Mode$^\wedge$
Acacia colletioides	8	Fabaceae	Shrub	Native	254.20	320.63	-28.7	-25.4	-32.7053	18.3	-0.01651	0.382824	SWATT	CO
Acacia inaequiloba	9	Fabaceae	Shrub	Native	301.49	311.94	-30.1	-25.7	-69.5183	135.1	-0.00277	0.355652	SWATT	CO
Acacia ligulata	6	Fabaceae	Shrub	Native	161.55	428.35	-29.7	-25.6	-28.1455	3.0	-0.17268	0.634633	TREND	CO
Acacia ligulata	10	Fabaceae	Shrub	Native	257.04	304.44	-30.3	-26.0	-20.0565	-26.2	0.019205	0.309724	SWATT	BH
Acacia myrtifolia	6	Fabaceae	Shrub	Native	506.13	979.90	-31.7	-28.9	-29.3252	-1.2	-0.21356	0.746391	TREND	IS
Acacia oswaldii	5	Fabaceae	Shrub	Native	168.43	413.97	-29.2	-25.8	-27.2605	-2.2	-0.30252	0.8072	TREND	US
Acacia paradoxa	7	Fabaceae	Shrub	Native	546.30	838.25	-32.1	-27.3	-20.4516	-12.9	0.389888	0.079224	TREND	BH
Acacia pycnantha	12	Fabaceae	Tree	Native	413.97	979.90	-32.0	-26.4	-25.5028	-6.0	0.381831	0.019059	TREND	BH
Acacia tetragonophylla	12	Fabaceae	Shrub	Native	161.55	307.00	-30.1	-26.1	-28.9119	4.3	-0.06733	0.59227	TREND	CO
Acacia victoriae	10	Fabaceae	Shrub	Native	168.24	283.29	-28.8	-25.5	-24.0683	-12.9	0.147138	0.148773	TREND	BH
Acacia yorkraekinensis	8	Fabaceae	Shrub	Native	294.35	347.62	-29.3	-26.5	-28.0317	-0.4	-0.16661	0.986886	SWATT	US
Acaena echinata	9	Rosaceae	Per. Forb	Native	653.04	980.00	-32.0	-28.7	-25.119	-6.8	0.304552	0.071498	TREND	BH
Acer pictum	4	Sapindaceae	Tree		555.22	709.95	-30.3	-28.8	-27.6035	-3.4	-0.31223	0.646195	NECT	IS
Agropyron michnoi	3	Poaceae	Per. Grass		350.54	400.52	-26.8	-24.6	-9.55413	-43.0	0.99972	0.007537	NECT	BH
Alectryon oleifolius	8	Sapindaceae	Tree	Native	236.02	428.35	-28.6	-26.5	-26.7751	-3.2	-0.03432	0.414614	TREND	US
Allium mongolicum	6	Amaryllidaceae	Per. Forb		200.20	400.52	-28.1	-22.6	-18.5673	-22.1	0.751131	0.015979	NECT	BH
Allium ramosum	11	Amaryllidaceae	Per. Forb		145.05	384.15	-26.6	-23.8	-23.2966	-7.0	0.553386	0.005241	NECT	US
Allocasuarina campestris	9	Casuarinaceae	Shrub	Native	299.07	341.72	-30.9	-26.0	-18.7617	-28.4	0.003596	0.344193	SWATT	BH
Allocasuarina muelleriana	4	Casuarinaceae	Tree	Native	399.11	841.06	-31.6	-28.6	-27.8939	-2.7	-0.29809	0.63311	TREND	US
Allocasuarina spinosissima	17	Casuarinaceae	Shrub	Native	283.07	341.72	-29.4	-25.5	-18.9108	-26.9	0.207671	0.037726	SWATT	BH
Allocasuarina verticillata	9	Casuarinaceae	Tree	Native	413.97	980.00	-30.7	-25.4	-23.5534	-8.1	0.493037	0.021011	TREND	BH

Amphipogon caricinus	16	Poaceae	Per. Grass	Native	257.04	340.90	-31.1	-27.2	-25.2196	-12.3	0.01816	0.277367	SWATT	BH
Amyema miquelii	10	Loranthaceae	Epiphyte	Native	249.16	807.80	-31.6	-29	-29.8167	-1.6	0.088006	0.208823	TREND	US
Anagallis arvensis	8	Primulaceae	Ann. Forb	Alien	288.631	829.052	-33.5	-27.6	-27.2814	-5.4	0.171304	0.168784	TREND	BH
Artemisia frigida	12	Asteraceae	Per. Forb		227.09	400.52	-29.5	-26.3	-24.5627	-9.6	0.297632	0.038655	NECT	US
Artemisia sacrorum	5	Asteraceae	Per. Forb		380.18	621.93	-30.2	-26.9	-22.4772	-12.4	0.838831	0.018529	NECT	US
Artemisia scoparia	3	Asteraceae	Per. Forb		376.77	418.94	-30.4	-26.9	-27.3177	-3.7	-0.99505	0.968315	NECT	IS
Arthropodium strictum	7	Asparagaceae	Per. Forb	Native	399.11	980.00	-30.5	-29.0	-28.8601	-1.4	0.101443	0.251847	TREND	IS
Asparagus asparagoides	6	Asparagaceae	Vine	Alien	357.87	807.80	-32.8	-26.0	-24.5118	-8.2	0.100294	0.280116	TREND	BH
Asparagus dauricus	7	Asparagaceae	Per. Forb		145.05	582.84	-30.1	-23.8	-22.4841	-12.7	0.881881	0.001071	NECT	US
Aster altaicus	3	Asteraceae	Per. Forb		316.03	380.18	-27.4	-25.9	-34.433	22.1	0.876668	0.159761	NECT	CO
Astroloma conostephioides	6	Ericaceae	Shrub	Native	399.11	729.13	-31.3	-28.6	-30.0137	0.3	-0.24893	0.956181	TREND	IS
Astroloma humifusum	11	Ericaceae	Shrub	Native	376.29	838.25	-31.8	-28.6	-29.3979	-1.3	-0.04797	0.480249	TREND	IS
Austrostipa elegantissima	6	Poaceae	Per. Grass	Native	293.91	688.07	-29.2	-26.8	-26.6449	-3.0	0.018128	0.354956	TREND	US
Austrostipa elegantissima	14	Poaceae	Per. Grass	Native	283.07	320.63	-27.0	-24.4	-21.2089	-14.6	-0.01287	0.378879	SWATT	BH
Austrostipa mollis	4	Poaceae	Per. Grass	Native	506.13	729.13	-29.9	-27.3	-22.0189	-10.8	0.686508	0.110615	TREND	BH
Austrostipa nitida	4	Poaceae	Per. Grass	Native	234.26	357.87	-29.2	-27.1	-24.0306	-15.2	0.511089	0.178989	TREND	BH
Austrostipa scabra	4	Poaceae	Per. Grass	Native	293.91	688.07	-26.9	-23.0	-23.5723	-4.4	-0.21248	0.56219	TREND	US
Avena barbata	6	Poaceae	Ann. Grass	Alien	357.87	807.80	-30.8	-28.0	-26.3427	-5.4	0.718839	0.020607	TREND	BH
Beyeria sulcata	9	Euphorbiaceae	Shrub	Native	283.07	336.69	-26.6	-25.0	-23.8895	-5.9	-0.11751	0.70216	SWATT	US
Bossiaea prostrata	4	Fabaceae	Shrub	Native	653.04	829.05	-30.1	-28.1	-23.081	-8.3	0.461548	0.199355	TREND	BH
Brachyscome ciliaris	12	Asteraceae	Per. Forb	Native	161.55	399.11	-34.0	-28.4	-27.9537	-11.7	0.264848	0.050032	TREND	BH
Brassica tournefortii	4	Brassicaceae	Ann. Forb	Alien	161.54	445.25	-28.3	-24.3	-26.1092	-4.0	-0.35656	0.690769	TREND	US
Briza maxima	6	Poaceae	Ann. Grass	Alien	653.04	980.00	-31.8	-27.8	-26.1838	-5.0	-0.06622	0.453037	TREND	BH
Bromus diandrus	4	Poaceae	Ann. Grass	Alien	428.35	807.80	-31.2	-29.5	-31.4728	2.4	-0.02414	0.436757	TREND	CO

Brunonia australis	5	Goodeniaceae	Per. Forb	Native	661.55	980.00	-32.4	-30.4	-29.9582	-1.6	-0.25043	0.685842	TREND	US
Bulbine alata	5	Xanthorrhoeaceae	Ann. Forb	Native	161.55	399.11	-29.7	-26.1	-27.1322	-2.2	-0.29605	0.788094	TREND	US
Bulbine bulbosa	4	Xanthorrhoeaceae	Per. Forb	Native	428.35	829.05	-32.9	-29.9	-27.0796	-6.6	0.858905	0.048193	TREND	BH
Burchardia umbellata	4	Colchicaceae	Per. Forb	Native	688.07	980.00	-28.7	-26.7	-22.4051	-6.7	0.580707	0.151194	TREND	BH
Bursaria spinosa	14	Pittosporaceae	Shrub	Native	306.97	829.05	-30.2	-25.2	-28.1824	-0.4	-0.08092	0.872802	TREND	IS
Caesia calliantha	8	Xanthorrhoeaceae	Per. Forb	Native	288.63	980.00	-31.9	-28.6	-28.4424	-3.1	0.194354	0.152175	TREND	US
Calandrinia eremaea	4	Portulacaceae	Ann. Forb	Native	277.64	445.25	-27.1	-24.4	-24.0735	-6.9	-0.22824	0.574354	TREND	BH
Callitris glaucophylla	8	Cupressaceae	Tree	Native	253.13	482.13	-27.3	-25.6	-25.2324	-3.6	0.08163	0.249898	TREND	US
Callitris preissii	15	Cupressaceae	Tree	Native	299.07	347.70	-27.8	-24.8	-22.6291	-12.3	-0.03303	0.47055	SWATT	BH
Calotis hispidula	9	Asteraceae	Ann. Forb	Native	161.55	293.91	-32.6	-28.6	-29.9295	-3.5	-0.11343	0.680021	TREND	US
Calothamnus gilesii	7	Myrtaceae	Shrub	Native	294.35	340.08	-30.2	-27.4	-33.5269	16.2	-0.11049	0.553451	SWATT	CO
Calytrix tetragona	9	Myrtaceae	Shrub	Native	379.55	729.13	-31.2	-25.4	-22.9932	-8.9	0.157059	0.158537	TREND	BH
Caragana microphylla	8	Fabaceae	Shrub		151.04	371.17	-27.2	-24.6	-24.1125	-5.4	0.228332	0.13024	NECT	IS
Caragana stenophylla	7	Fabaceae	Shrub		145.18	263.23	-25.0	-23.9	-23.7106	-3.8	-0.07402	0.478358	NECT	IS
Carex pediformis	3	Cyperaceae	Sedge		555.22	568.96	-31.2	-29.4	44.28438	-132.7	0.98023	0.0634	NECT	BH
Carex sp.	3	Cyperaceae	Sedge		554.73	582.84	-31.0	-28.9	-70.652	71.0	0.445756	0.352935	NECT	CO
Carrichtera annua	7	Brassicaceae	Ann. Forb	Alien	234.26	413.97	-29.2	-26.0	-28.2159	1.8	-0.18878	0.836614	TREND	CO
Cassinia complanata	4	Asteraceae	Shrub	Native	283.29	506.13	-30.9	-28.8	-27.9398	-5.8	0.301816	0.268875	TREND	BH
Cassinia laevis	6	Asteraceae	Shrub	Native	306.97	560.78	-31.3	-27.4	-23.5237	-13.3	0.591647	0.045409	TREND	BH
Cassytha glabella	4	Lauraceae	Vine	Native	661.55	841.06	-28.0	-26.9	-25.3075	-2.5	-0.2179	0.566335	TREND	US
Casuarina pauper	7	Casuarinaceae	Tree	Native	173.75	531.37	-28.3	-26.2	-26.0223	-4.0	0.273092	0.131094	TREND	US
Chamaescilla corymbosa	4	Asparagaceae	Per. Forb	Native	293.91	841.06	-31.5	-28.0	-26.0471	-5.9	0.736276	0.092154	TREND	BH
Cheilanthes austrotenuifolia	11	Pteridaceae	Fern	Native	413.97	980.00	-32.0	-27.4	-28.5834	-0.7	-0.10206	0.791902	TREND	IS
Cheilanthes lasiophylla	6	Pteridaceae	Fern	Native	236.02	482.13	-30.5	-28.2	-30.1107	3.4	-0.08854	0.484117	TREND	CO

Cheilanthes sieberi	7	Pteridaceae	Fern	Native	306.97	661.55	-30.0	-26.7	-26.0513	-4.2	-0.03046	0.406001	TREND	US
Chenopodium acuminatum	3	Chenopodiaceae	Chenopod		365.25	371.17	-29.1	-27.9	-68.9212	110.2	-0.41888	0.637576	NECT	CO
Chenopodium desertorum	4	Chenopodiaceae	Chenopod	Native	250.34	506.13	-30.8	-28.1	-32.5472	7.2	0.256805	0.289692	TREND	CO
Chrysocephalum apiculatum	4	Asteraceae	Per. Forb	Native	399.11	506.13	-32.1	-28.1	-28.141	-3.9	-0.48201	0.890489	TREND	US
Chrysocephalum semipapposum	5	Asteraceae	Per. Forb	Native	288.63	428.35	-30.5	-25.9	-33.1562	13.3	-0.14779	0.536293	TREND	CO
Clematis microphylla	6	Ranunculaceae	Vine	Native	445.25	980.00	-32.5	-23.9	-23.791	-7.2	0.085216	0.292656	TREND	BH
Convolvulus remotus	7	Convolvulaceae	Per. Forb	Native	168.43	729.13	-30.1	-26.5	-26.9976	-4.1	0.37811	0.083617	TREND	US
Corylus heterophylla	6	Betulaceae	Shrub		554.73	709.95	-31.9	-27.9	-23.0681	-11.0	0.029556	0.343515	NECT	US
Craspedia variabilis	5	Asteraceae	Ann. Forb	Native	482.13	829.05	-33.1	-32.0	-33.0419	0.5	-0.29962	0.79839	TREND	IS
Daucus glochidiatus	10	Apiaceae	Ann. Forb	Native	164.63	653.04	-32.4	-27.8	-29.0278	-3.0	-0.0124	0.373147	TREND	US
Dianella revoluta	15	Xanthorrhoeaceae	Per. Forb	Native	376.29	980.00	-30.7	-26.4	-23.7719	-7.4	0.656377	0.000152	TREND	BH
Dianella revoluta	23	Xanthorrhoeaceae	Per. Forb	Native	254.20	745.70	-31.3	-24.3	-22.78	-10.9	0.402277	0.000689	SWATT	BH
Dioscorea nipponica	3	Dioscoreaceae	Per. Forb		568.96	709.95	-32.1	-28.8	-18.7073	-18.7	0.572575	0.305944	NECT	US
Dodonaea viscosa	16	Sapindaceae	Shrub	Native	163.05	807.80	-29.7	-26.0	-25.7536	-4.2	0.493381	0.00145	TREND	US
Drosera auriculata	4	Droseraceae	Per. Forb	Native	546.30	838.25	-29.9	-27.4	-28.9407	0.5	-0.49291	0.931272	TREND	IS
Drosera whittakeri	5	Droseraceae	Per. Forb	Native	546.30	841.06	-29.2	-27.1	-30.3434	2.6	-0.10933	0.493129	TREND	CO
Echium plantagineum	7	Boraginaceae	Ann. Forb	Alien	306.97	829.05	-30.4	-28.0	-30.804	1.9	0.050529	0.302681	TREND	CO
Einadia nutans	8	Chenopodiaceae	Chenopod	Native	161.55	506.13	-30.8	-26.6	-28.5928	-4.1	0.009129	0.341975	TREND	US
Enchylaena tomentosa	14	Chenopodiaceae	Chenopod	Native	161.54	445.25	-30.7	-23.7	-25.2196	-6.8	0.073697	0.179281	TREND	BH
Enchylaena tomentosa	8	Chenopodiaceae	Chenopod	Native	265.07	299.65	-28.8	-24.9	-41.7841	54.8	0.352665	0.070685	SWATT	CO
Eremophila deserti	4	Scrophulariaceae	Shrub	Native	283.29	379.55	-29.5	-27.0	-25.5325	-9.3	-0.28327	0.619882	TREND	BH
Eremophila freelingii	6	Scrophulariaceae	Shrub	Native	168.24	293.91	-27.4	-25.5	-24.0067	-9.0	0.40698	0.103059	TREND	BH
Eremophila longifolia	4	Scrophulariaceae	Shrub	Native	168.43	428.35	-29.4	-25.7	-26.2003	-4.1	-0.37523	0.711592	TREND	US

Eremophila maculata	4	Scrophulariaceae	Shrub	Native	161.55	293.91	-30.7	-25.9	-36.2721	35.7	0.991807	0.002735	TREND	CO
Eremophila scoparia	9	Scrophulariaceae	Shrub	Native	283.07	320.63	-28.3	-24.7	-13.2296	-45.4	0.252523	0.095732	SWATT	BH
Eriochiton sclerolaenoides	6	Chenopodiaceae	Chenopod	Native	168.24	376.29	-30.4	-28.4	-27.3716	-7.9	0.431489	0.093721	TREND	BH
Erodium cicutarium	4	Geraniaceae	Ann. Forb	Alien	161.54	506.13	-29.5	-24.4	-25.185	-7.3	-0.14321	0.512289	TREND	BH
Erodium crinitum	6	Geraniaceae	Ann. Forb	Native	168.24	293.91	-31.4	-28.1	-30.7865	5.4	-0.17313	0.635646	TREND	CO
Eucalyptus fasciculosa	7	Myrtaceae	Tree	Native	546.30	829.05	-30.7	-27.9	-29.3112	0.3	-0.19904	0.95198	TREND	IS
Eucalyptus flindersii	5	Myrtaceae	Tree	Native	283.29	399.11	-28.1	-25.1	-32.4667	16.1	0.36208	0.168256	TREND	CO
Eucalyptus intertexta	6	Myrtaceae	Tree	Native	261.87	399.11	-29.7	-26.4	-25.9705	-8.4	-0.12005	0.533122	TREND	BH
Eucalyptus leptopoda	13	Myrtaceae	Tree	Native	265.65	347.62	-28.4	-26.0	-21.2712	-19.0	0.281053	0.036142	SWATT	BH
Eucalyptus obliqua	5	Myrtaceae	Tree	Native	678.44	980.00	-31.3	-28.3	-25.822	-4.5	-0.14146	0.528844	TREND	US
Eucalyptus odorata	4	Myrtaceae	Tree	Native	413.97	506.13	-28.1	-25.6	-20.8859	-13.2	-0.27747	0.61483	TREND	BH
Eucalyptus rigidula	10	Myrtaceae	Tree	Native	297.33	347.70	-27.4	-25.3	-28.6192	7.0	-0.08358	0.595379	SWATT	CO
Euryomyrtus maidenii	9	Myrtaceae	Shrub	Native	297.33	347.70	-28.7	-26.6	-22.2341	-18.4	0.039465	0.286877	SWATT	BH
Eutaxia microphylla	7	Fabaceae	Shrub	Native	376.29	729.13	-30.9	-26.7	-25.5383	-6.3	0.368744	0.087233	TREND	BH
Exocarpos aphyllus	6	Santalaceae	Shrub	Native	277.64	413.97	-28.7	-23.8	-23.9855	-10.1	-0.1387	0.565691	TREND	BH
Exocarpos aphyllus	10	Santalaceae	Shrub	Native	283.07	347.70	-28.5	-26.5	-27.8882	1.4	-0.12302	0.908431	SWATT	IS
Exocarpos cupressiformis	8	Santalaceae	Shrub	Native	506.13	980.00	-32.1	-27.0	-26.6272	-5.5	0.248751	0.118376	TREND	BH
Fraxinus chinensis	4	Oleaceae	Tree		555.22	582.84	-31.6	-30.1	-22.9754	-13.3	-0.43343	0.78933	NECT	US
Glischrocaryon behrii	5	Haloragaceae	Per. Forb	Native	661.55	980.00	-31.5	-29.3	-27.7445	-3.4	0.038043	0.360681	TREND	US
Glycine rubiginosa	8	Fabaceae	Vine	Native	234.26	531.37	-32.7	-23.8	-24.2221	-11.8	0.157009	0.179857	TREND	BH
Gonocarpus elatus	8	Haloragaceae	Per. Forb	Native	428.35	729.13	-32.7	-28.6	-25.6318	-7.3	0.306283	0.089588	TREND	BH
Gonocarpus tetragynus	8	Haloragaceae	Per. Forb	Native	560.78	980.00	-32.0	-29.1	-29.3191	-1.5	-0.10482	0.583308	TREND	IS
Goodenia blackiana	7	Goodeniaceae	Per. Forb	Native	506.13	841.06	-31.3	-26.4	-21.0005	-11.4	0.575576	0.029325	TREND	BH
Goodenia fascicularis	9	Goodeniaceae	Per. Forb	Native	161.55	293.91	-31.3	-27.9	-28.4575	-4.6	-0.09794	0.609145	TREND	BH

Grevillea didymobotrya	20	Proteaceae	Shrub	Native	294.35	347.70	-27.6	-25.3	-22.5672	-13.5	0.036272	0.206794	SWATT	BH
Grevillea hookeriana	6	Proteaceae	Shrub	Native	301.19	306.26	-26.3	-24.5	17.41797	-140.9	-0.09336	0.491194	SWATT	BH
Hakea erecta	11	Proteaceae	Shrub	Native	297.33	347.70	-28.1	-23.9	-16.5372	-29.1	0.158476	0.123734	SWATT	BH
Hakea francisiana	10	Proteaceae	Shrub	Native	265.07	329.20	-28.1	-22.7	-21.8045	-12.5	-0.08772	0.614737	SWATT	BH
Hakea rostrata	5	Proteaceae	Shrub	Native	678.44	980.00	-31.5	-28.1	-24.2248	-6.8	0.135211	0.292118	TREND	BH
Hakea rugosa	5	Proteaceae	Shrub	Native	288.63	807.80	-30.7	-26.2	-28.5716	0.8	-0.31781	0.862864	TREND	IS
Halgania cyanea	5	Boraginaceae	Shrub	Native	288.63	531.37	-31.4	-27.4	-26.7572	-6.3	-0.14486	0.532829	TREND	BH
Hibbertia crinita	11	Dilleniaceae	Per. Forb	Native	376.29	980.00	-31.9	-27.5	-27.4439	-3.4	0.125972	0.152616	TREND	US
Hibbertia exutiacies	8	Dilleniaceae	Per. Forb	Native	506.13	980.00	-32.4	-28.4	-28.7652	-1.8	-0.11299	0.609977	TREND	US
Hypochaeris glabra	5	Asteraceae	Ann. Forb	Alien	288.63	807.80	-31.8	-29.0	-30.78	0.5	-0.32492	0.898965	TREND	IS
Iris lactea	3	Iridaceae	Per. Forb		316.03	370.36	-25.3	-24.3	-28.4674	10.0	-0.36089	0.617526	NECT	CO
Jacksonia nematoclada	10	Fabaceae	Shrub	Native	297.33	347.70	-28.8	-25.6	-28.2529	2.7	-0.12076	0.866286	SWATT	IS
Keraudrenia velutina	14	Malvaceae	Shrub	Native	265.07	306.26	-29.0	-25.1	-22.5981	-15.6	-0.02268	0.415379	SWATT	BH
Klasea yamatsutana	4	Asteraceae	Per. Forb		263.23	382.19	-29.17	-26.1	-21.1761	-17.9	0.303883	0.267933	NECT	US
Lagenophora huegelii	4	Asteraceae	Per. Forb	Native	560.78	807.80	-32.7	-29.9	-39.1659	10.8	0.591963	0.146785	TREND	CO
Leiocarpa semicalva	7	Asteraceae	Per. Forb	Native	250.34	482.13	-31.7	-29.8	-30.9333	-0.5	-0.19469	0.887314	TREND	IS
Leiocarpa websteri	5	Asteraceae	Per. Forb	Native	161.55	186.68	-31.3	-27.6	-9.50444	-118.3	0.309811	0.193122	TREND	BH
Lepidium papillosum	4	Brassicaceae	Ann. Forb	Native	261.87	293.91	-30.7	-28.7	-32.3166	8.3	-0.47705	0.876305	TREND	CO
Lepidobolus preissianus	11	Restionaceae	Per. Forb	Native	294.35	449.67	-30.2	-26.1	-25.6544	-6.7	-0.04895	0.483771	SWATT	BH
Lepidosperma rigidulum	11	Cyperaceae	Sedge	Native	296.00	340.90	-29.5	-24.4	-20.1109	-20.4	-0.06892	0.565838	SWATT	BH
Lepidosperma sanguinolentum	14	Cyperaceae	Sedge	Native	301.19	347.70	-28.0	-24.5	-14.72	-35.9	0.202109	0.06048	SWATT	BH
Lepidosperma semiteres	6	Cyperaceae	Sedge	Native	678.44	980.00	-31.3	-27.4	-28.0042	-1.7	-0.22409	0.785539	TREND	US
Leptomeria preissiana	9	Santalaceae	Shrub	Native	299.07	347.70	-29.1	-27.8	-31.3651	9.1	-0.00659	0.362749	SWATT	CO
Leptospermum fastigiatum	12	Myrtaceae	Shrub	Native	296.41	306.26	-29.3	-26.2	-15.5897	-38.8	-0.08363	0.705693	SWATT	BH

Leptospermum myrsinoides	4	Myrtaceae	Shrub	Native	678.44	980.00	-29.9	-29.1	-29.6929	0.3	-0.47973	0.883756	TREND	IS
Lespedeza bicolor	5	Fabaceae	Shrub		554.73	635.30	-33.0	-28.0	-8.22728	-39.7	0.277788	0.209349	NECT	BH
Lespedeza davurica	9	Fabaceae	Per. Forb		367.00	621.93	-29.6	-25.7	-22.2828	-11.8	0.721464	0.002313	NECT	US
Leymus chinensis	9	Poaceae	Per. Grass		263.23	418.94	-26.7	-25.0	-26.4792	1.4	-0.13235	0.806183	NECT	IS
Lissanthe strigosa	4	Ericaceae	Shrub	Native	661.55	838.25	-31.0	-28.6	-24.83	-6.2	-0.11635	0.494267	TREND	BH
Lolium rigidum	4	Poaceae	Ann. Grass	Alien	506.13	688.07	-31.8	-28.7	-30.6501	0.9	-0.49419	0.93778	TREND	IS
Lomandra densiflora	8	Asparagaceae	Per. Forb	Native	482.13	980.00	-31.4	-27.5	-26.4266	-3.7	0.140175	0.193719	TREND	US
Lomandra micrantha	5	Asparagaceae	Per. Forb	Native	653.04	980.00	-28.4	-25.8	-24.8384	-2.4	-0.20402	0.609989	TREND	US
Lomandra multiflora	14	Asparagaceae	Per. Forb	Native	261.87	980.00	-28.0	-22.8	-26.6306	-0.1	-0.08284	0.942047	TREND	IS
Lysiana exocarpi	6	Loranthaceae	Epiphyte	Native	173.75	546.30	-29.7	-26.5	-25.3416	-7.6	0.691009	0.025118	TREND	BH
Maireana pyramidata	5	Chenopodiaceae	Chenopod	Native	161.55	250.34	-26.8	-24.8	-23.3499	-13.6	0.071723	0.335583	TREND	BH
Maireana trichoptera	12	Chenopodiaceae	Chenopod	Native	283.07	299.65	-28.6	-25.4	2.416295	-99.0	0.118388	0.146588	SWATT	BH
Marrubium vulgare	4	Lamiaceae	Per. Forb	Alien	253.13	428.35	-30.8	-28.7	-27.4026	-7.8	0.158675	0.337341	TREND	BH
Medicago ruthenica	6	Fabaceae	Per. Forb		263.23	400.52	-27.7	-25.1	-22.4451	-11.0	0.254001	0.175541	NECT	US
Melaleuca calyptroides	11	Myrtaceae	Shrub	Native	299.65	347.70	-29.3	-26.4	-24.2489	-11.8	-0.04926	0.484904	SWATT	BH
Melaleuca cordata	16	Myrtaceae	Shrub	Native	294.35	347.70	-28.6	-26.9	-25.4836	-7.6	0.002937	0.324183	SWATT	US
Melaleuca hamata	9	Myrtaceae	Shrub	Native	283.07	332.29	-29.1	-25.8	-15.8426	-38.0	0.11482	0.196492	SWATT	BH
Minuria cunninghamii	4	Asteraceae	Per. Forb	Native	164.63	261.87	-31.6	-27.7	-28.1771	-9.0	-0.40128	0.743454	TREND	BH
Monachather paradoxus	11	Poaceae	Per. Grass	Native	257.04	319.11	-31.7	-27.0	-31.818	10.6	-0.08192	0.633981	SWATT	CO
Nicotiana simulans	6	Solanaceae	Ann. Forb	Native	163.05	283.29	-30.6	-25.4	-35.3627	32.5	0.622688	0.03834	TREND	CO
Olearia decurrens	8	Asteraceae	Shrub	Native	283.29	560.78	-29.6	-25.9	-25.1485	-7.2	0.372202	0.063722	TREND	BH
Olearia muelleri	12	Asteraceae	Shrub	Native	283.07	320.63	-28.3	-24.8	-22.366	-13.8	-0.06771	0.594445	SWATT	BH
Olearia ramulosa	7	Asteraceae	Shrub	Native	482.13	841.06	-32.9	-28.4	-27.0218	-5.1	0.085364	0.266954	TREND	BH
Oxalis perennans	11	Oxalidaceae	Ann. Forb	Native	250.34	980.00	-33.8	-29.4	-30.6111	-2.3	0.097611	0.182959	TREND	US

Persoonia coriacea	12	Proteaceae	Shrub	Native	297.33	347.70	-27.7	-25.1	-27.3757	2.6	-0.09644	0.860517	SWATT	IS
Philadelphus tenuifolius	3	Saxifragaceae	Shrub		555.22	709.95	-31.0	-28.7	-20.9252	-14.6	0.658894	0.271025	NECT	US
Phlomis maximowiczii	3	Lamiaceae	Per. Forb		568.96	709.95	-32.7	-31.9	-30.4411	-3.1	-0.32363	0.604904	NECT	IS
Pimelea humilis	6	Thymelaeaceae	Shrub	Native	653.04	980.00	-31.1	-27.1	-23.8133	-7.3	0.174688	0.224696	TREND	BH
Pimelea linifolia	5	Thymelaeaceae	Shrub	Native	560.78	980.00	-31.2	-27.9	-24.1159	-7.2	0.667809	0.057354	TREND	BH
Pimelea microcephala	4	Thymelaeaceae	Shrub	Native	173.75	413.97	-28.9	-27.5	-28.9401	2.5	-0.21769	0.566171	TREND	CO
Pimelea simplex	4	Thymelaeaceae	Shrub	Native	163.05	168.43	-28.4	-24.7	-94.3918	410.6	0.201268	0.316251	TREND	CO
Pittosporum angustifolium	4	Pittosporaceae	Tree	Native	306.97	445.25	-28.6	-25.6	-34.6777	20.9	0.882797	0.039862	TREND	CO
Plantago drummondii	5	Plantaginaceae	Ann. Forb	Native	161.55	413.97	-31.2	-25.9	-26.9773	-6.1	-0.19643	0.59911	TREND	BH
Platylobium obtusangulum	4	Fabaceae	Shrub	Native	699.09	980.00	-31.6	-29.3	-31.7531	1.3	-0.4625	0.841879	TREND	IS
Platysace trachymenioides	12	Apiaceae	Shrub	Native	283.07	341.72	-29.8	-25.9	-9.583	-58.2	0.719632	0.000299	SWATT	BH
Poa crassicaudex	7	Poaceae	Per. Grass	Native	482.13	980.00	-30.8	-28.9	-28.1957	-2.3	0.382891	0.081813	TREND	US
Polycalymma stuartii	4	Asteraceae	Ann. Forb	Native	161.54	168.24	-29.3	-26.2	-17.3161	-64.9	-0.46132	0.839413	TREND	BH
Polygonum divaricatum	4	Polygonaceae	Per. Forb		376.77	400.52	-28.1	-27.5	-27.391	-1.4	-0.49567	0.946286	NECT	IS
Potentilla acaulis	3	Rosaceae	Per. Forb		316.03	370.36	-27.1	-26.2	-27.0676	1.7	-0.98604	0.94676	NECT	IS
Potentilla chinensis	7	Rosaceae	Per. Forb		263.23	621.93	-29.4	-25.2	-23.0483	-10.9	0.577024	0.029058	NECT	US
Prostanthera striatiflora	4	Lamiaceae	Shrub	Native	261.87	376.29	-29.5	-27.2	-24.6923	-12.5	0.012715	0.415355	TREND	BH
Psammomoya choretroides	8	Celastraceae	Shrub	Native	297.33	340.90	-29.8	-25.6	-21.0521	-20.3	-0.09057	0.541561	SWATT	BH
Pteridium aquilinum	4	Dennstaedtiaceae	Fern		555.22	709.95	-33.3	-29.6	-17.4941	-21.9	0.943348	0.019066	NECT	BH
Pterocaulon sphacelatum	9	Asteraceae	Per. Forb	Native	161.55	283.29	-29.5	-26.5	-24.6759	-13.1	0.319996	0.06537	TREND	BH
Ptilotus nobilis	4	Amaranthaceae	Per. Forb	Native	236.02	428.35	-29.2	-25.0	-20.4374	-20.4	0.804555	0.067421	TREND	BH
Ptilotus obovatus	9	Amaranthaceae	Per. Forb	Native	236.02	379.55	-30.6	-27.5	-27.8147	-4.1	-0.10786	0.652464	TREND	US
Ptilotus obovatus	13	Amaranthaceae	Shrub	Native	254.20	299.65	-29.3	-23.7	-49.5416	82.8	0.473463	0.005586	SWATT	CO
Pultenaea daphnoides	4	Fabaceae	Shrub	Native	829.05	980.00	-32.3	-28.3	-29.4914	-0.3	-0.49976	0.987307	TREND	IS

Pultenaea largiflorens	5	Fabaceae	Shrub	Native	560.78	841.06	-30.3	-29.0	-31.0827	2.1	-0.04117	0.426531	TREND	CO
Quercus mongolica	7	Fagaceae	Tree		554.73	709.95	-30.5	-27.6	-35.9966	11.9	0.200149	0.174589	NECT	CO
Rhagodia parabolica	7	Chenopodiaceae	Chenopod	Native	161.54	445.25	-31.0	-25.2	-24.4088	-13.9	0.716111	0.010152	TREND	BH
Rhagodia spinescens	6	Chenopodiaceae	Chenopod	Native	161.54	250.34	-30.3	-25.9	-25.1505	-15.7	-0.13916	0.566528	TREND	BH
Rhodanthe moschata	5	Asteraceae	Ann. Forb	Native	161.54	168.24	-31.6	-25.3	92.36396	-734.4	0.5807	0.083411	TREND	BH
Rhodanthe pygmaea	8	Asteraceae	Ann. Forb	Native	163.05	293.91	-32.2	-29.9	-30.6376	-0.9	-0.16033	0.862302	TREND	IS
Rhyncharrhena linearis	7	Apocynaceae	Vine	Native	256.21	287.66	-29.9	-26.7	-15.3926	-47.3	-0.05143	0.438939	SWATT	BH
Santalum acuminatum	14	Santalaceae	Tree	Native	257.04	341.72	-30.8	-26.8	-21.1775	-23.5	0.040923	0.236233	SWATT	BH
Scaevola albida	4	Goodeniaceae	Shrub	Native	688.07	980.00	-30.8	-28.3	-24.6165	-5.8	0.234268	0.300349	TREND	BH
Scaevola spinescens	10	Goodeniaceae	Shrub	Native	256.21	320.63	-28.3	-25.5	-26.0233	-3.5	-0.11611	0.807121	SWATT	US
Schismus barbatus	4	Poaceae	Ann. Grass	Alien	250.34	838.25	-31.3	-27.8	-30.2272	0.5	-0.489	0.914359	TREND	IS
Schoenus apogon	4	Cyperaceae	Sedge	Native	653.04	829.05	-31.7	-29.5	-26.0741	-6.2	-0.03896	0.445597	TREND	BH
Schoenus hexandrus	10	Cyperaceae	Sedge	Native	297.33	347.70	-28.4	-26.0	-23.0814	-13.0	-0.02758	0.409181	SWATT	BH
Schoenus subaphyllus	7	Cyperaceae	Sedge	Native	301.19	329.20	-29.9	-24.9	-15.8156	-37.9	-0.12507	0.588897	SWATT	BH
Sclerolaena brachyptera	4	Chenopodiaceae	Chenopod	Native	164.63	277.64	-26.8	-24.3	-25.4303	-2.5	-0.47891	0.88142	TREND	US
Sclerolaena diacantha	7	Chenopodiaceae	Chenopod	Native	161.55	357.87	-28.4	-26.8	-27.641	-0.6	-0.19315	0.872067	TREND	IS
Sclerolaena diacantha	11	Chenopodiaceae	Chenopod	Native	283.07	320.63	-28.8	-24.6	-14.9055	-40.0	0.095553	0.185377	SWATT	BH
Sclerolaena obliquicuspis	4	Chenopodiaceae	Chenopod	Native	173.75	250.34	-28.6	-25.3	-32.6846	25.3	0.121548	0.356288	TREND	CO
Senecio pterophorus	5	Asteraceae	Per. Forb	Alien	653.04	980.00	-31.5	-28.5	-25.415	-5.7	0.03509	0.362957	TREND	BH
Senecio quadridentatus	6	Asteraceae	Per. Forb	Native	283.29	807.80	-33.4	-27.9	-28.6644	-4.7	0.010048	0.363276	TREND	US
Senecio spanomerus	4	Asteraceae	Per. Forb	Native	173.75	261.87	-31.1	-26.4	-39.3428	47.0	0.703131	0.104407	TREND	CO
Senna artemisioides	27	Fabaceae	Shrub	Native	168.24	428.35	-29.7	-24.2	-26.7233	-2.2	-0.02786	0.591624	TREND	US
Senna artemisioides	24	Fabaceae	Shrub	Native	254.20	320.63	-29.3	-20.5	-35.626	32.9	0.118254	0.05561	SWATT	CO
Sida fibulifera	15	Malvaceae	Per. Forb	Native	161.55	293.91	-30.8	-26.2	-29.0805	4.1	-0.03755	0.494855	TREND	CO

Sida petrophila	9	Malvaceae	Shrub	Native	236.02	306.97	-30.5	-25.8	-17.2863	-39.8	0.206344	0.122699	TREND	BH
Solanum ellipticum	7	Solanaceae	Per. Forb	Native	161.55	482.13	-29.4	-26.5	-25.2568	-8.6	0.335369	0.101048	TREND	BH
Solanum lasiophyllum	18	Solanaceae	Shrub	Native	254.20	299.65	-31.3	-25.0	-40.4366	43.2	0.161319	0.055376	SWATT	CO
Solanum petrophilum	5	Solanaceae	Per. Forb	Native	250.34	413.97	-29.7	-28.1	-29.5101	1.9	-0.29544	0.786381	TREND	IS
Solanum quadriloculatum	10	Solanaceae	Per. Forb	Native	163.05	306.97	-30.4	-26.0	-26.8477	-6.7	-0.06089	0.506542	TREND	BH
Sonchus oleraceus	15	Asteraceae	Ann. Forb	Alien	161.54	807.80	-32.1	-25.5	-28.7563	-1.7	-0.04448	0.536153	TREND	US
Spyridium parvifolium	4	Rhamnaceae	Shrub	Native	506.13	699.09	-30.0	-27.6	-27.3317	-2.5	-0.41342	0.759754	TREND	US
Stipa sareptana	10	Poaceae	Per. Grass		200.20	398.38	-26.4	-23.1	-22.1278	-9.3	0.423658	0.024688	NECT	US
Stipa tianschanica	4	Poaceae	Per. Grass		145.05	155.12	-25.4	-23.3	-1.52971	-155.0	0.453571	0.202683	NECT	BH
Tetragonia eremaea	6	Aizoaceae	Ann. Forb	Native	168.24	277.64	-29.6	-23.7	-22.5332	-25.4	0.123119	0.26202	TREND	BH
Thalictrum squarrosum	3	Ranunculaceae	Per. Forb		371.17	400.52	-26.6	-25.4	-22.3214	-9.4	-0.8959	0.853468	NECT	US
Thysanotus patersonii	4	Asparagaceae	Vine	Native	546.30	980.00	-32.0	-29.6	-29.0502	-1.8	-0.35863	0.693004	TREND	US
Tilia amurensis	3	Tiliaceae	Tree		554.73	568.01	-31.5	-30.1	-24.8745	-10.7	-0.97333	0.926322	NECT	US
Tilia mandshurica	3	Tiliaceae	Tree		555.22	709.95	-31.3	-28.5	-21.8351	-13.0	0.01165	0.496292	NECT	US
Ulmus davidiana	5	Ulmaceae	Tree		384.15	621.93	-29.9	-26.6	-21.8058	-12.1	0.375013	0.162399	NECT	US
Vittadinia cuneata	4	Asteraceae	Ann. Forb	Native	413.97	688.07	-31.3	-29.8	-33.1795	5.1	0.552207	0.162461	TREND	CO
Vittadinia sulcata	4	Asteraceae	Ann. Forb	Native	234.26	293.91	-32.3	-27.6	-21.7222	-32.5	-0.16104	0.524634	TREND	BH
Wahlenbergia luteola	5	Campanulaceae	Per. Forb	Native	283.29	661.55	-31.8	-28.9	-28.3033	-4.3	0.011256	0.381796	TREND	US
Wahlenbergia stricta	7	Campanulaceae	Per. Forb	Native	531.37	980.00	-31.4	-29.5	-31.0874	1.1	-0.14088	0.632396	TREND	IS
Xanthorrhoea quadrangulata	11	Xanthorrhoeaceae	Grass Tree	Native	283.29	729.13	-29.8	-25.2	-24.8643	-6.1	0.242681	0.070559	TREND	BH
Xanthorrhoea semiplana	9	Xanthorrhoeaceae	Grass Tree	Native	546.30	980.00	-29.1	-26.1	-25.2304	-3.7	0.076972	0.237645	TREND	US

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[*] Chinese (NECT) species descriptions, including authorities, are available in the online Flora of China (http://www.floraofchina.org/). TREND species descriptions, including authorities, are available in Barker *et al.* (2016). SWATT species descriptions, including authorities, are available in the online FloraBase (http://www.florabase.dpaw.wa.gov.au). ^ Biotic Homeostasis (BH), Contrary (CO), Universal Scaling (US), IS (Insensitive).

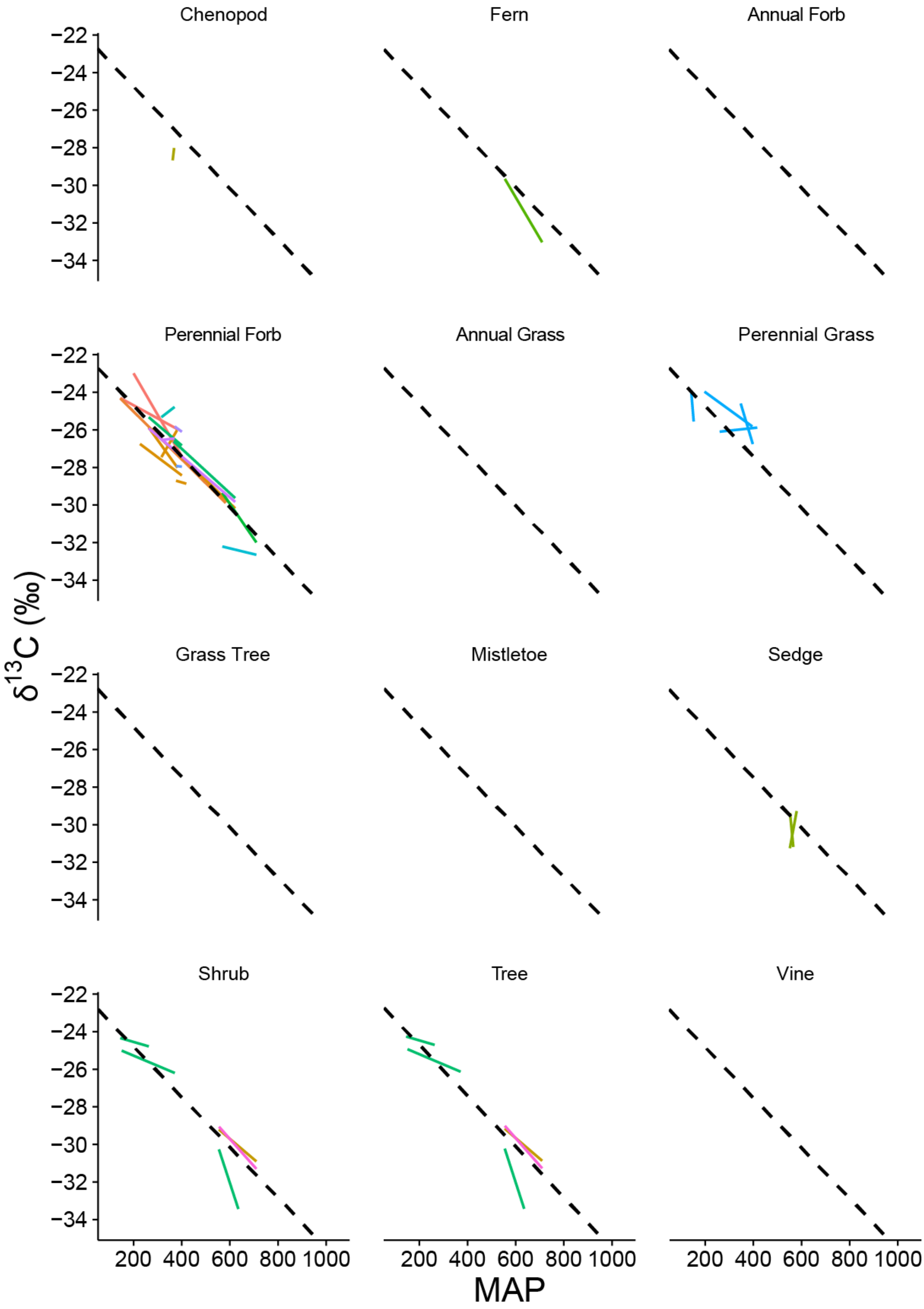
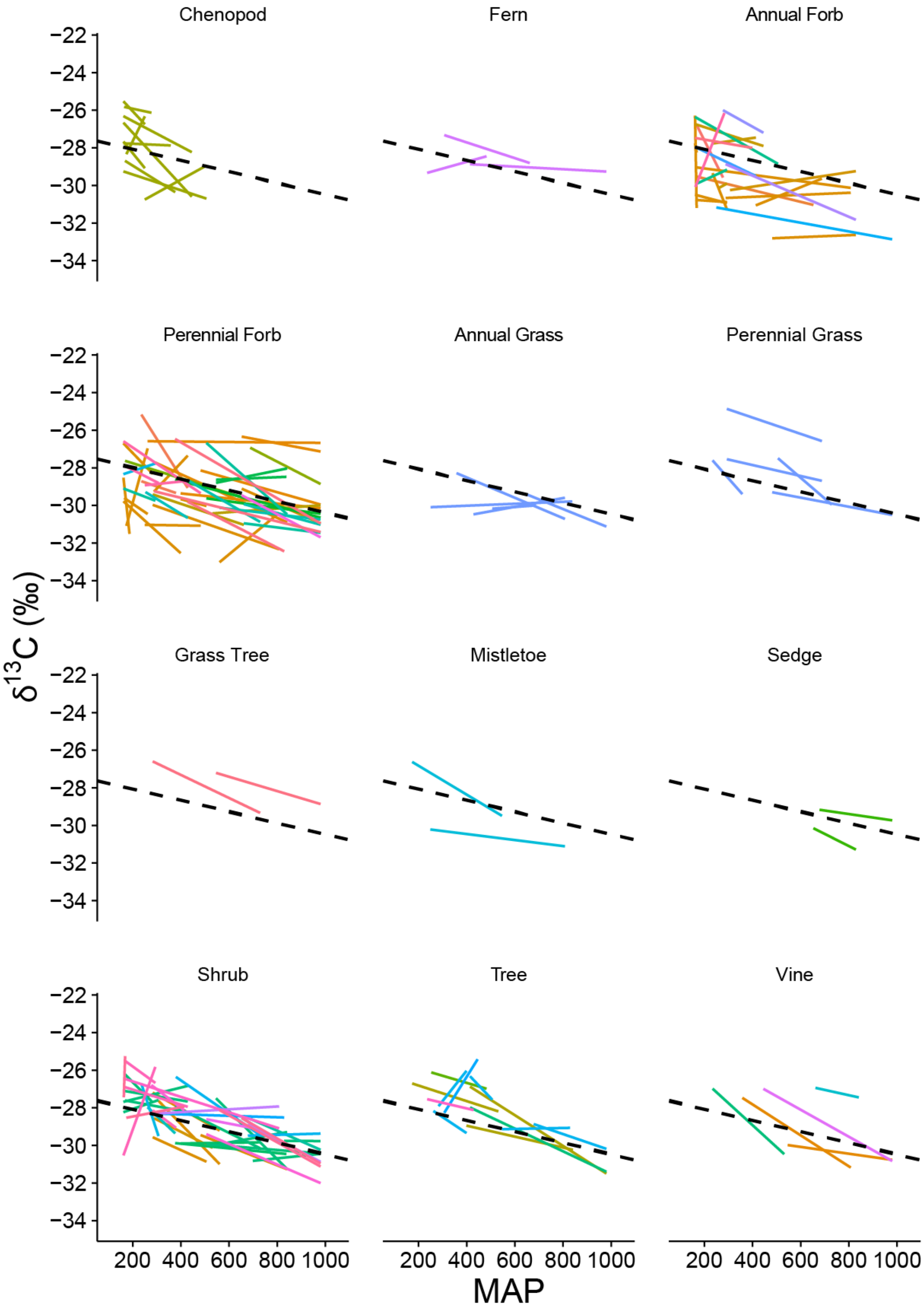


Figure S1: Species leaf δ^{13}C~MAP regressions (m_s) for 36 NECT species and NECT common regression (m_c). Colours represent 21 different families to demonstrate the phylogenetic diversity within growth form classes.



904

905 **Figure S2**: Species leaf δ^{13}C~MAP regressions (m_s) for 150 TREND species and TREND

906 common regression (m_c). Colours represent 44 different families to demonstrate the

907 phylogenetic diversity within growth form classes.

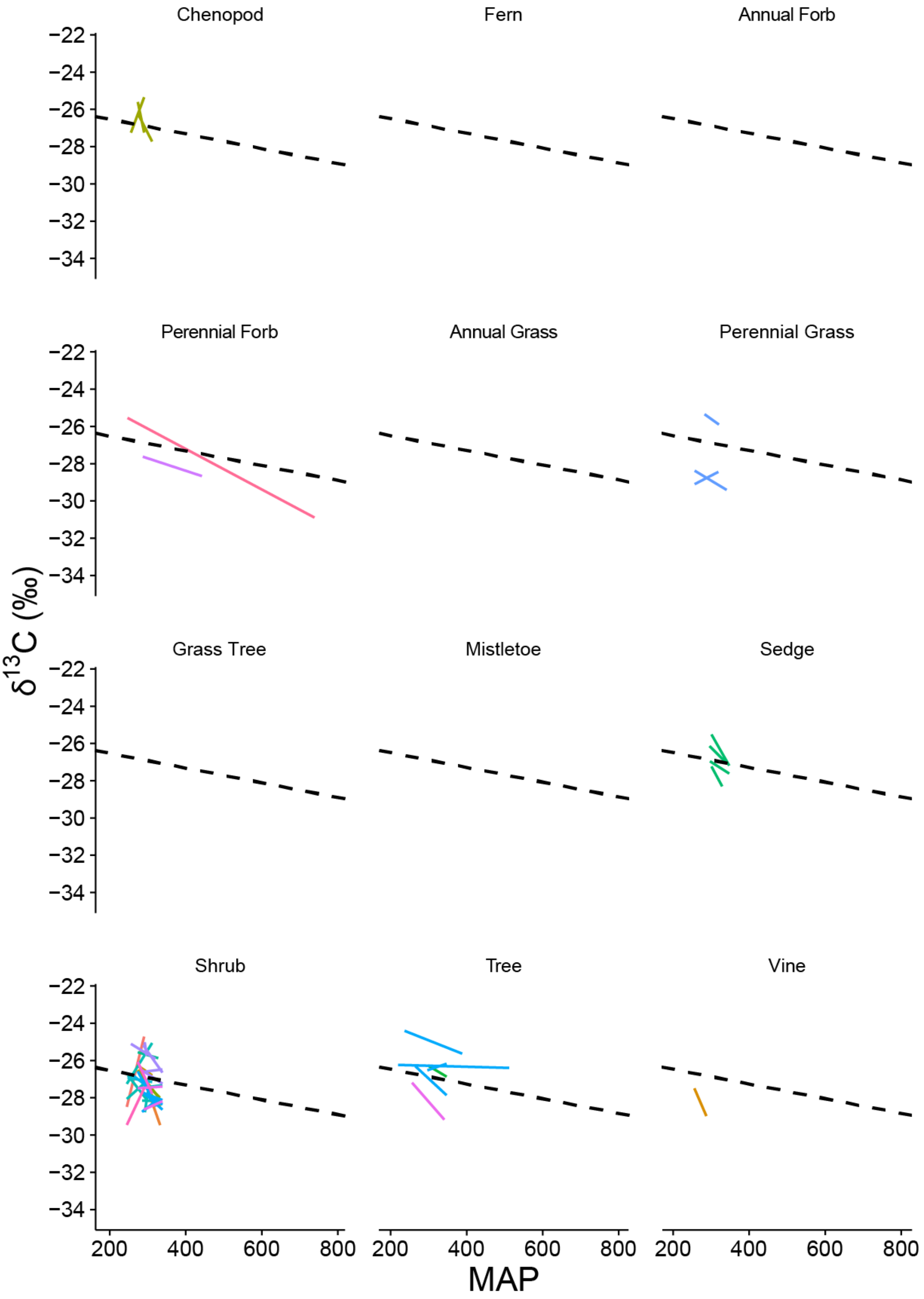


Figure S3: Species leaf δ^{13}C~MAP regressions (m_s) for 47 SWATT species and SWATT common regression (m_c). Colours represent 21 different families to demonstrate the phylogenetic diversity within growth form classes.

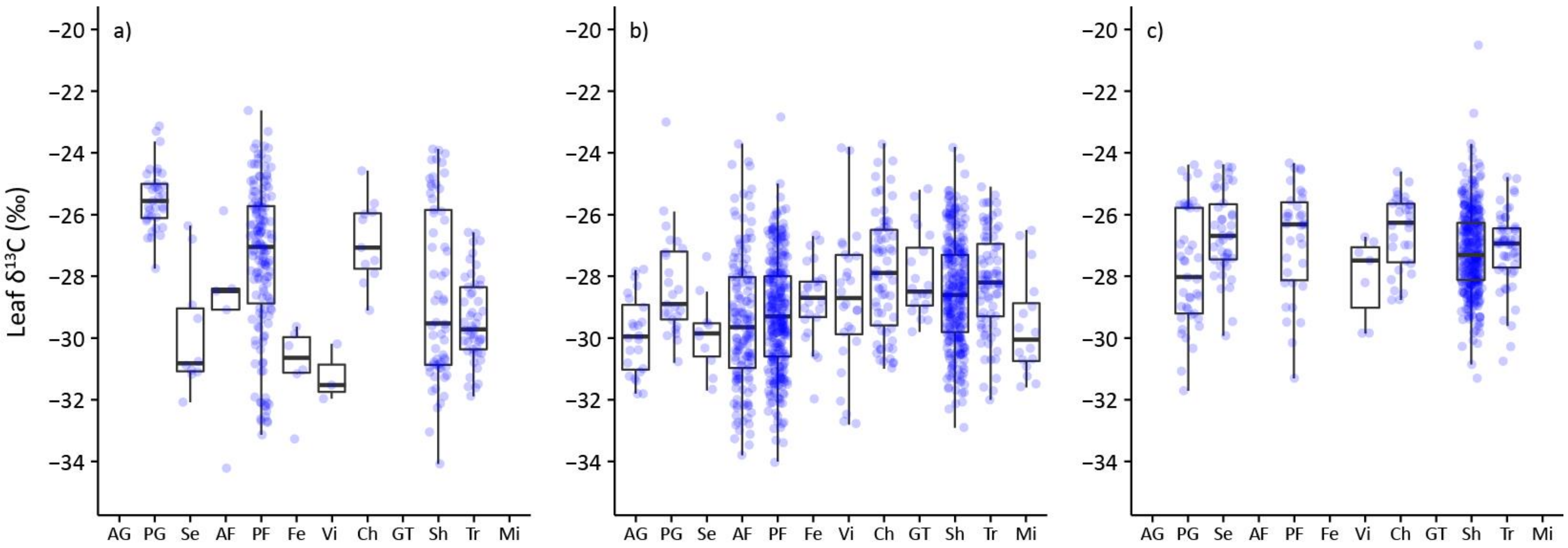


Figure S4: Stable carbon isotope ratios ($\delta^{13}C$) for plants of each growth form on the NECT (a), TREND (b) and SWATT (c). AG=annual grass, PG=perennial grass, Se=sedge, AF=annual forb, PF=perennial forb, Fe=fern, Vi=vine, Ch=chenopod, GT=grass tree, Sh=shrub, Tr=tree, Mi=mistletoe.

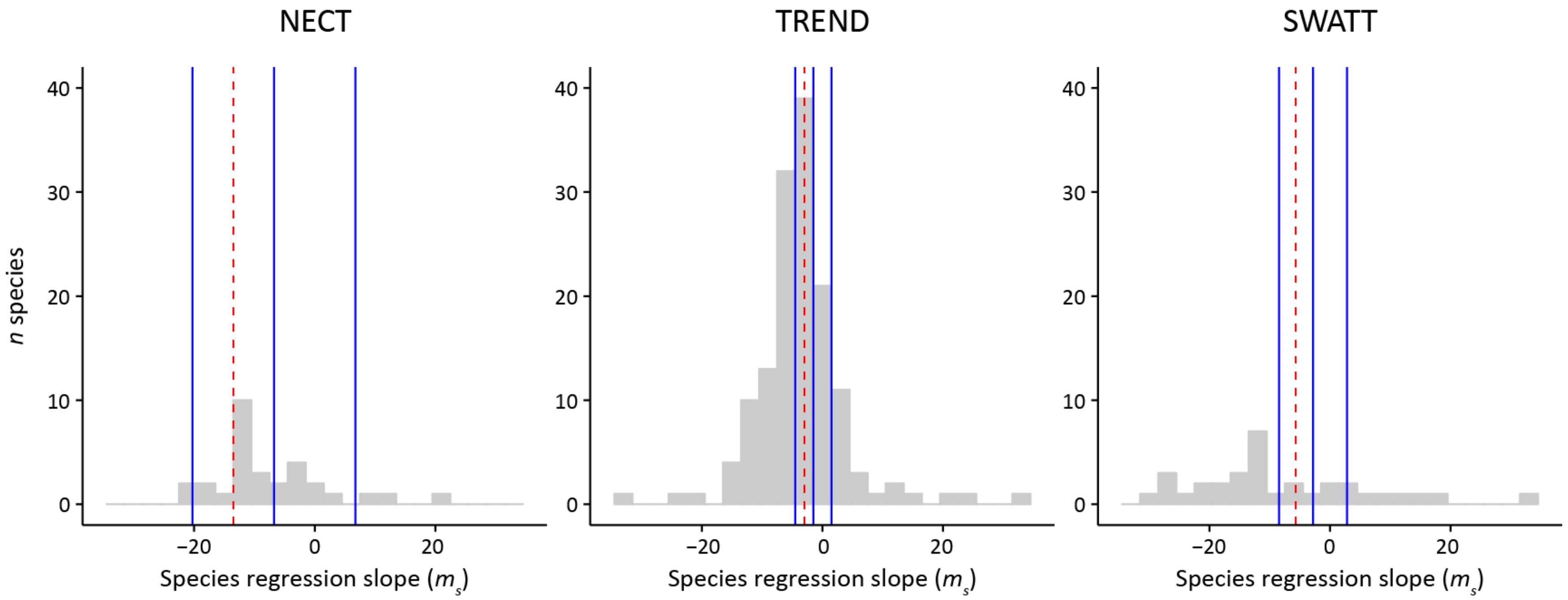


Figure S5: Frequency histogram of species MAP/δ^{13}C (m_s) regression slopes for plants on the NECT (36 species), TREND (150 species) and SWATT (47 species). Dashed red line = Transect common slope (m_c). Solid blue lines delineate response modes (biotic homeostasis m_s<1.5(m_c), universal scaling m_s=m_c±0.5(m_c), insensitivity m_s=0±0.5(m_c), contrary m_s>-0.5(m_c))). Bin size = 3‰. To allow data to be visualised, outliers (m_s <-35‰ or >35‰) were removed (27 species).

Chapter 5: Vegetation change across a mediterranean to arid gradient is robust to survey methodology

Title of Paper	Vegetation change across a mediterranean to arid gradient is robust to survey methodology
Publication Status	○ Published ○ Accepted for Publication ○ Submitted for Publication ☑ Publication Style
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Name of Principal Author (Candidate)	Stefan Caddy-Retalic		
Contribution to the Paper	Designed the study, led the field work in South Australia, conducted data analysis and wrote the manuscript.		
Overall percentage (%)	75		
Signature		Date	10 Oct 2017

Name of Co-Author	Prof Glenda M. Wardle		
Contribution to the Paper	Supervised SCR. Designed the study, provided input in to data analysis and manuscript. Reviewed and edited final manuscript.		
Signature		Date	23 Oct 2017

Name of Co-Author	Dr Greg R. Guerin		
Contribution to the Paper	Designed the study, undertook field work, plant identification and contributed plant data set. Provided coding support and direction on analytical direction. Reviewed and edited final manuscript.		
Signature		Date	11 Oct 2017

Name of Co-Author	Emrys J. Leitch		
Contribution to the Paper	Undertook field work and plant identification. Reviewed and edited final manuscript.		
Signature		Date	10 Oct 2017

Name of Co-Author	Francesca A. McInerney		
Contribution to the Paper	Supervised SCR. Provided input on analytical approach. Reviewed and edited final manuscript.		
Signature		Date	11 Oct 2017

Name of Co-Author	Prof Andrew J. Lowe		
Contribution to the Paper	Supervised SCR. Designed the study. Reviewed and edited final manuscript.		
Signature		Date	2 Nov 2017

Title: Landscape scale analysis of a mediterranean-arid zone bioclimatic gradient reveals scale-dependent ecotone patterning

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Abstract

Questions:

How do the rate and main drivers of vegetation change across a Mediterranean-arid zone gradient?

Is species turnover and vegetation structural change monotonic, or are there one or more disjunctions which may represent ecological thresholds for vegetation change?

Do different survey methodologies lead to similar descriptions of vegetation change?

Location:

127 sites spanning ca. 800 km and 160-980 mm MAP from the temperate Fleurieu Peninsula to the arid Stony Plains of southern Australia.

Methods:

Overlapping transects were established across a bioclimatic gradient using two methodologies; a nested approach of five small (30x30 m) plots at 17 locations; and a non-nested approach using 42 single, large (100x100 m) plots across an extended gradient. We related change in vegetation composition and structure to soil, landscape and climate to determine the strongest environmental associations. Ordinations and TITAN threshold analysis were used to detect potential ecological disjunctions associated with environmental thresholds. Site groupings based on Bray-Curtis classification were aligned with landscape classifiers (bioregions, sub-regions and agro-climatic zones) to test for congruence. Results from the two transects were compared to test the effects of the different sampling methodology and spatial sampling on pattern detection.

Results:

We found similar trends for both transects. Ordinations and regressions for both transects indicate vegetation changes linearly with the environmental gradient. Species richness and total cover were positively correlated with rainfall but declined with temperature. Species turnover was very high, with low nestedness, indicating species replacement was very high for this region. NMDS ordinations indicated that vegetation was structured along the primary (latitudinal) climate gradient and a weaker (longitudinal) soil gradient. We also identified strong structural change, with woody cover positively correlated with rainfall. TITAN analysis of the nested (TREND-Guerin) transect indicated an ecotone between 400-600 mm MAP, however little support for an ecotone was found on the longer TREND-AusPlots transect. Classification of sites was reasonably aligned with a coarse landscape classifier (agro-climatic zones) but poorly aligned with bioregions and subregions.

Conclusions:

Climate is the major driver of broad-scale vegetation change in South Australia, while topographic and edaphic variables drive vegetation change at a more local scale. At the subcontinental scale, tested survey methodologies revealed similar vegetation patterning, suggesting biotic change is readily recovered by a variety of survey methods. TITAN identification of a threshold within the shorter, nested transect but not the longer transect which extended in to the arid zone, indicates that while smaller-scale vegetation disjunctions may be present, change spanning the entire mesic to arid zone is largely monotonic.

Nomenclature:

Census of South Australian Plants. Available at www.flora.sa.gov.au/census.shtml

Abbreviations:

EC = Soil Electrical conductivity; MAP = mean annual precipitation; NMDS = Non-metric dimensional scaling; TITAN = Threshold Indicator Taxa Analysis; VCE = visual cover estimate

Introduction

Plot networks that are spatially aligned with environmental gradients (e.g. climatic or soil gradients) allow species turnover to be linked to potential environmental drivers, which is critical to understanding how and why ecosystems change in time and space. Locations on such environmental gradients at which species turnover is higher than for the rest of the gradient may indicate the presence of an abiotic threshold, at which point non-linear biotic change occurs. By linking vegetation change to known environmental gradients and understanding if and where non-linear disjunctions occur, we can better predict how vegetation will response to perturbations (including climate change) and promote biodiversity conservation and management.

Drivers of species turnover can include abiotic factors such as climate and soil, as well as biotic effects, such as shade or associations with other organisms. Because these factors rarely change in isolation, most studies examine complex gradients comprised of several overlapping factors (e.g. temperature, rainfall, soil pH). These variables potentially affect vegetation independently, as well as together. Complex gradients can therefore lead to cumulative, dampening or otherwise interacting effects of multiple variables (Conover and Schultz 1995; Powers and Reynolds 1999).

Because environmental drivers co-vary on complex gradients, isolating the effects of a single driver (including spatial distance) on species turnover can be difficult (Warren *et al.* 2014). Nevertheless, generalizable trends of how vegetation responds to common gradients is of interest (e.g. the transition between temperate and arid zones is represented in Australia, Europe, Africa and the Americas). When observations of change from independent taxa and locations are combined, it is possible to determine if a generalised response can be expected, or if responses to environmental change are likely to be peculiar to a specific location (Caddy-Retalic *et al.* 2017).

Rapid global change, particularly in climate, has seen increased interest in bioclimatic transects, where vegetation and environment vary together, as a tool to identify whether space can be used as a proxy for time in predicting how vegetation might change in the future (Blois *et al.* 2013; Caddy-Retalic *et al.* 2017). In addition to determining the primary drivers and mechanisms of change, a goal of this approach is to establish whether species turnover and vegetation structure are linearly linked to the environment, or if there are thresholds at which non-linear or abrupt change occurs. Abrupt vegetation transitions have been detected on gradients of elevation (Crausbay and Hotchkiss 2010; Díaz-Varela *et al.* 2010) and climate (Allen and Breshears 1998; Kutiel *et al.* 1995) but other gradients have revealed continuous change with no obvious threshold (e.g. Auerbach and Shmida 1993). If thresholds of vegetation change exist and can be detected, they may be able to be used to predict whether vegetation will respond to ongoing climate change gradually, or experience one or more dramatic transitions as thresholds are breached (Kreyling *et al.* 2014; Lenton 2011).

Should thresholds exist on a large-scale gradient, they may occur at the intersection of different physical environments (i.e. prevailing climate, landform, etc.) because different environments will impose different filtering processes on the species that can persist. In order to divide large spatial areas, such as continents, into units that are both environmentally meaningful (i.e. of similar environment) and an appropriate scale to be useful for management, landscape classification processes have been undertaken for many parts of the globe. In Australia, the agro-climatic classification (Hutchinson *et al.* 2005; hereafter known as agro-climatic zones) and Interim Biogeographic Regionalisation for Australia (Thackway and Cresswell 1995; hereafter known as IBRA bioregions and subregions) represent the two most commonly used landscape classification systems. Both of these classification systems have been undertaken at a continental scale, but it is unclear as to whether vegetation transition across zones will relate to ecotones at a finer spatial scale.

The transition between the Mediterranean and arid zones is an ideal study location for vegetation gradient research, because this transition occurs at many locations around the globe, providing opportunities for replication. Additionally, the mediterranean climate zone is usually associated with intensive human use, particularly for agriculture, yet is predicted to shift globally over the next century (Klausmeyer and Shaw 2009). The mediterranean biome is megadiverse (Abbott and Le Maitre 2010), and the combined threats of climate change and land use change make it one of the world's most vulnerable biomes (Underwood *et al.* 2009). Understanding how vegetation might change in response to such perturbation is therefore important for human use and biodiversity conservation in the mediterranean zone.

Our efforts to detect vegetation response to environmental change (including thresholds) may be influenced by the survey method chosen to describe a system. For example, the impacts of a design using many small plots or fewer larger plots has been debated (Vittoz and Guisan 2007). Large plots better represent the spatial patterns of association among plants, particularly for larger growth forms such as trees, which are typically more sparsely distributed than herbaceous plants. On the other hand, smaller plots are likely to deliver more complete species inventories because there is less area to search (Bonham 2013). Similarly, within equal resource constraints, field measures of plant cover can emphasise accuracy, through a larger number of qualitative measures; or precision, through fewer, more quantitative measures. The choice between few large or many smaller plots is common to all vegetation surveys and must be guided by the survey environment and scientific questions being addressed, usually requiring a compromise between competing interests (e.g. plot homogeneity and survey intensity) (Vittoz and Guisan 2007).

Here, we leverage the data available from two programs undertaken in southern Australia, where the mediterranean zone is projected to contract towards the southern coast (Klausmeyer and Shaw 2009). The first program surveyed the vegetation on the Adelaide geosyncline, a protracted linear basin with uplifted ranges extending inland from the Fleurieu peninsula, to establish the Transect for Environmental Monitoring and Decision making (TREND), a ca. 550 km transect of vegetation plots traversing the gradient between the mediterranean zone and arid zone (Guerin *et al.* 2014; Guerin *et al.* 2016). The TREND was subsequently resampled and expanded under the auspices of the Australian Transect Network (Caddy-Retalic *et al.* 2017). We use the data provided by these programs to address the following aims:

Aim 1: Describe the vegetation change across a bioclimatic gradient from the mediterranean zone to the arid zone of South Australia and determine the degree to which plant composition and structural change is associated with climate.

Aim 2: Test whether vegetation composition and structural change on the gradient is monotonic or if there are one or more disjunctions which may represent ecological thresholds for vegetation change.

Aim 3: Determine whether survey methodology affects our ability to relate changes in species richness, cover and composition to the environment.

Aim 4: Determine the degree to which vegetation communities on a gradient align with coarse scale environmental classifications.

Methods

Field surveys

Two field campaigns were undertaken using different survey methods. The initial survey, hereafter referred to as the TREND-Guerin transect, was undertaken in the austral spring (August to December) of 2011 by Guerin *et al.* (2014), using a nested design of five 900 m^2 (30x30 m) plots spread across 17 locations, spanning a 550 km transect from Deep Creek, on the Fleurieu Peninsula to Mt Hack in the Northern Flinders Ranges (Figure 1, Supplementary Table S1, Table S2). The survey was undertaken in order to assess the study region as a spatial analogue for climate change through the linking of vegetation composition with spatial environmental change (Guerin *et al.* 2014). The survey methodology included making a plant species inventory at each site with cover estimates for all species.

The second field campaign, to establish the TREND-AusPlots transect, was undertaken during spring from 2012 to 2014 using the AusPlots Rangelands survey method (White *et al.* 2012). Ideally, all surveys would have been undertaken in a single trip, but logistical challenges in gaining access to remote sites and the exhaustive survey of large plots made this impossible. Rather than extend a single trip over multiple seasons (i.e. winter to spring or spring to summer), we maximised the quantity and quality floral material by undertaking three spring surveys.

The TREND-AusPlots surveys were undertaken as an investment in ecological infrastructure by Australia’s Terrestrial Ecosystem Research Network using a methodology designed to promote quantitative and repeatable surveillance monitoring of rangeland biomes (White *et al.* 2012). One hectare (100 x100 m) plots were situated to overlap a TREND-Guerin plot in 17 locations, with an additional 8 plots situated to fill gaps in the gradient between TREND-Guerin plot locations, and 17 plots to extend the transect 150 km north to the Gammon Ranges and Stony Plains (Figure 1, Supplementary Table S1). At each AusPlot, collections were made of all vascular plant species, and structure, including cover, measured using a point intercept method. All plants present at 1010 intercept points on N-S and E-W transects across the plot were recorded, providing a quantitative measurement of vegetation structure (White *et al.* 2012).

To minimise the potential of sampling transitional or heavily degraded vegetation, all sites were chosen in large areas of homogenous “best on offer” native vegetation on public or private conservation reserves, except the northernmost Stony Plains AusPlots (Mt Lyndhurst and Murnpeowie), which are on pastoral stations. Surveys were conducted in years which experienced rainfall near to or more than the long-term mean (Figure S1). Vouchers were identified following the nomenclature of Barker et al. (2016) and deposited in the State Herbarium of South Australia (AD).

Environmental data

Elevation was extracted from the 9s Digital Elevation Model v3 (Hutchinson *et al.* 2008). Site values for Mean Annual Temperature (MAT; Bio01); Mean temperature of warmest quarter (MaxT; Bio10); Mean temperature of coldest quarter (MinT; Bio11); Mean Annual Precipitation (MAP; Bio012); Precipitation of wettest week (MaxP; Bio13), Precipitation of driest week (MinP; Bio14); Mean Annual Moisture Index (MAMI; Bio28); Mean moisture index of the wettest quarter (MaxMI; Bio32) and Mean moisture index of the driest quarter (MinMI; Bio33) were extracted from BioClim layers of a 1960-2014 long-term average at 9 second (approx. 250m resolution) extracted from ANUCLIM 6.1 (Xu and Hutchinson 2013) with the GEODATA 9 second DEM (Hutchinson *et al.* 2008) derived by CSIRO (Harwood *et al.* 2014). Monthly climate data for four permanent weather stations (Arkaroola (107099), Hawker SA (019017), Clare Calcannia (021075) and Pawara Sharon

(023761) was provided by the Australian Bureau of Meteorology (http://www.bom.gov.au/climate/data).

Surface soil pH and EC were measured for all sites using portable probes on samples suspended in demineralised water. For the TREND-Guerin surveys only, the sand, nitrate, ammonium, potassium and phosphorus content of surface soils were measured in the laboratory and estimates made of percentage surface strew and outcrop.

Data analysis

All data analyses were undertaken in R (R Core Team 2017). In order to assess the completeness of species detection in our surveys, species accumulation curves were calculated using `vegan` (Oksanen *et al.* 2016). For the TREND-AusPlots sites, cover for each species was calculated by dividing the number of point intercepts at which each species was encountered by the number of points sampled (1010). Due to the potential for multiple species to occur at different strata at the same intercept point, cover values of a growth form or an entire plot can exceed 100%. To account for aspect being a circular variable (i.e. there is only 1° difference between 0° and 359°), aspect was converted to the linear variables "eastness": $\sin(\frac{\text{aspect} \times \pi}{180})$ and "northness": $\cos(\frac{\text{aspect} \times \pi}{180})$.

In order to test the spread of sites in environmental space and determine the dominant environmental variables across space, we ran a Principal Component Analysis (PCA) on environmental variables for both transects. Variables were scaled to ensure variables with larger scales did not dominate the analysis. Bray-Curtis dissimilarity indices for each transect were calculated based on plant composition, and non-metric dimensional scaling (NMDS) and canonical correspondence analysis (CCA) ordinations undertaken in `vegan`. We assessed which environmental variable showed the strongest association with community change by correlating all environmental variables against the NMDS ordination. Mean annual precipitation (MAP) and mean annual moisture index showed the highest correlation to ordinations, and MAP was selected to represent the gradient for subsequent analyses due to its clear control on plant productivity, particularly in the arid zone. Species richness and cover values were regressed against MAP to visually inspect for non-linear relationships. The cover and species richness of herbaceous and woody species richness were regressed to test for potential suppressive influence on herbaceous species through shading. To quantify the rate of species turnover with environmental change, we measured the species turnover ($ß_{SIM}$) and nestedness ($ß_{SNE}$) of assemblages on both transects using `betapart` (Baselga and Orme 2012) using the spatial order of sites along the transects from north to south.

In order to compare the structure of species assemblages across the gradient, we built species composition dendrograms based on Bray-Curtis dissimilarities and reordered the leaves by latitude to respect the spatial structure of the plots. Finally, we attempted to identify the region of highest biotic change on each transect using Threshold Indicator Taxa Analysis (TITAN; Baker and King 2010). Datasets were filtered to exclude species found at <3 sites and MAP used to represent the gradient. TITAN analysis was undertaken using `TITAN2` (Baker *et al.* 2015), with 1000 bootstrap replicates for each data set.

Results

Environmental gradient analysis

Principal Component Analysis (PCA) of the TREND-Guerin and TREND-AusPlots transects confirmed a primary temperature-rainfall gradient corresponding to the first PCA axis, and an orthogonal soil (EC, NO3 and K) gradient (Figure 2). Hutchinson agro-climatic zones are clearly delineated on these ordinations, with the exception of some overlap between

E1 and E2 zones for the TREND-AusPlot sites. The E6 (semi-arid) and E2 (dry mediterranean) zones occupied the most environmental space, with more mesic (D5, cool, wet; E1, mediterranean) and arid (G, desert) sites confined to the periphery (Figure 2).

Most tested environmental variables correlated with the environmental PCA (Figure 2), indicating a dominant gradient characterised by moisture availability (precipitation and moisture index), temperature (mean annual temperature and minimum temperature of the coolest month) and soil pH; with a minor orthogonal gradient of soil and topographic variables (elevation, sand content, slope and soil electroconductivity. This correlation of many environmental variables indicates that our study gradient exemplifies a complex gradient.

The variables best able to represent the gradient (based on R^2 scores for NMDS ordinations of both transects) were mean annual precipitation (MAP) and mean annual moisture index (Table 1). Temperature, precipitation and moisture values were highly correlated with the ordination, with TREND-AusPlots showing greater overall R^2 values than TREND-Guerin plots due to the greater environmental extent of that transect. Except for soil pH, edaphic variables did not correlate strongly with the PCAs, suggesting that while a weak edaphic gradient is present, soil factors vary considerably locally across the two transects.

Vegetation patterning

Across all sites, a total of 4,861 plants were identified, comprising 794 species from 82 families. 698 species were detected on the TREND-AusPlot transect, with 417 species identified on the TREND-Guerin transect. An average of 45.9 (95% CI ±3.7) species were found in each one-hectare AusPlot and 33.2 (95% CI ±2.2) species in the smaller (0.09 ha) TREND-Guerin plots. 96 species were found in TREND-Guerin plots exclusively, roughly half of which were herbaceous grasses, sedges or small forbs (particularly orchids), which may have not been present when AusPlot surveys were being undertaken. Most of the larger trees and shrub species (e.g. Eucalyptus and Acacia species) that were present in TREND-Guerin plots but not TREND-AusPlot were present in low cover (i.e. scattered individuals) in plots that did not overlap the co-located AusPlot. Based on species accumulation curves (Figure S2), the TREND-Guerin transect was more completely sampled than the TREND-AusPlots transect.

The most widespread species were the shrubs *Dodonaea viscosa* (Sapindaceae; 16 locations) and *Senna artemisioides* (Fabaceae, 16 locations) and the forbs *Sonchus oleraceus* (Asteraceae, 16 locations), *Dianella revoluta* (Xanthorrhoeaceae, 15 locations) and *Sida fibulifera* (Malvaceae, 15 locations).

Woody and total (woody+herbaceous) species richness were correlated with MAP for both TREND-AusPlot and TREND-Guerin plots but herbaceous richness was correlated with MAP for the TREND-Guerin transect only (Figure 3). For the entire mediterranean zone (agro-climatic zones D5, E1 & E2), total species richness was 51.9±6.7/ha for the TREND-AusPlot transect, and increased linearly with MAP (Table 2; Figure 3). Total cover also increased linearly with MAP for both TREND-Guerin plots and TREND-AusPlot, although TREND-Guerin cover values (summed VCEs for each species) were consistently higher than the TREND-AusPlot cover values (calculated from point intercept data) (Figure 3). The correlation between vegetation cover and rainfall was driven by woody vegetation, particularly trees and shrubs (Figure S3). No relationship was found between herbaceous cover and MAP (Figure 3; Figure S3) or between woody and herbaceous cover (Figure 4).

Entire-transect beta diversity (measured as Sørensen dissimilarity) was 0.976 (TREND-Guerin) and 0.966 (TREND-AusPlots) with low nestedness (0.059 for both transects, Table 3), consistent with results reported for vegetation on a similar gradient in Western Australia (Gibson *et al.* 2017).

Environmental correlates with vegetation change

Constrained correspondence analysis of species cover values in each plot (Figure 5) revealed vegetation corresponded to the temperature-rainfall gradient shown in Figure 2. Soil chemistry data were only available for TREND-Guerin plots, but revealed weaker gradients of soil potassium (K), phosphorus (P), ammonium (NH_4), nitrate (NO_3), as well as the cover values for rock outcrop and surface strew, all aligned with the primary temperature-rainfall gradient.

NMDS ordination of sites based on species cover values showed a primary north-south gradient for both transects (Figure 6). Tests for correlations between the ordinations and environmental variables showed all tested climatic variables were strongly correlated to vegetation composition, but topographic and edaphic variables were much more weakly correlated (Table 1). Mean Annual Precipitation (MAP) and Mean Annual Moisture Index (MAMI) were the strongest correlates with both datasets (Table 1). MAP was therefore used to represent the gradient in subsequent biotic analyses.

Biotic change appears to mirror MAP change along the gradient, with no immediately discernible disjunctions based on cover or richness correlations (Figure 3), or species composition and cover ordinations (Figure 6). The TREND-Guerin NMDS showed some overlap between sites at different locations (e.g. REMU and TOTR, and HORS and MONT, and similar spread for sites within and between several survey locations (Figure 6b).

Threshold analysis indicated that the area of highest species turnover occurred between ca. 400-600mm MAP for the TREND-Guerin transect, but in a much broader interval of 160-600mm MAP for the broader AusPlot transect (Figure S4).

Congruence of vegetation groupings with landscape classifications

Classification of sites based on species composition and cover was broadly aligned with agro-climatic zones (Figure S5; Figure S6). The first division within the TREND-AusPlot classification (Figure S6) was between the far north (Gammon Ranges and Stony Plains sites) and the rest of the transect. Subsequent divisions are congruent with semi-arid and temperate agro-climatic zones, but an important exception was the mallee woodland sites (PEDB, TOTR, REML, PENG, CLEM and ARK3). Mallee woodlands are characteristic of the semi-arid zone in Australia, but extend into the arid and temperate zones. Mallee woodlands dominated by *Eucalyptus odorata* cluster together in the E2 climatic zone despite REML being an E6 site, and similarly a *Eucalyptus socialis* dominated mallee clade in the E6 zone includes the E2 PENG and CLEM sites. The REML sites formed a similar outlier cluster in the classification of TREND-Guerin plots (Figure S5). The Horsnell Gully (HORS) group of sites sit within the D5 (cool, wet) agro-climatic zone, rather than the mediterranean zone, but were not separated by the dendrogram from sites from the surrounding mediterranean zone (Figure S5).

Less alignment was visible between the classifications and IBRA bioregions or subregions (Figure S7; Figure S8), particularly for the longer TREND-AusPlots transect which traversed more bioregions. Only two branches sat completely within any one IBRA bioregion, but most bioregions were represented in more than one group (Figure S8).

Discussion

By examining two overlapping transects, we described turnover in vegetation structure and composition from the arid zone to the mediterranean zone, and found that vegetation responds to a complex gradient of environmental variables. Our results provide limited support for an ecotonal region between 400-600 mm MAP as reported by Guerin et al. (2014), but this ecotone was not apparent in the analysis of the TREND-AusPlots transect, which extended further in to the arid zone. The lack of evidence for an ecotone in the longer transect indicates that while there may be a zone of elevated species turnover within the northern Mt Lofty ranges and southern Flinders Ranges, when a slightly greater climatic range is considered, this putative ecotone is not a major vegetation delineator. Here, we discuss the patterns of vegetation patterning with climate on the TREND gradient and the consistency of results between the TREND-Guerin and TREND-AusPlots transects.

Environmental gradient detection

The correlated variables of temperature and rainfall dominated both the TREND-Guerin and TREND-AusPlot transects, transitioning from the cool, wet south to the hot, dry north. Most other tested variables (soil nutrients and pH, outcrop and surface strew) corresponded weakly with the climatic gradient, and together form a complex bio-edaphic gradient. Complex gradients comprised of many environmental variables can be represented by ordination axes but can be difficult to intuitively conceptualise. As MAP was the putatively strongest driver of vegetation change, this was how we represented the complex gradient, but the covariance of most tested variables, including temperature, rainfall, soil pH and soil nutrients makes it impossible to isolate a single variable as the primary driver, particularly given that there are causative relationships between many variables. For example, increased rainfall leads to leaching of basic ions from soils and a corresponding decrease in soil pH, particularly in sandy soils. A relationship between MAP, soil pH and percentage sand is therefore expected. The dominance of climate (rather than soil or topography) as the driver of change on this large-scale gradient aligns with results that suggests that globally, plant community composition is largely edaphically driven at a local scale, but becomes climatically driven at increasing spatial scales (Siefert *et al.* 2012).

Because we used MAP to represent the complex gradient, we investigated the consistency of the survey years with long-term averages for our study region. Overall, rainfall was reasonably representative of the long term average. 2011 and 2012 had some exceptions, recording high autumn rainfall on the northern half of the transect, and a low winter-spring rainfall around Clare. Higher than average rainfall is likely to have prompted the growth of some herbaceous species in the arid zone at the northern end of the transect. Conversely, the lower than average rainfall in 2011 and 2012 may have depressed these species, particularly short-lived annuals.

For this study, we sampled nine second (~250 m) resolution climate layers, which have similar cell size to the survey plots (30-100 m). High resolution layers give more accurate climate data than the 1 km interpolated data used in other studies (e.g. Guerin et al. (2014)), particularly for sites in the Mt Lofty, Flinders and Gammon Ranges. These Ranges are topographically complex, with elevation and aspect - and thus climate - varying substantially within 1 km cells. Downscaling to nine second cells therefore substantially improves our ability to resolve the environmental drivers of vegetation change.

Survey methodology and biases

All survey methodologies bring inherent strengths, weaknesses and biases, and ideally each survey should be optimised to answer one or more specific questions (Nichols and Williams 2006). Field ecology is time consuming and expensive, however, and there is increasing call to re-use ecological data (Kapfer *et al.* 2016; Zimmerman 2008). The TREND-Guerin plot survey methodology was developed for the location-specific purpose of testing the influence of an environmental gradient on species turnover (Guerin *et al.* 2014), while the TREND-AusPlot methodology was designed to measure a suite of popular ecological attributes while maximising flexibility and applicability across many ecosystem types to support continental-scale investigations by many researchers (White *et al.* 2012).

The three primary biases of vegetation resurvey are plot relocation, observer bias and seasonality bias (Kapfer *et al.* 2016). Because specific plots were not resurveyed in this study, we discuss the potential impacts of observer and seasonality bias, as well as the additional potential bias of plot design.

In order to emphasise accuracy at each location, small (30 m x 30 m; 0.09 ha) TREND-Guerin plots were surveyed with a nested design to allow variation in species richness, abundance and composition to be quantified. TREND-AusPlot are designed to be used in a variety of vegetation types, particularly sparse rangeland communities, and therefore use a large (1 ha) plot size, which reduces variability in vegetation analysis (Otypková *et al.* 2006). The smaller size of the TREND-Guerin plots likely means a less complete representation of the local species were present, even across a grouping of five plots (0.45 ha) compared to one AusPlot. Because the TREND-Guerin plots were permanently marked, we were able to ensure that the TREND-AusPlot sites completely encompassed a

TREND-Guerin plot, but because TREND-AusPlots sites were larger, a larger total area was surveyed. The TREND-Guerin plots and co-located TREND-AusPlot site were located in large areas of homogenous vegetation. Assuming the vegetation was homogenous at the scale of the plots, plot design seems unlikely to substantially influence the ability to detect vegetation patterns across the gradient.

The data used in this study were collected over several years, but confined to spring in order to minimise variation across seasons and maximise the flowering material available to aid in species determinations. The northern end of the transect received higher than normal rainfall in February-March 2011 and 2012, but these sites were not surveyed until 2013. Other rainfall fluctuations were relatively minor and unlikely to have substantially affected the presence or cover of vegetation. Our surveys consisted of a single visit to each site, meaning we were only able to sample species present at that time. It is likely that we did not detect some species, particularly short-lived annual forbs and grasses, and species that can be difficult to distinguish in the field (e.g. some species of *Eucalyptus*), particularly when only sterile material is available. The limitation of a single visit is likely to have limited our detection of species particularly in the arid zone, where many species may only emerge following specific environmental cues (Dickman *et al.* 2014). While it is likely that revisits to the sites over a year would increase the number of species detected,this was not possible within the resourcing constraints of our study. Species accumulation curves indicated most species within the sampled habitat and season were detected in both surveys (Figure S2).

Observer bias can lead to major differences in species detection and cover values (Lepš and Hadincová 1992), but it is difficult to quantify observer differences for different methodologies. Species recorded in the TREND-Guerin sites that were not detected in the later TREND-AusPlot sites may have been absent, or not detected despite similar observer skill and effort because the TREND-AusPlots were more than 10x larger than the TREND-Guerin sites. Cover for each species was estimated for the TREND-Guerin plots, which is a known source of potential observer bias, particularly for species with low cover (Kennedy and Addison 1987). Additionally, the TREND-Guerin methodology used 1% as the lowest possible cover value (Guerin *et al.* 2014), potentially inflating the dominance of rare species. The AusPlots methodology is designed to replicate sites at a scale larger than our study location, and emphasises precision and repeatability of cover estimates by employing a point intercept method to minimise observer error (Lepš and Hadincová 1992; White *et al.* 2012). By comparing the visual estimate data from the TREND-Guerin sites and contrasting it with quantitative vegetation cover derived from the TREND-AusPlots, we were able to determine that patterns of composition and structure were not significantly affected by plot design.

Indeed, the fact that our results were consistent across two independent survey campaigns reinforces that our results were reasonably robust for the sampled region and time period, and that any difference between the surveys can be attributed to the greater spatial extent of the TREND-AusPlots transect or temporal change between the surveys or the methodology used. The consistency of our results provides evidence that data from different survey programs can be reasonably combined in larger analyses.

Vegetation response to environment

Our finding of smooth vegetation transition between the mediterranean and arid zones is relevant because globally the extent of the mediterranean zone is projected to change substantially. Over the next century, the entire mediterranean zone will expand by 6-11%, but contract in Australia, North America and South Africa, primarily through conversion to more arid systems (Klausmeyer and Shaw 2009). To the extent that our spatial gradient can be used to infer a future temporal response, we may therefore expect aridification will lead to a smooth transition to a more arid-adapted flora, rather than a stepped change, highlighting the relevance of our study to global biodiversity change. Our description of spatial floristic change also provide a platform on which to test similar gradients elsewhere to see whether this trend is uniform for all mediterranean systems, or unique to southern Australia (Caddy-Retalic *et al.* 2017). Additionally, this work forms a starting point for comparisons with other taxa, such as the examination of flora-fauna community congruence (Caddy-Retalic *et al.* in prep).

Species richness

Patterns of species richness on the TREND gradient are similar to those recorded elsewhere. Plant species richness is positively correlated with rainfall across much of the world (Pausas and Austin 2001), and an increase in species richness, woody cover and total cover with MAP (Figure 3) is consistent with similar bioclimatic gradients in Western Australia (Gibson *et al.* 2017) and Israel (Aronson and Shmida 1992)). Similarly, the mean species richness for AusPlots in the mediterranean zone (D1, E1 and E2 agro-climatic zones) was 51.9 ± 6.7 species/ha (Table 2), which is consistent with the values published for other mediterranean regions: 68 ± 20 /ha (SW Australia); 70 ± 21 /ha (South Africa); 70 ± 54 /ha (Mediterranean Basin) and (31 ± 10/ha (California) (Cowling *et al.* 1996). The higher species richness for TREND-AusPlot compared to TREND-Guerin plots is likely to be a factor of the larger size of the TREND-AusPlot (1 ha compared to 0.09 ha). Without the development of species-area curves for each group of sites, it was not possible to standardise species richness values to a common area for all plots.

Herbaceous species richness was far more variable than woody species richness (Figure 3), presumably due to the potential for a community that has a homogenous overstory to harbour a far more heterogeneous understory, with many species showing low abundance and patchy distribution. Our finding that herbaceous species richness was related to MAP in TREND-Guerin plots but not TREND-AusPlot (Figure 3) may be related to the extension of that transect in to the arid zone, which displays higher herbaceous species richness than some sites in the wetter half of the transect. High herbaceous species richness in the arid zone is often related to facilitative effects, with small plants often found in association with larger protective grasses, chenopods or shrubs, presumably due to the "nurse plant" role larger plants can have in protecting smaller plants from harsh climatic conditions (Flores *et al.* 2003).

It is notable that we detected increasing species richness and cover with MAP for woody species but not for herbaceous species, which is consistent with results from a mediterranean-arid transect in Israel (Aronson and Shmida 1992) and an elevational transect in the Himalayas (Bhattarai and Vetaas 2003). Although a significant relationship between MAP and herbaceous species richness was detected for TREND-Guerin plots, this was not present in the wider TREND-AusPlot gradient, which displayed high herbaceous species richness (>30 spp/ha) in some arid sites and lower richness (<20 spp/ha) in a few mesic sites (Figure 3). High herbaceous species richness in the arid zone could be related to facilitative effects, as small plants were often found in association with larger, presumably protective grasses, chenopods or shrubs. High herbaceous species richness in the arid and semi-arid zones may be related to the lack of shading from dominant woody vegetation, which was low for those sites, or involve a shift from perennial to short-lived species. Our surveys were undertaken in years which experienced close to the long-term rainfall average, but high interannual rainfall variability means that our surveys were undertaken in “good” years rather than “bad” (dry) years, which is likely to have encouraged the germination of annual forbs and grasses (Aronson and Shmida 1992).

Community composition

We found no obvious evidence of community disjunctions on the gradient. For example, there was no obvious clustering of sites for the AusPlots in NMDS ordinations, with a smooth transition between the Stony Plains, Flinders Ranges, Mt Lofty Ranges and Fleurieu Peninsula sites (Figure 6). The lack of appreciable clustering was probably related to the high degree of species replacement and low nestedness (Table 3), indicating that most species were not shared between many sites.

Vegetation cover

A positive correlation was present between vegetation cover and MAP (Figure 3), suggesting water availability is a primary driver of these ecosystem attributes. The difference in cover values between TREND-AusPlot and TREND-Guerin plots is probably due to the different estimation methods used. TREND-AusPlot used a point intercept method to quantitatively and repeatably obtain cover for each species (White *et al.* 2012), emphasising measurement precision. TREND-Guerin plot surveys used visual cover estimates, which can be varied in their consistancy and bias (Zhou *et al.* 1998), but the survey of five plots in a cluster allowed estimates of accuracy to be generated. The higher woody species cover in TREND-Guerin plots is probably related to a tendency for the cover of overstory species to be overestimated visually (Vanha-Majamaa *et al.* 2000). The lack of a clear trend in herbaceous cover may be related to a lack of rainfall and/or soil fertility at the northern end of the gradient and the suppressive effect of overstorey shading at the southern end. Individual growth forms displayed uniformly low cover (forbs), a patchy distribution that does not appear to be driven by MAP (e.g. grasses), or a combination (i.e. restricted to the arid or mesic end of the gradient but not otherwise showing an obvious relationship with MAP, e.g. sedges (also displayed by woody grass trees and arid-adapted chenopods).

Direct comparison did not reveal a relationship between woody and herbaceous cover or species richness (Figure 4). The lack of correlation between woody and herbaceous plant metrics indicates that while the overstory may have a supressive effect on the understory through light or soil moisture competition at individual sites, any such effects do not occur in a consistent manner across the gradient.

Threshold detection

The detection of ecotones in natural systems is informative because they may represent thresholds at which future environmental change (e.g. climate change) will prompt a transformative biotic response. Modelling of species turnover based on species presence data from 3567 historical survey plots was used to postulate an arid-mesic ecotone for our study region between 400-600 mm MAP (Guerin *et al.* 2013). Analysis of indicator species scores based on species cover for the TREND-Guerin plots supported a 400-600 mm MAP ecotone (Figure S4a) roughly congruent with the E2 agro-climatic zone, but a much broader ecotone (ca. 160-600mm MAP) for the TREND-AusPlot (Figure S4b). Agreement of an upper MAP threshold of ca. 600 mm provides strong support for its actuality, but the lower threshold is more dubious. For the TREND-Guerin transect, there were only two groups of plots with MAP <600 mm, suggesting that more intensive sampling at the arid end of the transect may have altered the results. The TREND-AusPlot sampled heavily on the arid end of the transect (31 of 42 plots had MAP <600 mm), yet failed to find a lower limit to a putative ecotone (160 mm MAP was the limit of the dataset). The lack of an obvious break in linear regressions (Figure 3) or ordinations (Figure 6) supports any such transition zone as either absent, very subtle or inadequately sampled. If a threshold were present in our data, for example a sharp shift from woodlands dominated by trees to a grassland dominated by herbaceous species, there would be a non-linear relationship between both herbaceous and woody cover and MAP. While the ongoing establishment of TREND-AusPlot in the semi-arid and arid zones of South Australia may lead to a more conclusive analysis of the presence of one or more ecotones in our study region, the “E2 ecotone” is plausible, as it represents the environmental extremity for several characteristic arid and temperate zone species (e.g. *Xanthorrhoea semiplana* and *Senna artemisioides* respectively).

Relationship between woody and herbaceous growth forms

Dominant woody vegetation can suppress the growth and occurrence of herbaceous understory species through shading and competition for soil nutrients and moisture. However, the lack of a negative relationship between woody and herbaceous cover or species diversity (Figure 4) suggests that interaction between woody and herbaceous growth forms is more complex. While both tree and shrub cover increased with latitude, herbaceous cover did not (Figure S3). The apparent lack of a suppressive effect could be explained by a facilitative relationship between woody and herbaceous vegetation. Facilitative relationships, in which species display positive interactions which improve one or both species’ ability to persist, are generally more common at the more stressful end of an environmental gradient (Maestre *et al.* 2009). The presence of a competitive interaction at the benign end of a the transect could transform to a facilitative interaction at the more stressful end. For example, while shading reduces photosynthetic potential, it also decreases heat stress and dessication. Thus, dominant woody species could suppress herbaceous species at the mesic end of our transects, while simultaneously providing hospitable microhabitats which allow similar species to persist at the arid end of the transects.

Another important consideration is that the traits of woody plants may be a stronger driver of species turnover than cover alone implies. Some woody plants may be able to exert positive influences on herbaceous species that outweigh their suppressive effect. For example, nitrogen fixing *Acacia* trees and shrubs were present in 88% of TREND-AusPlot, with a mean cover value of 3.8%. While dominant nitrogen-fixers probably do shade out some species, the nitrogen that they introduce in to the environment is likely to promote others, either directly, or by fostering soil fungi that benefit many species through improving nutrient cycling, water holding potential and mycorhizzal associations (Callaway and Walker 1997).

Site groupings and congruence with landscape classifications

Classification of sites was reasonably consistent between TREND-Guerin plots (Figure S5) and TREND-AusPlots (Figure S6), although some plots grouped in different clusters between surveys. The REML site is an *E. odorata* dominated mallee woodland, which clustered with other *E. odorata* mallee woodlands, TOTR and PEDB in AusPlot surveys (Figure S6), but was a first-level outlier in the TREND-Guerin surveys (Figure S5). The HALE AusPlot formed a cluster with MONT and HORS TREND-AusPlot, but the TREND-Guerin plot aligned more closely with BLCK and SAND. These differences persisted in a classification of only those TREND-AusPlot collocated with TREND-Guerin plots (not shown). The HORS site in the D5 agro-climatic zone did not separate from the mediterranean TREND-AusPlot (Figure S6) or TREND-Guerin Plots (Figure S5). The D5 zone within is a very small island in the Mt Lofty Ranges, surrounded by the mediterranean

(E1 and E2) zones. Due to the restricted nature of the D5 zone, it seems reasonable to assume that its flora would be similar to the surrounding mediterranean communities rather than reminiscent of vegetation in the nearest D5 zone (Victoria, ca. 360km SE).

The Interim Biogeographic Regionalisation for Australia (IBRA) classifies the Australian continent in to bioregions and subregions based on “dominant landscape scale attributes of climate, lithology, geology, landforms and vegetation” (Thackway and Cresswell 1995). One of the primary motivations to undertake the bioregionalisation process was the desire to develop ecological zones that were not governed by state boundaries (as distinct from Natural Resource Management regions) and could be used for cross-jurisdictional environmental management. IBRA boundaries have been periodically revised since IBRA’s inception, and are currently in their seventh iteration (IBRA 7). The agro-climatic zones of Australia are derived from extrapolations of elevation-dependent climate zones, aligned with the now superseded version IBRA 5.1 in order to inform on plant growth conditions (Hutchinson *et al.* 2005). We were interested in testing whether vegetation groupings align with these coarse-level landscape classifications in order to evaluate their management utility. The more arid regions (agro-climatic zones E6 and G; and the IBRA Stony Plains (STP) and northern Flinders Lofty Block (FLB) bioregions have been comparitively poorly studied and their likely response to climate change is still unclear (Sparrow *et al.* 2014).

The lack of clear clade groupings within agro-climatic zones (i.e. three of the four highest groupings contain sites from more than one zone) indicates that there are not clear delineations of vegetation between the agro-climatic zones (Figure S5; Figure S6), or the IBRA regions/subregions (Figure S7; Figure S8). We therefore conclude that these coarse level classifications are not suitable for classifying vegetation plots at the scale of our study.

TREND-Guerin plots had a better alignment with agro-climatic zones (Figure S5), but there were fewer, more spatially restricted plots located in fewer agro-climatic zones and IBRA subregions. Our inability to resolve an independent classification of vegetation plots based on species composition and cover with IBRA bioregions follows a similar failure with a larger, continent-wide network of TREND-AusPlot across the Australian rangelands (Baruch *et al.* in review).

The lack of matching between IBRA and our vegetation classifications is presumably due to IBRA (sub)regions being determined on the basis of a suite of factors, of which vegetation composition is only one. Similarly, as agro-climatic zones are based on climate aligned to IBRA regions, they are unlikely to match well to vegetation communities. Navigation of South Australia's vegetation map (https://data.environment.sa.gov.au/NatureMaps/Pages/default.aspx) indicates that several vegetation groups traverse IBRA bioregions, particularly mallee and *Eucalyptus* woodlands.

Limitations and future research

In addition to the biases introduced by methodologies, our results are also likely to have been heavily influenced by survey locations. We attempted to locate sites in areas representative of the broader landscape at each location, but substantial vegetation clearing, a paucity of large protected areas and a number of recent fires in the northern Mt Lofty and southern Flinders Ranges (-34 to -32º latitude) limited our ability to locate sites in these areas as densely as was possible at the northern and southern ends of the transects. Future studies on these transects should attempt to investigate remnant vegetation in this region. Potential locations include Mt Brown Conservation Park (-32.498º, 138.029º), Wirrabara State Forest (-33.060º, 138.182º) and The Pines Recreation Reserve (-34.299º, 138.856º).

By confining our analyses to only vegetation, we ignored a substantial proportion of biodiversity and potentially overlooked important factors controlling the presence or absence of some plant species. Given the importance of biotic interactions (e.g. plants and soil biota; plants and fauna) in maintaining ecosystem function, we recommend future studies should focus on examining multiple groups on bioclimatic transects. By investigating the degree to which environment influences taxa of different types, it will be possible to gain a more holistic understanding of community-level shifts and improve future precitions of biodiversity change.

The analyses presented here test for the presence of linear relationships between environmental variables and biotic responses such as species richness, cover and community composition. It is possible that these techniques are not sensitive enough to detect all possible responses, particularly if they are non-linear. A suite of other techniques, such as Generalised Dissimilarity Modelling (Ferrier *et al.* 2007) may be more sensitive in detecting such responses, however such approaches may be more appropriate for larger data sets such as the Biological Survey of South Australia (Guerin *et al.* 2013).

Conclusion

We find that the vegetation change on the transect traversing from the arid Stony Plains to the mesic Fleurieu peninsula is driven by a complex gradient of climate and edaphic variables, with climate controlling vegetation at larger scales and edaphic variables at a more local scale. Due to the relationships between tested variables and the descriptive methods used, we were not able to disentangle the effects of single drivers but found the gradient can be readily represented by MAP. Total species richness and cover increased monotonically with rainfall, but this trend was much more pronounced with woody vegetation than herbacious species.

Species composition changed linearly across the gradient. Threshold analysis of indicator species in TREND-Guerin plots supported previous findings of an ecotonal zone between 400-600 mm MAP but no lower threshold was supported for the longer TREND-AusPlots transect, suggesting there is no clear disjunction on this longer gradient. The consistency of results between the AusPlots and TREND-Guerin methodologies suggest that both are appropriate for describing vegetation within this region. Vegetation composition did change with IBRAs and arid zones, but there are no clear delineations in vegetation community composition or structure visible in our data. While zonation may be useful for environmental planning, we caution against treating zones as homogenous entities, as not all vegetation communities within an agro-climatic zone or IBRA (sub)region are likely to respond together. We recommend future work in this region focus on collecting vegetation in currently under-sampled areas, as well as investigations of multiple taxa to improve understanding of inter-species facilitation.

Data availability

All vegetation data used in this study are available through the AEKOS data repository. TREND-Guerin plots data (Guerin *et al.* 2015) are available at http://www.aekos.org.au/dataset/173971. TREND-AusPlot data are available at http://aekos.org.au/collection/adelaide.edu.au/trend.

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Table 1: Individual environmental variable correlations with NMDS ordinations of AusPlots and Guerin plots based on plant composition and cover† values.

Category	Variable	AusPlots	Guerin Plots
Topographic	Latitude	0.83***	0.77***
	Longitude	0.05*	0.28***
	Elevation	0.52*	0.12**
	Eastness		0.01 ns
	Northness		0.23***
	Slope		0.09*
Climatic	MAP	0.88***	0.86***
	MinP	0.85***	0.59***
	MaxP	0.80***	0.86***
	MAMI	0.89***	0.85***
	MinMI	0.84***	0.76***
	MaxMI	0.87***	0.72***
	MAT	0.86***	0.56***
	MinT	0.49***	0.41***
	MaxT	0.86***	0.86***
Edaphic	Surface pH	0.67***	0.49***
	Surface EC	0.11 ns	0.10**
	% Surface strew		0.39**
	% Outcrop		0.28***
	% Sand		0.35***
	P		0.17***
	K		0.37***
	NO_3		0.10**
	NH_4		0.35***

Mean Annual Temperature (MAT; Bio01); Mean temperature of warmest quarter (MaxT; Bio10); Mean temperature of coldest quarter (MinT; Bio11); Mean Annual Precipitation (MAP; Bio012); Precipitation of wettest week (MaxP; Bio13) and Precipitation of driest week (MinP; Bio14) were extracted from BioClim layers of a 1960-2014 long-term average at 9 second (ca. 30m resolution). †Cover values for AusPlots quantitatively measured using point intercepts; cover values for Guerin plots are coarse visual estimates. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, ns = not significant.

Table 2: Descriptions of agro-climatic zones and mallee woodland group, with characteristic species for each.

Group	Description*	Sites	Richness	Frequent woody species	Frequent herbaceous species
D5/E1	D5: Moisture availability high in winter-spring, moderate in summer, most plant growth in spring E1: Classic mediterranean climate with peaks of growth in winter and spring and moderate growth in winter	HORS (D5), DEEP, KYEE MTBI, SCOT (E1)	Species richness: 56.4±15.7	*Xanthorrhoeaea semiplana* (5) *Exocarpos cupressiformis* (4) *Olearia ramulosa* (4)	*Acaena echinata* (5) *Dianella revoluta* (4) *Gonocarpus tetragynus* (4) *Senecio pterophorus* (4)
E2	"Mediterranean" climate, but with drier cooler winters and less growth than E1	BLCK, BEEV, CLEM, HALE, KAIS, MONT, PEDB, PENG, SAND, SPRG, TOTR	Species richness: 49.8±8.5	*Acacia pycnantha* (7) *Gonocarpus elatus* (6) *Acacia paradoxa* (5) *Allocasuarina verticillata* (5) *Eucalyptus fasciculosa* (5) *Hibbertia crinita* (5)	*Dianella revoluta* (6) *Cheilanthes austrotenuifolia* (6) *Goodenia blackiana* (5)
E6	Semi-arid climate that is too dry to support field crops. Soil moisture tends to be greatest in winter	ARK1, ARK2, ARK3, ARK4, ARK5, BRAL, BRAU, DUTL, DUTU, MUR2, REML, REMU, VGR1, VGR2, VGR3, VGR5, WAR1, WAR2, WAR3, WILP	Species richness: 43.0±4.5	*Senna artemisioides* (13) *Dodonaea viscosa* (11) *Acacia tetragonophilla* (10)	*Sida fibulifera* (13) *Sonchus oleraceus* (10) *Ptilotus obovatus* (10)
G	Desert, supporting very little plant growth due to water limitation	LYN1, LYN2, MUR1, MUR3, MUR4	Species richness: 38.4±13.3	*Enchylaena tomentosa* (4)	*Calotis hispidula* (5) *Rhodanthe moschata* (5) *Eragrostis setifolia* (4) *Plantago drummondii* (4)

Mallee	Vegetation communities dominated by *Eucalyptus odorata* or *E. socialis*.	ARK3, CLEM, PEDB, PENG, REML, TOTR	Species richness: 49.2±9.2	*Enchylaeana tomentosa* (5) *Rhagodia parabolica* (5) *Alectryon oleifolius* (4) *Senna artemisioides* (4)	*Carrichtera annua* (4) *Rytidosperma caespitosum* (4)

[*] Agro-climatic zone descriptions from Hutchinson et al. (2005). Number of site occurrences for frequent species is noted in parentheses.

Table 3: Components of ß-diversity for two transects

	ß$_{\mathrm{SIM}}$	ß$_{\mathrm{SNE}}$	ß$_{\mathrm{SOR}}$
TREND-AusPlots	0.959	0.006	0.966
TREND-Guerin	0.970	0.006	0.976

ß$_{\mathrm{SIM}}$ = species replacement, ß$_{\mathrm{SNE}}$ = nestedness component of ß-diversity, ß$_{\mathrm{SOR}}$ = Sørensen pairwise dissimilarity

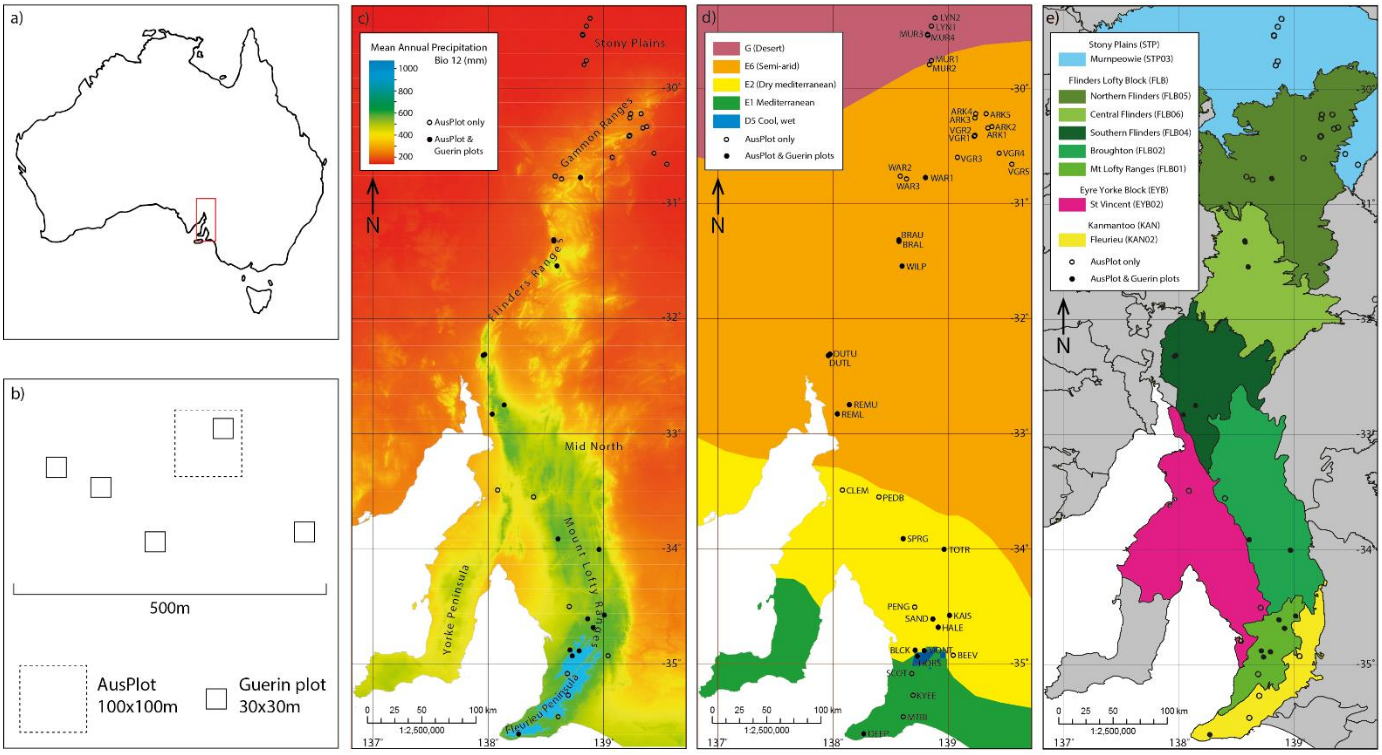

Figure 1: Plot locations in South Australia (a) traversing the mediterranean-arid zone. Guerin plots were co-located with an AusPlot (b) at 17 of 42 locations. c) The transect traverses 818mm mean annual precipitation from the Fleurieu Peninsula, through the Mt Lofty, Flinders and Gammon Ranges before terminating in the arid Stony Plains. d) The agro-climatic zones described by Hutchinson et al. (2005), broadly aligned to mean annual precipitation: Desert (<170mm); Semi-arid (170-500mm); Mediterranean dry (500-600mm); Mediterranean (600-920mm); Cool, wet (>920mm). e) IBRA bioregions and sub-regions surveyed.

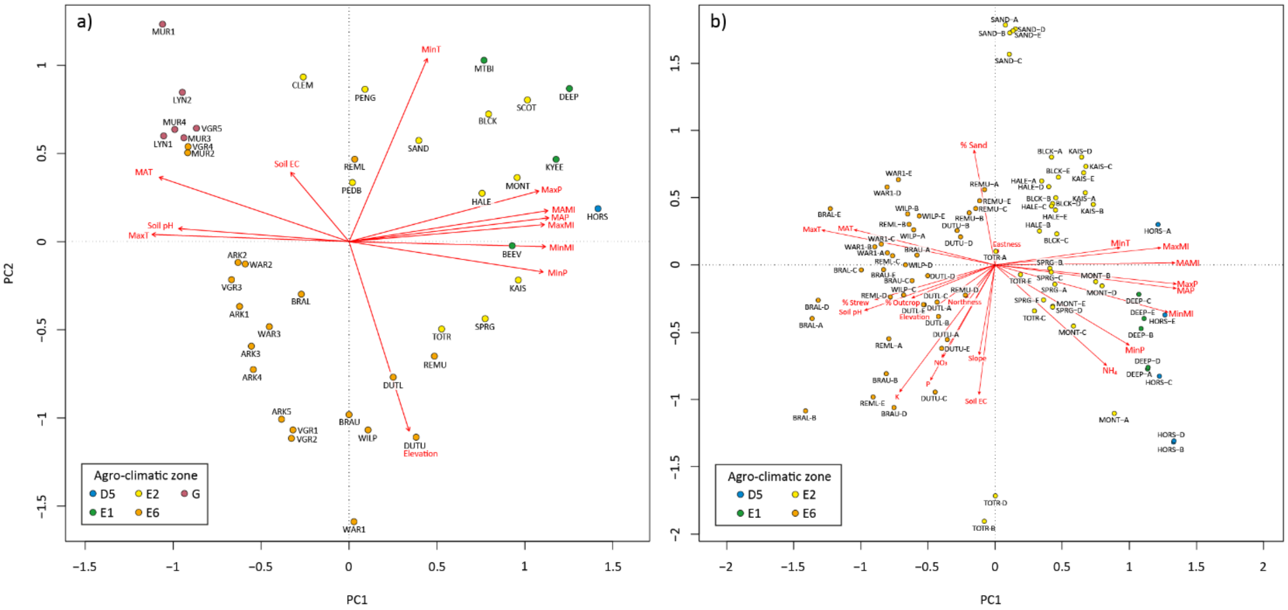

Figure 2: Principal Component Analysis of a) AusPlot and b) Guerin sites based on environmental variables (Table 1). Plots are coloured by agro-climatic zone. Note not all soil and landscape variables were available for AusPlots.

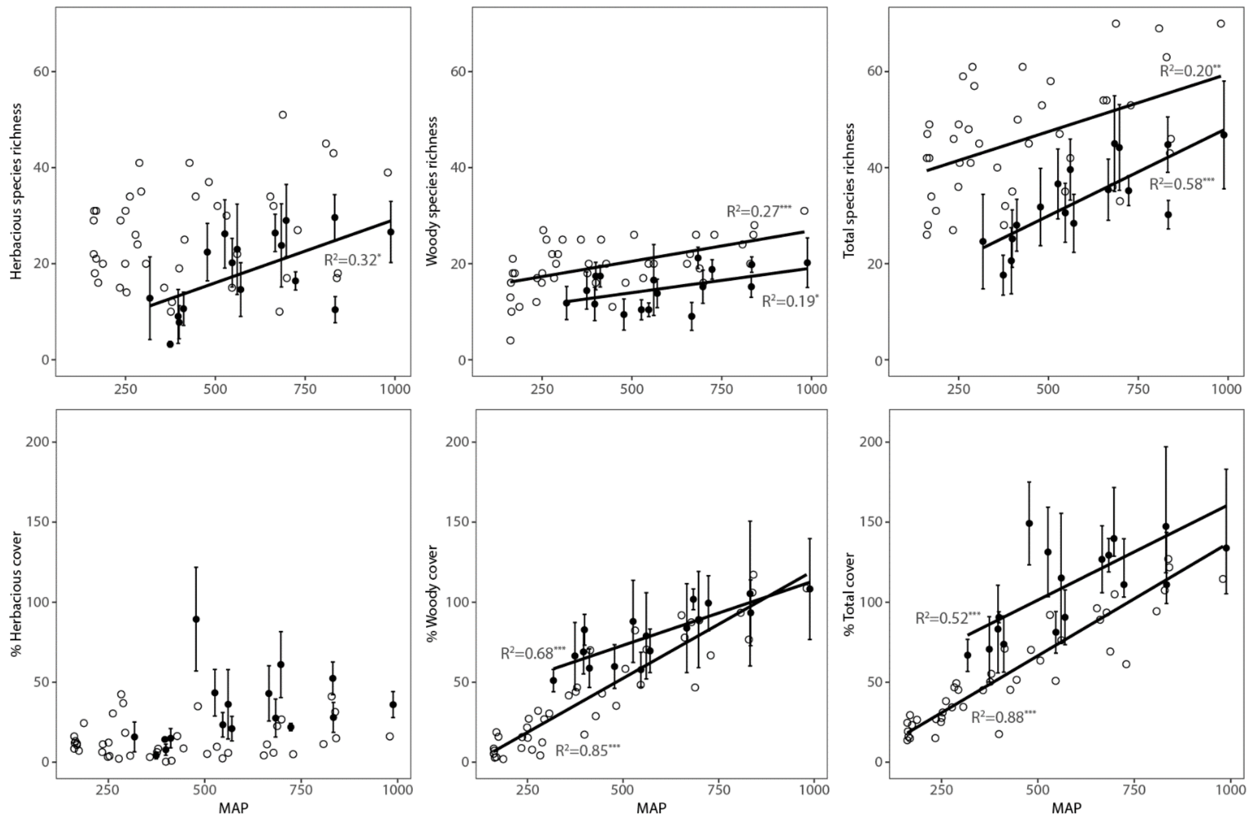

Figure 3: Total woody and herbaceous species richness and cover for AusPlots (open circles) and Guerin plots (filled circles) against mean annual precipitation (MAP). Woody values include chenopods, grass trees, mistletoes, shrubs and trees. Herbaceous values include ferns, forbs, grasses, sedges and vines. Cover values for AusPlots are calculated as summed cover values per species (point intercepts/1010). Cover values for Guerin plots are average summed visual cover estimates. Error bars represent 95% confidence interval across five sites. Solid lines represent statistically significant regressions (*p<0.05, **p<0.01, ***p<0.001).

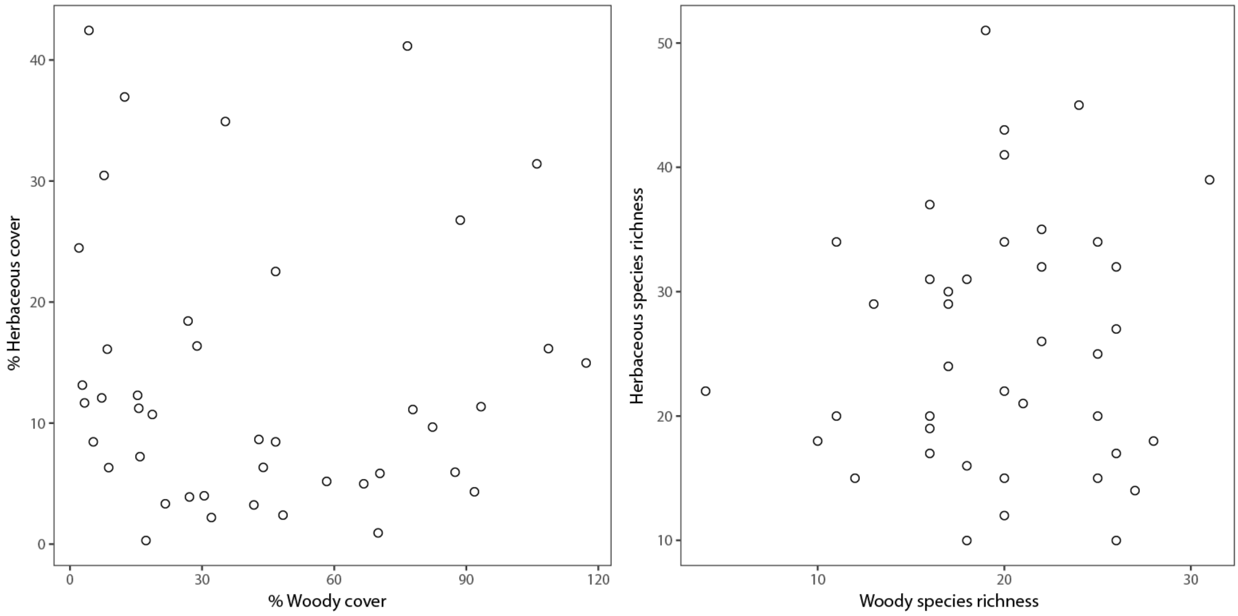

Figure 4: Total woody and herbaceous species cover; and woody and herbaceous species richness for AusPlots. No significant correlations were present across the tested gradient.

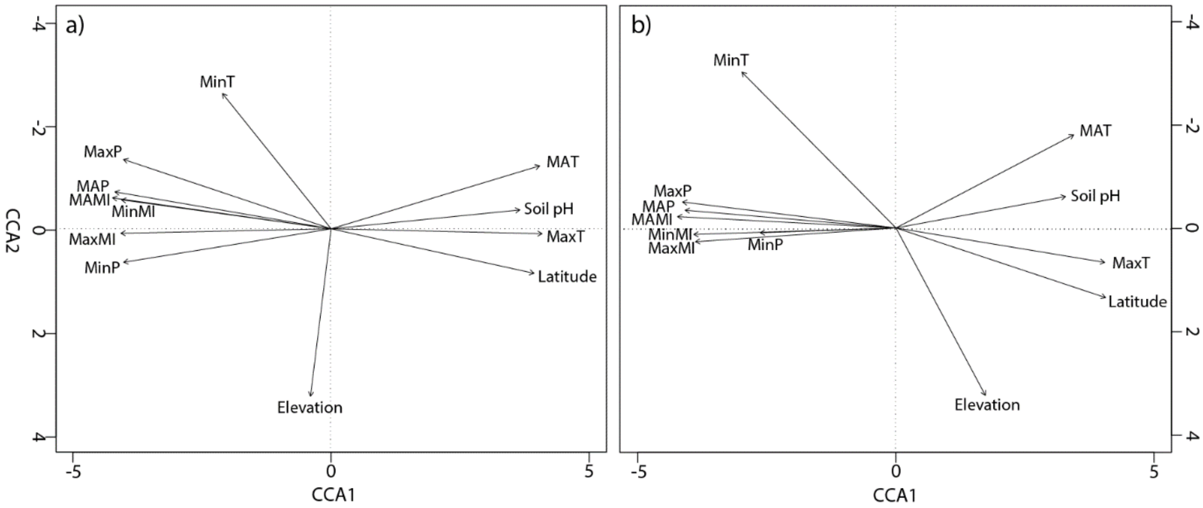

Figure 5: Environmental vectors based on Canonical Correspondence analysis of plant species cover for a) AusPlots and b) Guerin plots based on the environmental variables listed in Table 1. A primary temperature-rainfall gradient is evident corresponding with CCA1, with an orthogonal elevation axis (CCA2). Only environmental variables with R^2 values of >0.5 for at least one dataset are shown.

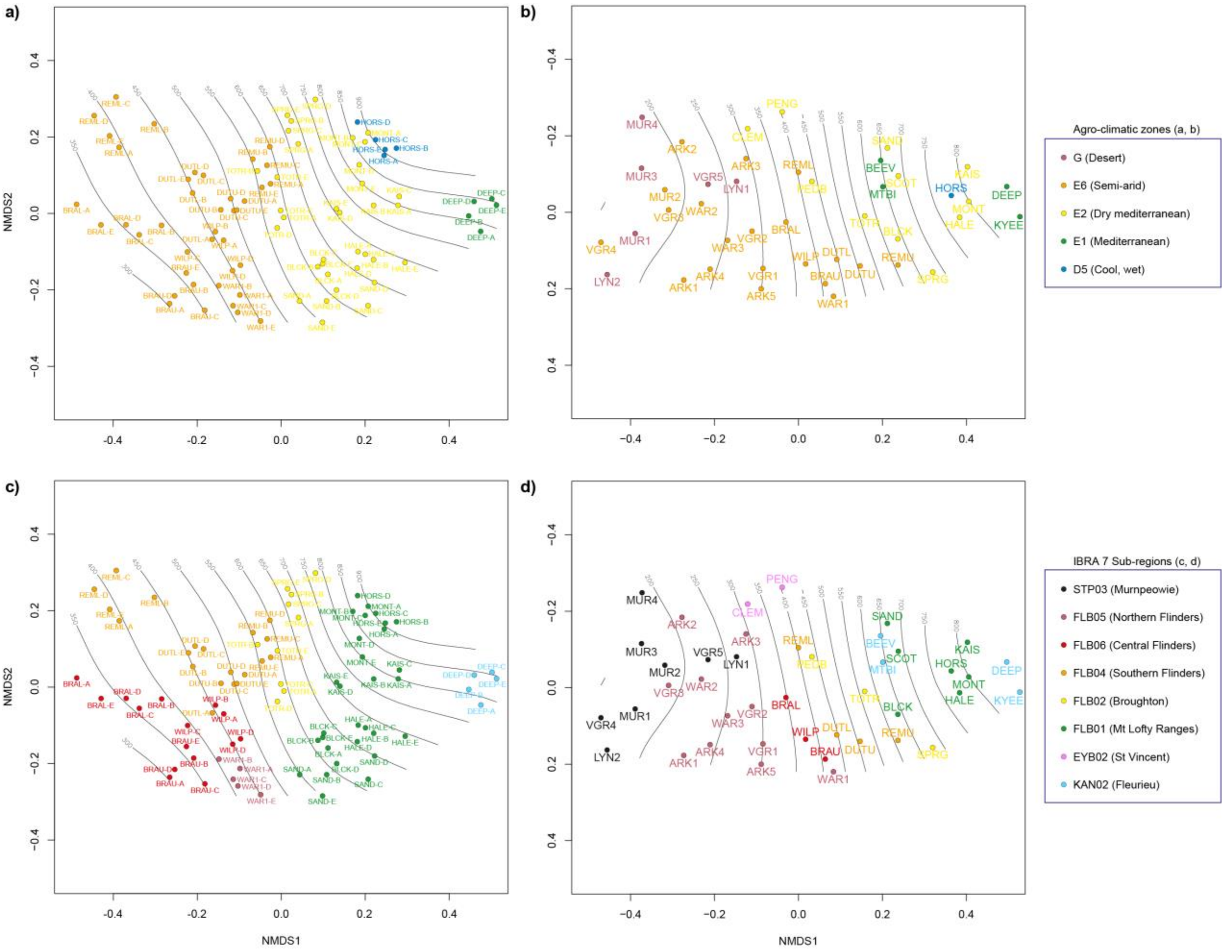

Figure 6: NMDS ordinations of TREND-Guerin (a, c) and TREND-AusPlots sites (b, d) based on species composition, with fitted surfaces of mean annual precipitation (grey isohyets). Sites are coloured by agro-climatic zones (a, b) and IBRA 7 sub-regions (c, d).

Supplementary Information

Table S1: Comparison of Guerin and AusPlot transect surveys

Design	Guerin plots	AusPlots
Total plots	85	42
Plot configuration	17 groups of 5 plots	Single plots
Plot size	900m^2 (30x30m)	1 ha (100x100m)
Total area sampled	7.65 ha	42 ha
Transect length	550 km 4.8º latitude	700 km 6.2º latitude
Environmental gradient	684 mm MAP (307-991 mm)	818 mm MAP (980-162 mm)
	4º C MAT (13.4-17.4º C)	7.2º C MAT (13.4-20.6º C)
Time of sampling	1 Sept – 9 Nov 2011 (all sites)	13-22 Aug 2012 (SATFLB0001-15, SATKAN0001-2). 6-17 Aug 2013 (SATFLB0016-25, SATSTP0001-8) 28 Oct – 6 Nov 2014 (SATEYB0001-2, SATFLB0027-28, SATKAN0003-4)
Observers	GRG	EJL, SCR & IF

Table S2: TREND site codes and location information including agro-climatic zone and Interim Bioregionalization of Australia (IBRA) subregion.

Location	Code	Guerin Code*	AusPlot Code	Agro-climatic zone	IBRA Subregion†	Latitude	Longitude
Mt Lyndhurst 2	LYN2		SATSTP0008	G	STP03	-29.3874	138.8819
Mt Lyndhurst 1	LYN1		SATSTP0005	G	STP03	-29.4562	138.8493
Murnpeowie 3	MUR3		SATSTP0006	G	STP03	-29.5301	138.8172
Murnpeowie 4	MUR4		SATSTP0007	G	STP03	-29.5356	138.8176
Murnpeowie 1	MUR1		SATSTP0003	G	STP03	-29.7548	138.8497
Murnpeowie 2	MUR2		SATSTP0004	E6	STP03	-29.791	138.8324
Radium Ridge	ARK5		SATFLB0018	E6	FLB05	-30.2186	139.3247
Arkaroola 4	ARK4		SATFLB0019	E6	FLB05	-30.2191	139.2334
Arkaroola 3	ARK3		SATFLB0020	E6	FLB05	-30.2552	139.2278
Arkaroola 2	ARK2		SATFLB0017	E6	FLB05	-30.3314	139.3742
Arkaroola 1	ARK1		SATFLB0016	E6	FLB05	-30.343	139.3402
Vulkathunha-Gammon Ranges Ridgetop 2	VGR2		SATFLB0022	E6	FLB05	-30.4056	139.2266
Vulkathunha-Gammon Ranges Ridgetop 1	VGR1		SATFLB0021	E6	FLB05	-30.4117	139.2205
Vulkathunha-Gammon Ranges Plains 1	VGR4		SATSTP0001	E6	STP03	-30.5612	139.4368
Nepabunna	VGR3		SATFLB0023	E6	FLB05	-30.5990	139.0741
Vulkathunha-Gammon Ranges Plains 2	VGR5		SATSTP0002	G	STP03	-30.6559	139.5479
Warraweena Plain	WAR2		SATFLB0024	E6	FLB05	-30.7604	138.5801
Warraweena Mt Hack	WAR1	WAR	SATFLB0006	E6	FLB05	-30.7752	138.7981
Warraweena Range	WAR3		SATFLB0025	E6	FLB05	-30.7846	138.6335
Brachina Upper	BRAU	BRA2	SATFLB0005	E6	FLB06	-31.315	138.5669
Brachina Lower	BRAL	BRA1	SATFLB0004	E6	FLB06	-31.3273	138.5679
Wilpena Pound	WILP	WIL	SATFLB0007	E6	FLB06	-31.5435	138.5952
Dutchman’s Stern Upper	DUTU	DUT2	SATFLB0009	E6	FLB04	-32.3101	137.9688
Dutchman’s Stern Lower	DUTL	DUT1	SATFLB0008	E6	FLB04	-32.3204	137.9549
Mt Remarkable Upper	REMU	REM2	SATFLB0011	E6	FLB04	-32.7480	138.1368
Mt Remarkable Lower	REML	REM1	SATFLB0010	E6	FLB04	-32.8281	138.0333
Clement’s Gap	CLEM		SATEYB0002	E2	EYB02	-33.4886	138.0807
Pedler’s Block	PEDB		SATFLB0028	E2	FLB02	-33.5526	138.3938
Spring Gully	SPRG	SPR	SATFLB0013	E2	FLB02	-33.9137	138.6043
Tothill Range	TOTR	TOT	SATFLB0014	E2	FLB02	-34.0047	138.9599
Pengilly Scrub	PENG		SATEYB0001	E2	EYB02	-34.5038	138.7038
Kaiserstuhl	KAIS	KAI	SATFLB0003	E2	FLB01	-34.5765	139.0067
Sandy Creek	SAND	SAN	SATFLB0001	E2	FLB01	-34.6085	138.8619
Hale	HALE	HAL	SATFLB0002	E2	FLB01	-34.6827	138.9090
Black Hill	BLCK	BLA	SATFLB0012	E2	FLB01	-34.8804	138.7088
Montacute	MONT	MON	SATFLB0026	E2	FLB01	-34.8873	138.7876
Mount Beevor	BEEV		SATKAN0004	E1	KAN02	-34.9267	139.0387
Horsnell Gully	HORS	HOR	SATFLB0015	D5	FLB01	-34.9330	138.7275
Scott Creek	SCOT		SATFLB0027	E2	FLB01	-35.0827	138.6796
Kyeema	KYEE		SATKAN0002	E1	KAN02	-35.2717	138.6907
Mount Billy	MTBI		SATKAN0003	E1	KAN02	-35.4605	138.6046
Deep Creek	DEEP	DEE	SATKAN0001	E1	KAN02	-35.6078	138.2618

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[*] Guerin codes align to those published in (Guerin *et al.* 2014). [†] EYB=Eyre Yorke Block (EYB02=St Vincent subregion), FLB=Flinders Lofty Block (FLB01=Mt Lofty Ranges, FLB02=Broughton, FLB04=Southern Flinders, FLB05=Northern Flinders, FLB06=Central Flinders subregions), KAN=Kanmantoo (KAN02=Fleurieu subregion), STP=Stony Plains (STP03=Murnpeowie subregion). Descriptions of agro-climatic zones in Table 2.

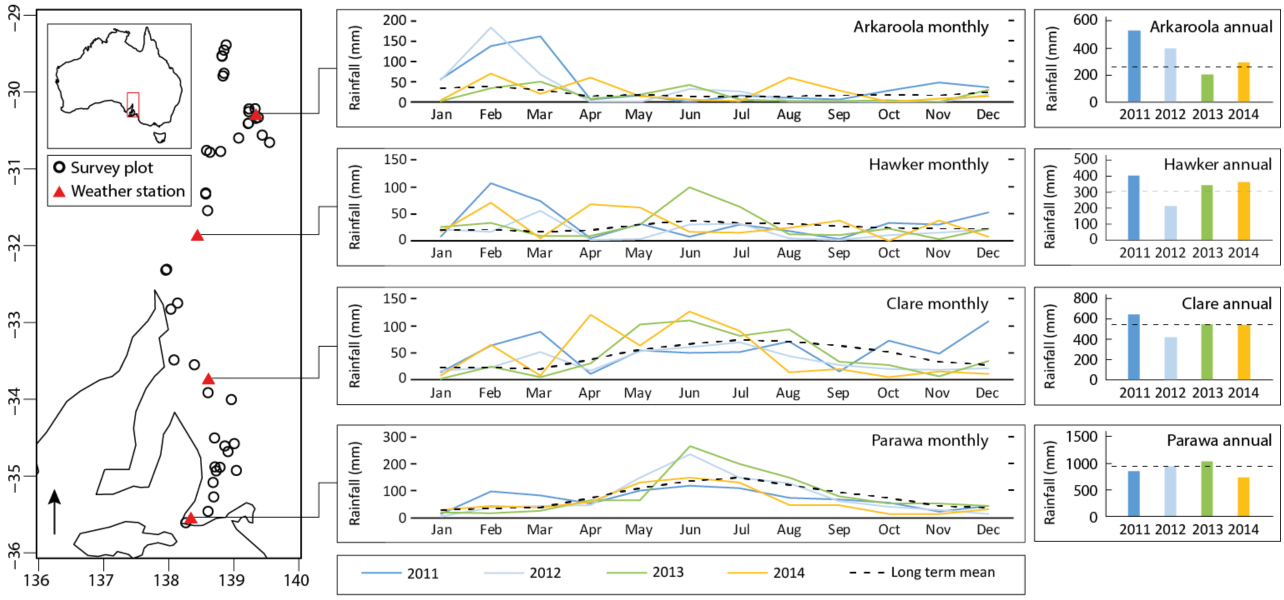

Figure S1: Monthly rainfall data for Bureau of Meteorology weather stations distributed on the TREND for 2011-2014. Dashed line represents long term average of recordings for that station.

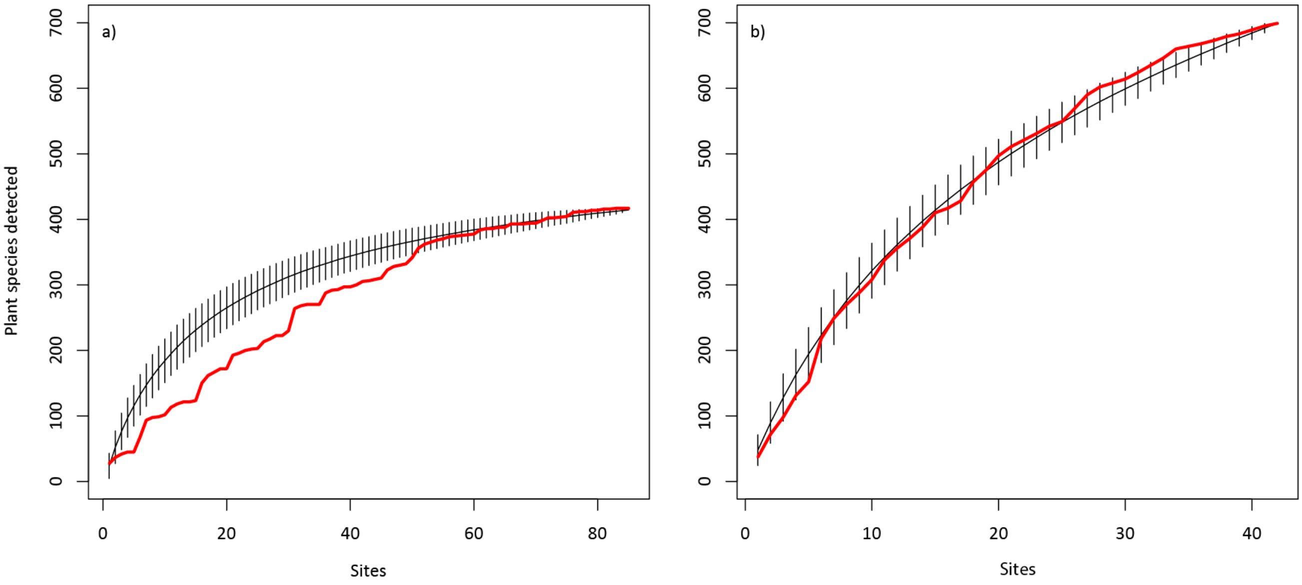

Figure S2: Species accumulation curves for the TREND-Guerin (a) and TREND-AusPlots (b) transects. Bold, red curves show species accumulation from north to south. Black curves show species accumulation from random site order with 95% confidence intervals.

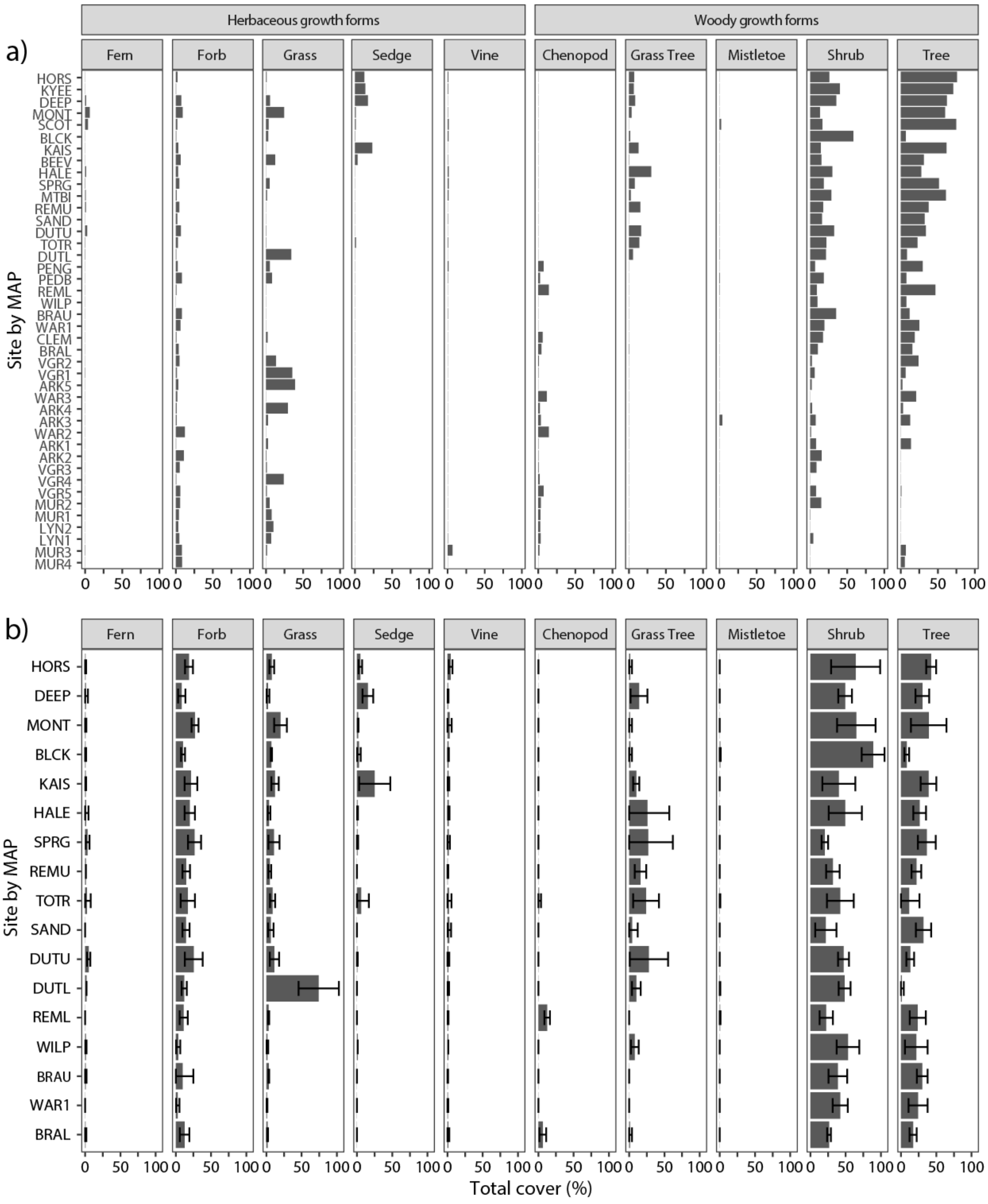

Figure S3: Total cover values by growth form for a) AusPlots and b) Guerin plots. Error bars for Guerin plots represent 95% confidence intervals across five sites.

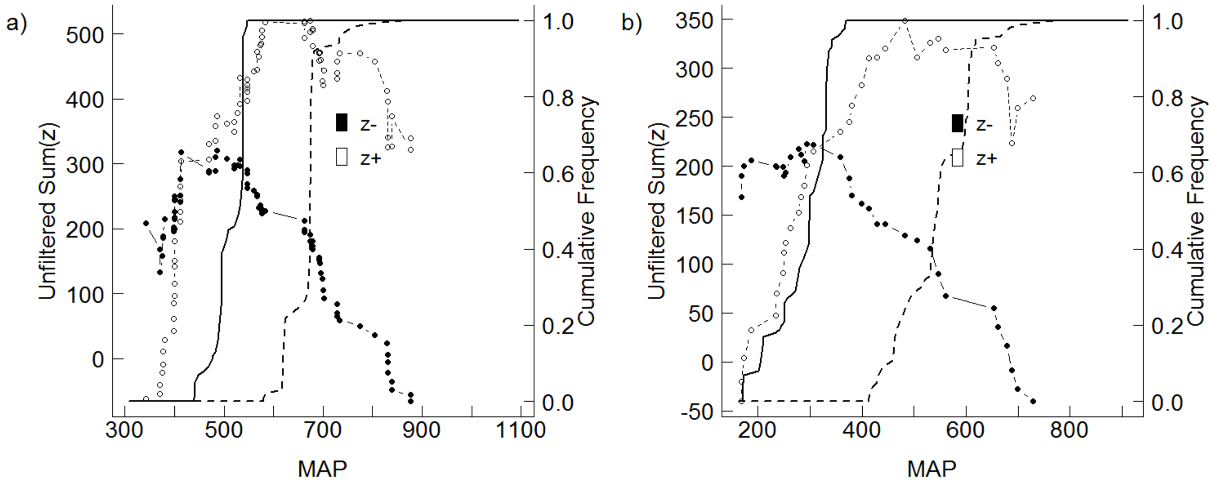

Figure S4: Plant community response from TITAN analysis of Guerin (a) and AusPlot (b) data sets, calculated for 100 bootstrap replicates. Unfilled circles signify change points calculated for species which increase with rainfall and filled circles signify change points calculated for species which decrease with rainfall.

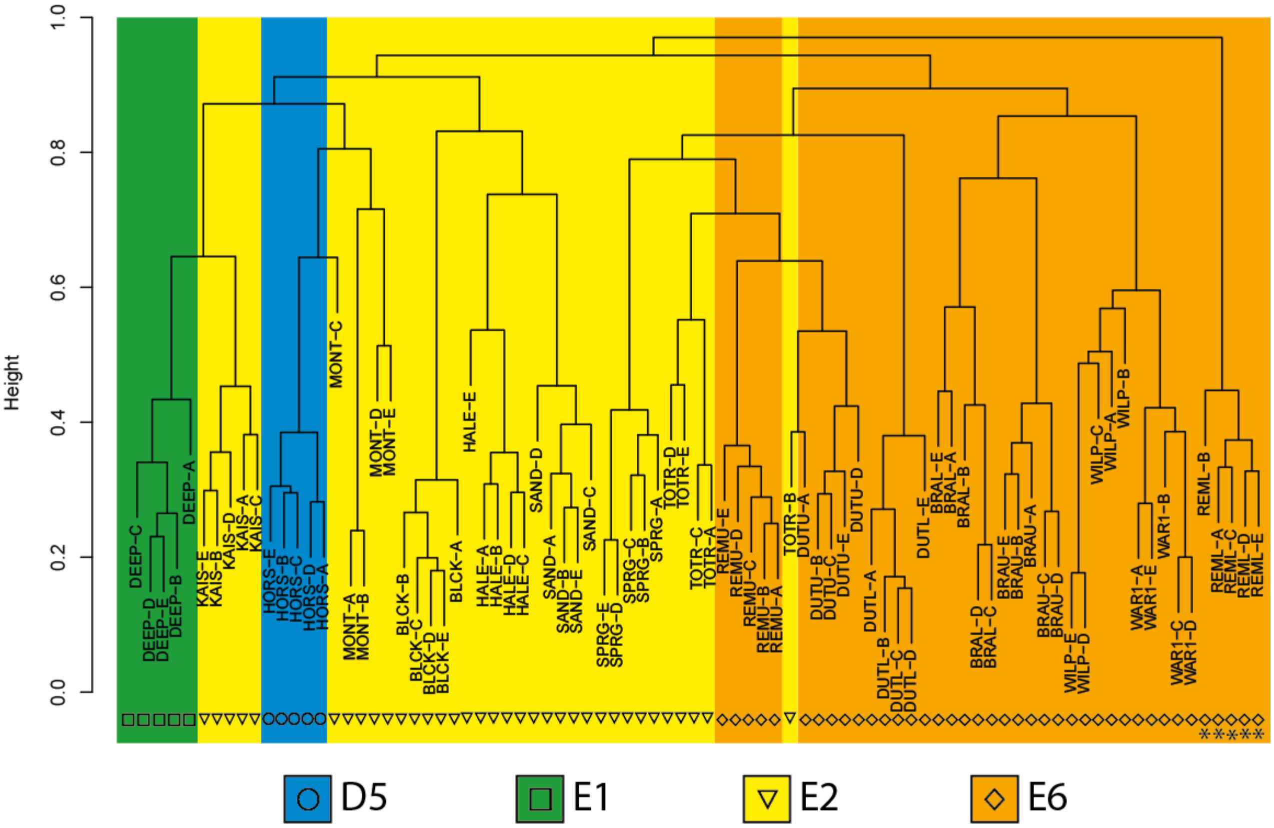

Figure S5: Species composition dendrogram of the Guerin transect with leaves ordered by latitude. Colours represent agro-climatic zones (Table 2; Hutchinson et al. 2005). Sub-sites cluster together closely, indicating sub-sites have sampled the same vegetation community. Sites marked with an asterisk are mallee woodlands dominated by *Eucalyptus odorata*.

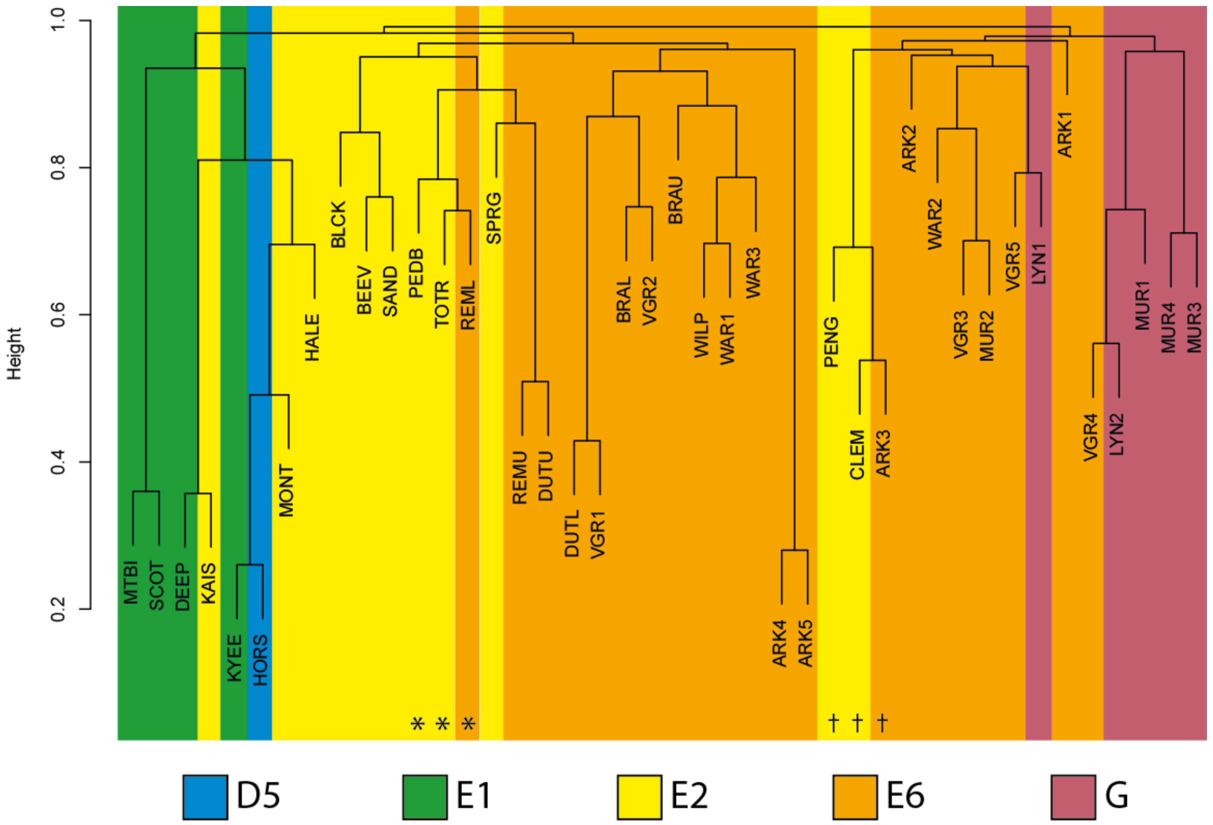

Figure S6: Species composition dendrogram of the AusPlot transect with leaves ordered by latitude. Colours represent agro-climatic zones (Table 2; Hutchinson et al. 2005).While there is a clear transition between agro-climatic zones, these do not correspond uniformly between high-level divisions of the dendrograms. Annotated sites are mallee woodlands dominated by *Eucalyptus odorata* (*) or *E. socialis* (†), generally characteristic of the arid and semi-arid plains of southern Australia.

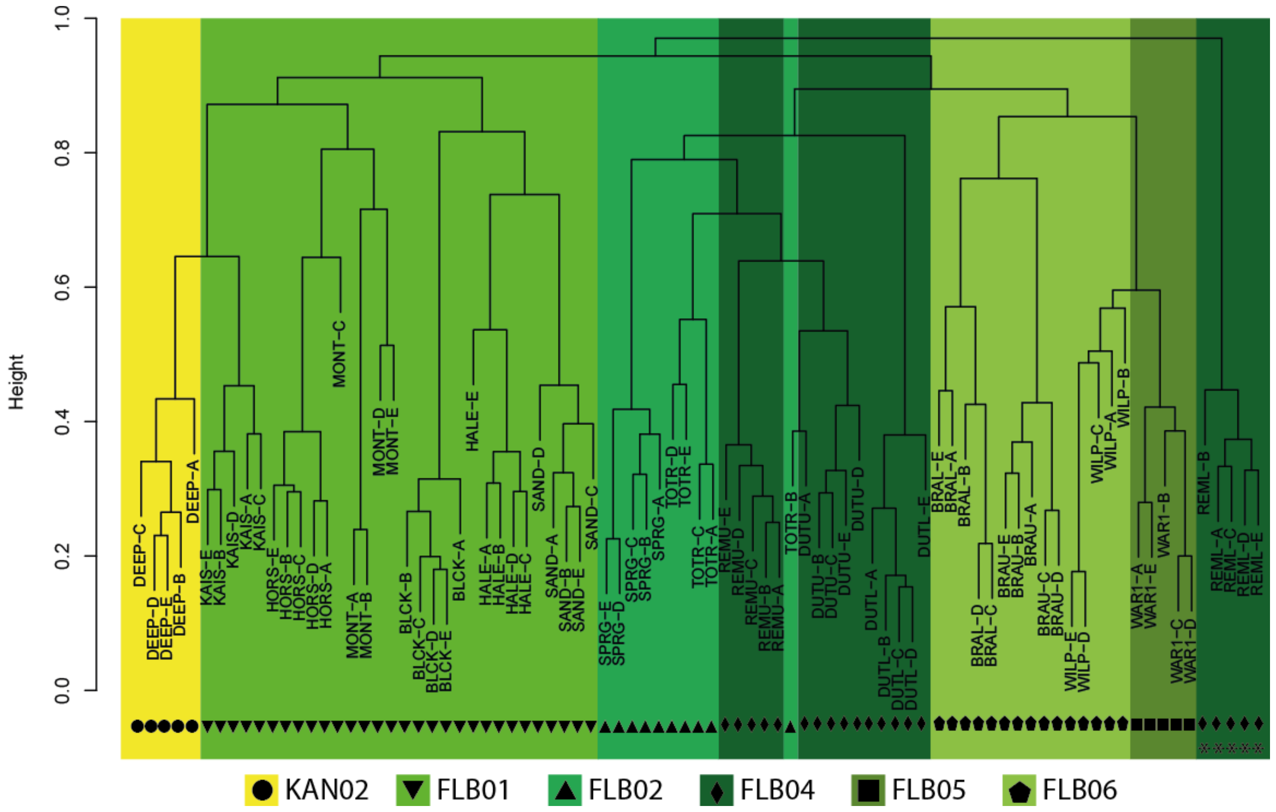

Figure S7: Species composition dendrogram of the Guerin transect with leaves ordered by latitude. Colours represent IBRA subregions (Hutchinson et al. 2005). There is limited congruence between IBRA subregions and high level division of groupings. Annotated sites are mallee woodlands dominated by *Eucalyptus odorata* (*), generally characteristic of the arid and semi-arid zone of southern Australia. FLB=Flinders Lofty Block (FLB01=Mt Lofty Ranges, FLB02=Broughton, FLB04=Southern Flinders, FLB05=Northern Flinders, FLB06=Central Flinders subregions), KAN=Kanmantoo (KAN02=Fleurieu subregion).

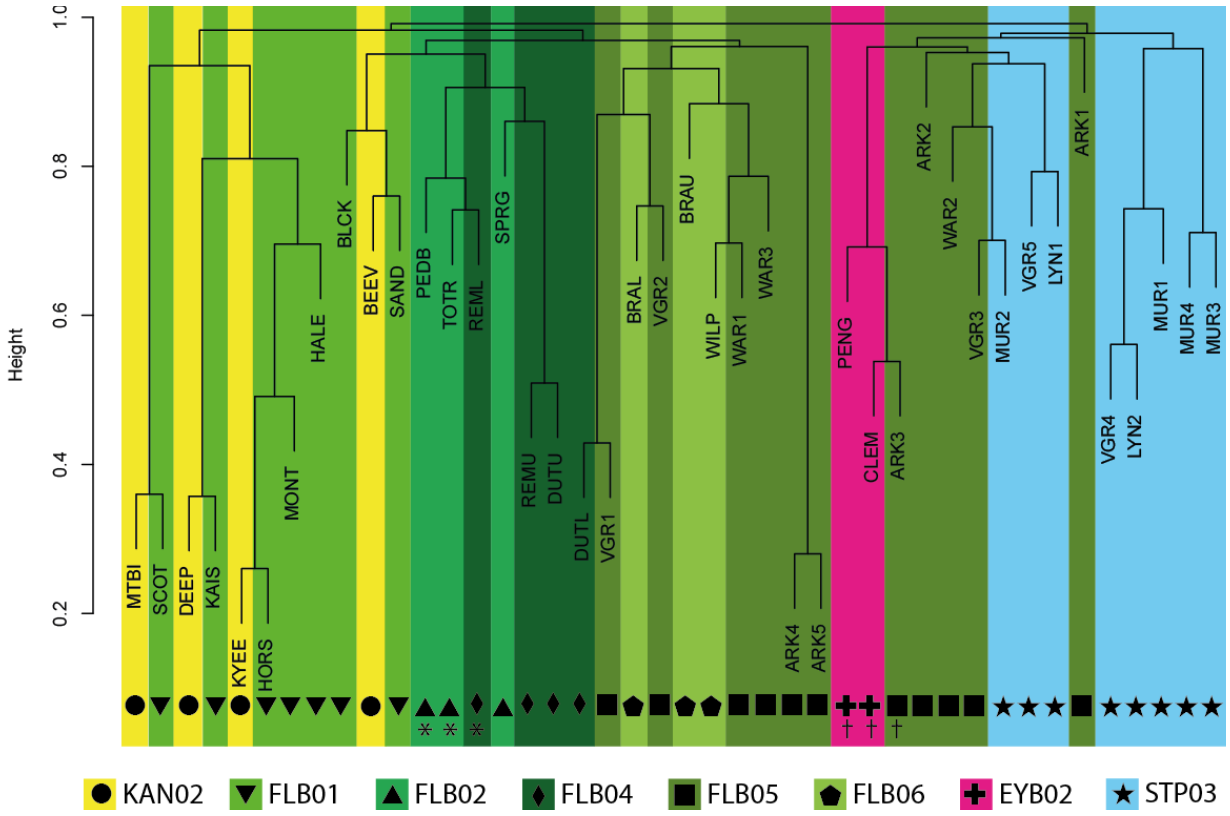

Figure S8: Species composition dendrogram of the AusPlot transect with leaves ordered by latitude. Colours represent IBRA subregions (Hutchinson et al. 2005). There is limited congruence between IBRA subregions and high level division of groupings. Annotated sites are mallee woodlands dominated by *Eucalyptus odorata* (*) or *E. socialis* (†), generally characteristic of the arid and semi-arid zone of southern Australia. EYB=Eyre Yorke Block (EYB02=St Vincent subregion), FLB=Flinders Lofty Block (FLB01=Mt Lofty Ranges, FLB02=Broughton, FLB04=Southern Flinders, FLB05=Northern Flinders, FLB06=Central Flinders subregions), KAN=Kanmantoo (KAN02=Fleurieu subregion), STP=Stony Plains (STP03=Murnpeowie subregion).

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Chapter 6: Plant and ant assemblages predicted to decouple under climate change

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Name of Principal Author (Candidate)	Stefan Caddy-Retalic		
Contribution to the Paper	Designed the study, led the field work in South Australia, conducted data analysis and wrote the manuscript.		
Overall percentage (%)	65		
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Contribution to the Paper	Led vegetation surveys. Provided input in to data analysis, including coding support. Reviewed and edited final manuscript.		
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Contribution to the Paper	Facilitated ant surveys and identified ants. Helped interpret results. Reviewed and edited final manuscript.		
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For submission to Global Change Biology

Title: Plant and ant assemblages predicted to decouple under climate change

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Abstract

Climate change is affecting ecological assemblages and has been responsible for widespread shifts in species distributions and ecosystem function. Different taxa are likely to respond to climate change differently due to different responses to climatic drivers, potentially leading to a breakdown of community structure and function. We aimed to determine the comparative sensitivity of flora and ant fauna to environmental change. We use analysis of plant and ant assemblages along a bioclimatic gradient in South Australia to establish assemblage-level responses to spatial climatic change, and then project future biotic response to climate change scenarios. Ant assemblages were up to 7.5 times more sensitive to projected climate change than were plant assemblages, suggesting a very substantial decoupling of these assemblages under a future climate. Our results suggest that a high degree of community reorganisation and change in ecosystem function should be expected under climate change.

Introduction

Most studies of ecological impacts of climate change have focussed on responses of individual species or biomes. However, climate change has important implications for community assembly that has received less scientific attention. Component species are likely to vary markedly in terms of the relative importance of climatic drivers (Foden et al., 2013), such that major community deconstruction and reconstruction might be expected (Gilman et al., 2010). Disproportionate species sensitivity to environmental drivers has important implications for community diversity, food web structure and species interactions (Sheldon et al., 2011). It is particularly important to understand the potential for decoupling of plant-animal associations under future climates. Marked differences in responses of plants and animals could have profound effects on community structure and function, with implications for habitat structure and resource availability for fauna, and the provision of faunal-mediated ecological services for plants (Van der Putten et al., 2010).

The composition of species assemblages across the landscape is strongly influenced by their environment, resulting in the familiar descriptions of biomes and vegetation types. However, climate change is rapidly altering both the abiotic environment, as well as the biotic environment, through modification of the dominant flora and fauna. The evidence for these changes is mounting (Parmesan, 2006, Chen et al., 2011, Corlett and Westcott, 2013, Pecl et al., 2017), but it is not yet clear how changing climatic conditions will affect the overall species composition of sites, and whether these responses will impact all groups (Wittmann and Pörtner, 2013, Bozinovic and Pörtner, 2015), and all places, equally (Carvalho et al., 2010, Garcia et al., 2014). In the face of this uncertainty, our ability to make well-informed decisions about how to address the current threats to biodiversity is severely limited, driving the need to develop new techniques to accurately predict future biodiversity changes on a large scale (Oliver and Morecroft, 2014, Urban et al., 2016).

Much work on predicting future biodiversity has focused on modelling species-level responses by establishing a bioclimatic envelope for each species and then projecting the required migration of that species in order to maintain conditions similar to its current climate in to the future (Araújo and Peterson, 2012). Such an approach is useful to infer the possible response of single species, but assumes that species require environments similar to their current distribution (which is particularly concerning given that climate change will result in many no-analogue enviroments), neglects biotic interactions (e.g. facilitation and competition), is resource intensive and, unless done on a massive scale, does not give substantial insight into the change of entire assemblages or landscapes (Reiss et al., 2009). Given the extent of change already underway, we do not have the luxury of taking the time to directly track changes across all species and landscapes, and we therefore need to find ways to gain some insight from current associations of species across sites that span a range of present day climates. While it would be convenient to be able to generalise the responses across taxonomic groups within ecosystems, there is limited understanding of how each group may, or may not, be concordant in responses to climate change, and the extent to which non-climatic environmental variables may constrain the ability of species to thrive in novel environments (Lavergne et al., 2010).

The current ecological breadth of species may provide guidance for the type of responses to expect. Some species have broad tolerances to temperature, moisture or other environmental variables (and therefore broad ecological niches), while others have more restricted requirements (narrower niches). The sensitivity of individual species to environmental change is variable, but is heavily constrained by physiology. For example, as ectotherms, invertebrates are sensitive to changes in temperature, as they are limited to behavioural rather than metabolic thermoregulation, but must maintain a thermal window warm enough to allow rapid movement for foraging and defence, but cool enough to avoid overheating. Noting that a wide range of abiotic variables can control species distributions, temperature is known to be a major driver of ant distributions (Andersen et al., 2015), while water availability is a primary driver for plants (Kreft and Jetz, 2007).

By surveying species composition on a linear gradient of climatic change, where environmental variation is maximised over a limited spatial extent, we can describe the association between species turnover and environment (Austin, 1987, Caddy-Retalic et al., 2017). This approach allows the degree to which species turnover is related to environmental change and geographic distance to be disentangled, essentially building an assemblage-level model of biotic sensitivity to the environmental change captured by the gradient.

By comparing species turnover models for multiple assemblages on the same gradient we can identify organism types that are more, or less, responsive to environmental change. For example, a comparison of species turnover in multiple functional groups of rainforest taxa on a land-use intensity gradient in Sulawesi revealed that ant and bee assemblages showed less response to the tested gradient than birds and vascular plant assemblages (Kessler et al., 2009). If assemblages show marked differences in sensitivity to ecosystem change, that might presage a decline in species co-occurrence across multiple functional groups within ecological communities on that gradient. In the Sulawesi example, the sensitivity shown by ants and bees might be reflective of the response that could be expected from all invertebrates, representing a shared sensitivity to the gradient due to a common trait (e.g. ectothermic metabolism). Disproportionate responses of some assemblages within an ecosystem could disrupt important interactions between species.

The many processes that characterise species interactions such as mutualism, commensalism, competition and predation; are critical for ecosystem function. Associations between generalist species can be established quickly, but specialist interactions take much longer to develop and may become obligate, meaning that one species is reliant on the presence of another for survival. Disproportionate response to environmental change from one assemblage (e.g. plants) will also alter ecosystem function through changed interactions between species across different assemblages.

Plants form the basis of the food web and are an important structural component of terrestrial ecosystems. Fauna are important consumers, and shape ecosystems through modification of the flora, herbivory and carnivory. Ants (Formicidae) are one of the most dominant and species-rich faunal groups, with near-ubiquitous distribution and provide major roles as ecosystem engineers and nutrient cyclers (Del Toro et al., 2012). Due to their ubiquity, ease of sampling and myriad of interactions with other biota, ants are often nominated as effective bioindicators of ecosystem function and/or disturbance (Gerlach et al., 2013). The relationships between ants and plants are broad. Plants provide shade and habitat, attract herbivore prey and are a food source, while ants help disperse seeds, defend against herbivores and cycle nutrients in the soil (Buckley, 2012). These joint contributions to ecosystem function make plants and ants attractive research foci, but it is unknown whether these keystone taxa are likely to show similar or different responses to climate change. Should plants and ants show similar response to extrinsic change, it is reasonable to assume that they will adapt, migrate or die together. On the other hand, if the response is uneven, we may see a breakdown of current assemblages and the loss of important interactions.

By linking change in plant and ant assemblages to spatial climatic change on a significant bioclimatic gradient, we attempt to quantify the climatic sensitivity of these groups to give us insight in to the biotic response we might expect from ongoing climate change. We observed plant and ant assemblages on a bioclimatic gradient traversing the Mediterranean to arid zone in South Australia. The spatial gradient spans a climatic change of approximately 5°C in mean annual temperature and 800mm in mean annual rainfall over 550 km, with cool, wet conditions in the south transitioning to warm, dry conditions in the north (Guerin et al., 2014), representing a similar change to that experienced between northern Portugal and Marrakesh, or Oregon and Baja California. The spatial gradient also encompasses the approximate temporal projections of global climate change (e.g. 1.1-4.8°C increase in mean annual temperature by 2100 (IPCC, 2013)). The vegetation on this gradient displays near-monotonic change in plant species turnover and vegetation structure (Caddy-Retalic et al., in review). Specifically, we address the following aims:

Aim 1: To examine the extent to which variation in the composition of plant and ant communities is differentially sensitive to climatic drivers, by describing variation in species richness, β-diversity and the composition of plant and ant communities in relation to climatic variation along the environmental gradient.

Aim 2: To forecast changes in plant and ant species composition under future climates, based on sensitivity to contemporary climatic variation in space.

Aim 3: Examine consistency of projected plant and ant community sensitivity given different climate projections.

Methods

Sampling

In order to assess the biotic change associated with spatial environmental change, a transect design was implemented, with groups of three sites established at 17 locations along the bioclimatic gradient present on the Adelaide Geosyncline in South Australia (Figure 1; Table 1). This nested design allowed us to quantify the biotic variability for each location, including that caused by disturbance. . Survey locations were established in areas of large, homogenous vegetation protected either in private or public biodiversity conservation reserves (Guerin *et al.* (2014). Plants were comprehensively surveyed in a 30 x 30 m plot at each of the 51 sites in the spring of 2011. For each species, growth form and a visual estimate of projected cover were recorded (minimum of 1% for very low cover, thereafter in 5% increments). Plants were identified to species (where possible) and vouchers lodged at the State Herbarium of South Australia. Other environmental variables (aspect, slope, estimated percentage rock outcrop and estimated percentage surface strew) were also recorded. Surface soil samples were collected and tested in the laboratory for sand content, pH, electroconductivity, and the concentration of ammonium (NH_4), nitrate (NO_3), phosphorous (P) and potassium (K). The circular variable “aspect” was transformed to the linear variables “eastness”: $\sin(\frac{\text{aspect} \times \pi}{180})$ and “northness”: $\cos(\frac{\text{aspect} \times \pi}{180})$ to allow these to be combined with other linear variables.

The same sites were sampled for ants from north to south in November 2012. Ants were trapped in pitfall traps in a 5 x 3 grid, with 10 m spacing. Pitfall traps were plastic containers (internal diameter of 42 mm), filled three quarters with 70% ethyl glycol as a preservative. Traps were left in the field for 48 hours. Ants were sorted to morphospecies (hereafter referred to as species), with undescribed species assigned a letter code applicable to this study only. For each trap, ant abundance was capped at 50 individuals of each species to reduce the influence of highly abundant species, or over-representation of species with nests situated close to a trap. A complete set of voucher specimens was lodged at the CSIRO Tropical Ecosystems Research Centre in Darwin.

Elevation was extracted from the GEODATA 9 second digital elevation model (Hutchinson et al., 2008). Site values for Mean Annual Temperature (MAT; Bio01); Mean temperature of warmest quarter (MaxT; Bio10); Mean temperature of coldest quarter (MinT; Bio11); Mean Annual Precipitation (MAP; Bio012); Precipitation of wettest month (MaxP;

Bio13) and Precipitation of driest month (MinP; Bio14) were extracted from BIOCLIM layers modelled at 9 second resolution extracted from ANUCLIM 6.1 (Xu and Hutchinson, 2013) with the GEODATA 9 second DEM (Hutchinson et al., 2008) derived by CSIRO (Harwood et al., 2014). Climate averages cover 30 years (1975 to 2005), centred on 1990 as a baseline.

Analysis

All data analyses were undertaken in R (R Core Team, 2017). To describe the rate of species change along our gradient, we calculated ß-diversity for plant and ant assemblages. ß-diversity can be partitioned into the species replacement ($ß_{SIM}$) and to species nestedness (the degree to which the species at one site are a subset of those from another site) (Baselga, 2012). To account for the contributions of species replacement and nestedness to ß-diversity, we calculated $ß_{SIM}$ and the nestedness fraction of dissimilarity ($ß_{SNE}$), as well as Sørensen pairwise dissimilarity ($ß_{SOR} = ß_{SNE} + ß_{SIM}$) using `betapart` (Baselga and Orme, 2012).

We used non-metric multidimensional scaling (NMDS) of plant and ant assemblages to describe and visualise the relationship between species composition and environmental variables, correlating environmental variables to the resultant ordination coordinates using `vegan` (Oksanen et al., 2016). Assemblages of plants and ants across the gradient were compared in two ways. First, we compared NMDS ordinations for plant and ant assemblages using a ‘Procrustes’ analysis, which fits one ordination onto another through scaling and symmetrical rotation to determine whether plant and ant assemblages are similarly distributed through biotic space across the gradient (Peres-Neto and Jackson, 2001). Second, we built dendrograms of both plant and ant assemblages, which were aligned using stepwise disentanglement of trees using `dendextend` (Galili, 2015). We were then able to visualise the alignment of plant and ant assemblage difference across the gradient and calculate "entanglement" between trees (i.e. the degree to which differences between the taxa and their drivers interferes with alignment of assemblages).

To make predictions of compositional change based on future climate projections, we used Canonical Correspondence Analysis (CCA) ordination to constrain compositional variation to that explained by environmental variables. To avoid "over-fitting" of the biological response, we used a separate stepwise model building process to select variables for plant and ant assemblages, where the most parsimonious set of explanatory variables were evaluated based on adjusted r^2 and p scores (Oksanen et al., 2016).

The baseline model was used to assess future change, and the workflow used to develop future predictions of plant and ant assemblages is illustrated in Figure 2. We obtained future values of the climatic variables from model simulations, and combined these with current soil and landscape variables to produce a predicted environmental variables matrix. Using the relationships established between environmental variables and assemblages in the baseline (current) CCA, we were able to generate a predicted CCA (pCCA) showing how the assemblages on the gradient are likely to migrate on the gradient in biotic space in response to the change in climate variables.

To represent biotic change over time and under different climate predictions, we generated 12 pCCAs using projected data for three time periods (2050, 2070 and 2090) from two climate models (the Australian Community Climate and Earth-System Simulator (ACCESS; Bi et al., 2013) and Geophysical Fluid Dynamics Laboratory (GFDL; Dunne et al., 2013)) and two Representative Concentration Pathways (RCPs; RCP4.5, where carbon emissions are rapidly controlled and stabilised by the end of the century with 1.1-2.6°C warming by 2100) and RCP8.5 (unchecked carbon emissions; 2.6-4.8°C warming by

2100) (IPCC, 2013). The climate scenario projections were generated and thermally-adjusted for topographic variation by the CSIRO (Harwood et al., 2014). All climate variables were extracted through ANUCLIM v6.1 (Xu and Hutchinson, 2013).

We then calculated the linear distance between the first two axes of the baseline (current) CCA and pCCA coordinates for each site, as a measure of predicted change between the baseline and a future climate scenario (Figure S1). This process was repeated for each pCCA, giving values of changes in Euclidean distances until 2090 under two climate models (ACCESS and GFDL) and two greenhouse gas concentration scenarios (RCP4.5 and RCP8.5); and allowed us to visualise whether linear distances (i.e. change in biodiversity) for plants and ants separately was projected to increase, decrease or remain static. In order to visualise the climatic change associated with biotic projections, we repeated the above process for climate data only using Principal Component Analysis (PCA) and projected PCA ordinations (Figure 2).

To determine how plant and ant assemblages are projected to change in relation to one another, a separate, but similar workflow was implemented (Figure 3). Linear distances between the coordinates of the first two axes for each site of CCAs established for current plant and ant assemblages measured and established as a baseline. The same process was then repeated using pCCAs generated using future environmental matrices derived for 12 future climates as described above. Euclidean distances were measured between sites for each pair of plant and ant assemblages at each time point under the 12 future climates (Figure S2).

Results

Surveys of sites across the entire bioclimatic gradient yielded a total of 363 plant species, primarily forbs and shrubs (40% and 33% of species respectively). We detected 227 ant species from eight subfamilies, primarily Formicinae and Myrmicinae (42% and 30% of species respectively). Species accumulation curves pooled across all sites for both the plant and ant datasets showed flattening consistent with sampling the majority of the regional species pool for sampled habitats, although ants appeared to be more completely sampled than plants (Figure S3). 145 ant species (66.8% of total) could not be identified to species and are likely to be undescribed.

Species diversity

Plant species richness was positively correlated with MAP while ant species richness was not (Figure 4). Total vegetation cover showed a strong positive correlation with MAP ($r^2=0.5159$, $p<0.001$), primarily driven by forbs and woody plants (Figure S4), but there was no relationship between total vegetation cover and either ant species richness or abundance (Figure S5).

ß-diversity was very high for both plants ($ß_{SOR}=0.963$) and ants ($ß_{SOR}=0.967$), with species replacement ($ß_{SIM}$) accounting for most species turnover in both cases (Table 2).

Species composition

NMDS ordinations correlated with all climate variables for both plants and ants. Plant assemblages were best associated with climatic (temperature, precipitation and moisture) variables and had weaker (but generally highly significant) correlations with most landscape and soil variables (Table 3). Ant assemblages were more-weakly correlated with climate, and did not show a significant relationship with any physical variables except latitude (which was correlated with climate) and slope. The strongest environmental correlates with community composition were MaxT (plants: $R = 0.87$, $p<0.001$; ants: $R = 0.18$, $p<0.05$), MaxP (plants: $R = 0.86$, $p<0.001$; ants: $R = 0.28$, $p<0.01$) and MAP (plants: $R = 0.85$, $p<0.001$; ants: $R = 0.28$, $p<0.01$). Precipitation variables were the strongest environmental correlate for both ant and plant assemblages. MaxP and MAP were highly correlated ($R = 0.996$, $p <0.001$) so despite being marginally less correlated than MaxP, MAP was chosen as the dominant environmental variable for subsequent analysis to facilitate ready comparison with other studies.

Based on plant community composition, sites fell in to three main clusters, mostly aligned with the Fleurieu Peninsula, Mid North and Flinders Ranges regions (Figure 5). There were two exceptions to this alignment: the Upper Dutchman's Stern (DUTU) sites were placed within the Mid-North cluster despite being located in the Flinders Ranges; and the lower Mt Remarkable (REML) sites formed an outlier group. Sites arranged by ant species composition displayed a similar pattern, with REML sites again forming an outlier group, but there was generally more variability than in plant communities, leading to a less clear geographic alignment of community composition. The Mt Remarkable Upper (REMU) and Dutchman's Stern (DUTU and DUTL) sites showed particularly high assemblage variability and were placed in all three geographic regions. Three Fleurieu sites (Kaiserstuhl-E (KAIS-E) Deep Creek-C (DEEP-C) and Montacute-C (MONT-C)) were placed in the Mid-North cluster despite being located in the Fleurieu. Alignment between plant and ant communities was high, with an entanglement of 0.108 (based on a scale of 0-1, where 0 indicates perfect alignment (Galili, 2015)), suggesting a high degree of similarity in the structuring of plant and ant communities in relation to environmental variation (Figure 5).

Procrustes correlation of plant and ant NMDS ordinations revealed a significant correlation (correlation= 0.36, p=0.004), again indicating substantial congruence between the plant and ant assemblages across the gradient.

Using variables selected through the stepwise model building process (Table 4), CCA ordinations of environmental variables were able to constrain 65% and 50% of observed variation in plant and ant species composition respectively. In order to visualise predicted change across the entire gradient, we represented each CCA and pCCA as a polygon of the ordination space occupied by all sites (Figure 6). For all four model-RCP scenario pairs, the biotic responses shifted far more markedly than the climatic space of the entire gradient, although this masks substantial climatic movement of individual sites (not shown). Plant communities were very sensitive to predicted climate change across all four tested scenarios but did not show ongoing movement along a single axis, instead migrating in a different direction between each time point. Ant communities showed a stronger and more consistent response, with the polygon representing the entirety of the gradient in ant composition space migrating extensively along a MaxT/MAP/MaxP axis. There was no consistent difference in biotic projections based on climate projections of RCP 4.5 and 8.5 scenarios from the ACCESS and GFDL models, but biotic projections based on GFDL models were less linear over time compared to ACCESS projections (Figure 6).

We represented the magnitude of compositional change (i.e. linear distance) over time for plant and ant assemblages compared to their current baseline, and for paired plant-ant assemblages through time (Figure 7). The projected compositional change for plant communities was much lower than for ant communities. The plant communities of Flinders Ranges and Fleurieu Peninsula sites generally appeared to be more sensitive than the Mid-North sites, although this was highly dependent on the model and emissions scenario. Projections for ant communities were similarly variable, with Fleurieu Peninsula and Mid-

North sites generally projected to show the greatest species change, regardless of emissions scenario. Many sites showed a decrease in linear distances after 2070, indicating movement back toward baseline and therefore partial ecological recovery. Such ecological recovery could be attributed to a climatic recovery for the RCP 4.5 scenarios, which assumes carbon emissions will peak around 2040, however this is not the case for the RCP 8.5 scenarios, which assume that emissions will not peak before the end of the century (Meinshausen et al., 2011). Because the ant community response was much greater than the plant community response, the total divergence (i.e. linear distance between a site's projected plant community and ant community) was dominated by the ant community response.

Ant assemblages were far more sensitive than plants irrespective of climate model or greenhouse gas scenario, but when the ratios of linear distances for plant and ant assemblage projected change by 2050 (Supplementary Figure S6) are compared, ants range from 3.4 times (ACCESS RCP4.5) to 7.5 times more sensitive than plants (GFDL RCP 8.5). When compared across models and greenhouse gas scenario pairs, climate model has a slightly larger effect than greenhouse gas scenario (Table 5).

While there was no systematic pattern of sensitivity to climate at a regional level, when looking at responses site-by-site (Supplementary Figure S6), some trends emerged. Mt Remarkable Lower (REML) appears to be the most consistently sensitive site for both plants and ants across all tested climate scenarios, indicating this area has a high likelihood of undergoing significant biodiversity shifts, while Kaiserstuhl (KAIS) is predicted to have comparatively low change. The lack of consistency in relative sensitivity of sites across different model-RCP scenario pairs indicates there is substantial uncertainty about which sites and assemblages will show the most response to future abiotic change.

Discussion

We used the sensitivity of ant and plant responses to spatial environmental change to predict whether these two focal taxa will show concordant responses future climate change. We found that variation in plant and ant assemblages were well-aligned across the spatial gradient, but there were differences in the environmental variables that explained the patterns of diversity. The general trend of warming and drying (with more rainfall variability) predicted for our study region is likely to have a greater impact on ant assemblages than plant assemblages.

Patterns of species richness

Determining whether patterns of species richness are consistent in different taxa and environments is important to understand the fundamental drivers of biodiversity. Our finding that species richness was correlated with MAP for plants but not ants indicates that there may be different processes driving diversity for these groups. The lack of change in ant species richness across a wide climate gradient is unusual (Dunn et al., 2009), but mirrors results from gradients from steppe to desert in central Asia (Pfeiffer et al., 2003) and tropical to arid zone savannas in northern Australia (Andersen et al., 2015). Consistent richness across the northern Australian gradient was attributed to a lack of temperature change, and/or the presence of a megadiverse ant fauna in the arid zone, which has been a source of species radiation back in to tropical savannas (Andersen et al., 2015, Andersen, 2016). On our southern Australian gradient, ant species richness was maintained despite temperature and rainfall changing together, suggesting that temperature is not driving ant diversity, or that temperature and rainfall acted on species richness diametrically. Both gradients move from wetter coastal environments to the arid interior, however, providing support that ant species diversity might be maintained by an unusually diverse species pool in the arid zone. Our finding of no association between total vegetation cover and ant species richness or abundance (Figure S5) is incongruent with other studies (e.g. Lassau and Hochuli, 2004, Vasconcelos et al., 2008), and suggests relationships between vegetation and ant richness may be region-dependant and driven by the vegetation type and species identities and functional roles of the regional ant fauna.

Species composition and environmental drivers

We found that variation in both plant and ant species composition was correlated with their environment, although there was a much stronger correlation between individual environmental variables and assemblages of plants than ants (Table 3). The finding that ant assemblages correlated more strongly with precipitation than temperature variables was contrary to our expectations that small ectotherms would be primarily temperature-driven as found in other studies (Sanders et al., 2007, Tiede et al., 2017). However, the included temperature variables were modelled air temperature rather than ground surface temperature, the latter being likely to be more relevant for ants (Lessard et al., 2009) and subject to small-scale microclimatic variation (Keppel et al., 2017). The correlation of slope and aspect variables to ant assemblages (Table 3) and the inclusion of those variables as predictors in our stepwise model building process (Table 4) reflects the importance of insolation as a key driver of ant species composition (Andersen, 1995).

When considered together, linear combinations of environmental variables were able to constrain 65% of assemblage variation for plants and 50% of assemblage variation for ants (Table 4). The weaker association of ant assemblages with individual environmental variables may point to the diversity of ant species, each of which may be more or less sensitive to a range of environmental drivers (temperature; moisture; soil type; cover, etc.), meaning that the assemblage as a whole does not correlate well with change in one driver. Our ability to explain 50% of assemblage variation with a suite of environmental drivers provides support for this hypothesis, but suggests that there are other important factors at play. Such factors could include untested variables, such as seasonality or climate variability, which can be an important driver of trait variation and species distributions, particularly for ants (Arnan et al., 2014). Incomplete sampling of assemblages (particularly for ants, which are more easily overlooked than plants) can also impede the signal between environment and biodiversity response. Potential nonlinear relationships between biodiversity occurrence and turnover and the environment could be further explored using other regression-based tools, such as generalised dissimilarity modelling (Ferrier et al., 2007) or fitting linear models to each species independently (Wang et al., 2012).

It is also likely that some species distributions are not driven by current environmental conditions. Indeed, stochastic factors may play an important role in observed distributions of species with restricted dispersal capacity, including many invertebrates and some plants. Short-range endemic invertebrate species are common in Australia, particularly in fragmented landscapes (Harvey, 2002). Non-environmentally driven species change could also be attributed to recent habitat clearance. This is plausible for our study region, which has experienced extensive clearing since European settlement and now comprises largely non-contiguous reserves, particularly in areas of high elevation surrounded by plains, such as Dutchman’s Stern (DUTL, DUTU), Mt Remarkable (REML, REMU) and the Tothill Range (TOTR) sets of sites (see Figure 1).

The relationship between the assemblages of different organisms provides an indication as to whether factors shaping species distributions (including environment, historic biogeography and even serendipity) have affected different taxa together or independently.

Our finding that plant and ant assemblages both exhibited high ß-diversity (Table 2) and similar spatial structuring over the environmental gradient (Figure 5), suggests that these assemblages have indeed been fashioned through similar ecological processes. Biotic interactions may also play a role in linking assemblages by the development of associations between species that strengthen local biotic networks. Close associations between plant and ant assemblages was also detected on an elevational gradient in Nevada (Sanders et al., 2003). Similarly, assemblage fidelity between plant and invertebrate assemblages was found in logged and unlogged forest sites (Oliver et al., 1998) and in riparian corridors in eastern Australia (Ives et al., 2011), suggesting congruence between plant and invertebrate taxa may be common.

Projecting changes in species composition

In addition to better understanding the drivers of current biodiversity, models of biotic response to spatial gradients allows projections to be made of how biodiversity will respond to future climate change. Any projections are subject to the limitations of the initial model. Nevertheless, despite the existence of drivers that impact on species distributions that we were not able to model, we were able to constrain nearly two thirds and half of observed variation in plants and ants respectively (Table 4). Nevertheless, because we do not know what is driving the unconstrained component of the observed species distributions, it is impossible to know whether unmodelled forces are extant or if they will accelerate or dampen the magnitude of any change in future climates. Moreover, when the predictive model is based on assemblage responses to correlated environmental variables, such as temperature and rainfall on our gradient, a decoupling of these variables (i.e. a novel environment) may lead to biotic change that cannot be predicted by the model. Noting these limitations, we have used “mild” (RCP 4.5) and “severe” (RCP 8.5) climate change scenarios from two global climate models to account for climate uncertainty and used these variables to predict how plant and ant assemblages will change by 2050, 2070 and 2090 (Figure 2; Figure 3).

The biotic predictions that we make using these scenarios are based on the environment-associated variability we were able to constrain and do not predict species occurrences directly, but rather the direction and magnitude of shift in “biodiversity space” over time. This approach has the benefit of accounting for the important, yet often neglected elements of species abundance and trait variability (McMahon et al., 2011, Ehrlén and Morris, 2015), but has several important limitations. Chief amongst these are the assumptions that: a) species are immutable (i.e. will not exhibit trait change that allows them to persist in currently unsuitable habitat); b) no new species will enter the system; c) all responses are environmentally driven and occur linearly with environmental change; d) all species are able to instantly disperse to locations with a suitable environment; and e) species absence indicates that the environment is not suitable. Unfortunately, none of these assumptions hold true in all cases, and will limit the accuracy of any predictions made. Nonetheless, strong environmental controls on species composition are evident across space, and this approach gives useful insight in to the trends we are likely to see in plants and ant fauna, and allows speculation on the comparative magnitude of biodiversity shifts we are likely to see in different taxa, locations and under different climate change scenarios.

The results of this analysis, that projected ant assemblages are far more responsive to projected environmental change than plant communities (Figure 6, Figure 7, Supplementary Figure S6), is surprising given both groups displayed similar species turnover (Table 2) and assemblage patterns (Figure 5). It is likely that the high proportion of ant species found at only one site (40.6%; compared to 23.5% of plant species) contributed to this uneven response, but it is unknown whether this current site-specificity represents an environmentally driven species response or a stochastic element that may not be responsive to future climate change. Surprisingly, the migration of the future plant assemblages through biotic space does not appear to be strongly aligned with any single dominant environmental variable (Figure 6). On the other hand, ant assemblages appear to be migrating on an axis of increasing MAP and MaxP and decreasing MaxT, despite the climate models generally predicting increased temperatures and rainfall variability, providing evidence that temperature and rainfall do drive ant assemblage change but each can mask the effect of the other. The approach we have used here of representing the entire gradient as a polygon in climate and biotic space may mask the rearrangement of individual sites within that polygon, but aggregating the response at a gradient level is likely to be more representative of the change we see in the future than site level change due to the reduced role of stochastic processes at the landscape level.

We have used linear distance to give a sense of the magnitude of projected biotic shifts, but it is important to realise that while related, linear distances do not necessarily scale to biodiversity change. Plant and ant assemblages at many sites show a peak in linear distance by 2050 or 2070, followed by a decrease by 2090 (Figure 7). It is tempting to interpret such a pattern as an “ecological recovery” following climate change being arrested. While this could be the case for the RCP4.5 scenarios, the presence of this response in assemblages under the RCP8.5 scenarios, for which there is no climatic recovery, suggests an alternative explanation. We found a high ß$_{SIM}$ (Table 2) and proportion of species detected at only one site (40.6% for ants, 23.5% for plants) for this gradient, suggesting a small environmental change is likely to result in the loss of several species from the system. The resulting increase the nestedness component of ß-diversity (ß$_{SNE}$) and homogenisation of assemblages across the gradient could result in a decline in linear distances despite increasing environmental change. Given that our modelling approach is unable to accommodate changes in traits or behaviours within species, or the migration of new species in to the study system, species loss is probably exaggerated. This suggests that decreases in linear distances between time periods are unlikely to be associated with real ecosystem recovery, and will instead be associated with increased species extirpation and immigration of new species.

It is likely that there will be a substantial lag in response to changed environment for some species. The generation time of both plants and ant colonies is variable, ranging from weeks to decades (Keller, 1998, Marbà et al., 2007), with some woody plants able to live substantially longer. Short-lived or rapidly dispersing species are likely to show the most rapid response to environmental change, with longer lived species and those with poor dispersal showing less response (Pearson and Dawson, 2003). It is therefore likely that some species (ants, short-lived plants) will show a more rapid environmental response to climate change, either dispersing to new locations or extirpated from the system. Longer-lived species, such as trees may persist far beyond their capacity to reproduce in a changed environment (Vellend et al., 2006), leading to an “extinction debt”.

In addition to this extinction debt, there may be an “immigration credit”, whereby the environment changes sufficiently to allow other species to migrate in to previously unsuitable habitat may also occur (Jackson and Sax, 2010). Extinction debts and immigration credits will have the effect of reducing shorter-term biodiversity response, but ecological inertia can continue to alter ecosystem assemblages for some time, even if climate change is arrested (Blonder et al., 2017). While we can therefore expect substantial changes in biodiversity and ecosystem function, the ecological lag of these effects may mean that these effects may take some time to materialise and accumulate to the point where second order changes (such as biotic-driven environmental change) can occur (Xu et al., 2015). In addition to the ecosystem services that the ecosystem engineering guilds of plants and ants provide, individual species associations play an important role in species persistence. Facilitative effects may allow some species to flourish in otherwise hostile environments (Gilman et al., 2010); while altered competition (Vergnon et al., 2017), predation (Beukema and Dekker, 2005) or other ecosystem dynamics may lead to species being extirpated from otherwise benign conditions.

Our finding that a given site's predicted biodiversity change can be in the top or bottom tertile depending on the RCP scenario or model selected underscores the bias and uncertainty climate model selection can introduce in to any biodiversity projections. The uneven sensitivity across taxa (Figure 6) and lack of clear regional trends (Figure 7) suggests simple space-for-time substitutions are unlikely to be accurate when predicting long-term biodiversity shifts for this study region, indicating such an approach should be approached with caution (Blois et al., 2013).

Despite evidence of strong congruence between plant and ant assemblage structuring under current climate conditions, our model suggest that climate change will place significant stress on this congruence. Taking into account the uncertainty in climate scenarios, a greater magnitude response to climate change of the ant fauna compared to the plant species along the gradient is likely to lead to decoupling of these keystone taxa. We interpret this to mean that other assemblages of organisms within communities are likely to show similarly uneven sensitivities to future climate change, which could lead to substantial change in species mix and function. These findings could be further augmented by analysis using newer techniques such as generalised dissimilarity modelling (Ferrier et al., 2007) which accommodates nonlinear relationships and has the potential to confirm and further refine the results we present here.

Conclusion

We found environment explained nearly two thirds of species composition for plants and half of species composition for ants, and that these two groups showed strong congruence over the tested bioclimatic gradient. We found that both groups are likely to display strong response to future climate change, but the magnitude of ants assemblage change by 2050 will be ca. 3.4 to 7.5 times greater than that for plants. This finding suggests that a decoupling of ant and plant assemblages are likely occur, which may have negative implications for the stability and function of these communities. This work provides exciting avenues for future research, including extension to include other important taxa, replication on other gradients to test for similar responses, and ongoing monitoring to test the accuracy of our predictions. The development of a global network of well-characterised plot networks through national and international ecological observatory networks such as the National Ecological Observatory Network in the United States, the Terrestrial Ecosystem Research Network in Australia and the International Long Term Ecological Research Network, provides a very strong infrastructure base on which this research could be undertaken.

Author contributions

SCR, GRG and GMW developed the concept with input from ANA and AJL. GRG led the plant surveys and BDH led the ant surveys. Ants were identified by ANA. SCR undertook the data analysis with coding support from GRG and input from GMW. The manuscript was drafted by SCR. All authors made substantial contributions to refinement of concepts, interpretation of results and editing the manuscript.

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Table 1: Survey site information for 51 sites ordered north to south.

Region	Site code	Site name	Reserve	Latitude	Longitude
Flinders Ranges	WARR-B	Warraweena B	Warraweena*	-30.7757	138.7975
	WARR-C	Warraweena C		-30.7757	138.7960
	WARR-E	Warraweena E		-30.7757	138.7929
	BRAU-D	Brachina Upper D	Ikara-Flinders Ranges National Park	-31.3132	138.5654
	BRAU-C	Brachina Upper C		-31.3136	138.5668
	BRAU-E	Brachina Upper E		-31.3155	138.5664
	BRAL-C	Brachina Lower C		-31.3246	138.5664
	BRAL-B	Brachina Lower B		-31.3262	138.5675
	BRAL-E	Brachina Lower E		-31.3277	138.5674
	WILP-A	Wilpena Pound A		-31.5439	138.5956
	WILP-C	Wilpena Pound C		-31.5463	138.5955
	WILP-E	Wilpena Pound E		-31.5485	138.5970
	DUTU-E	Dutchman’s Stern Upper E	Dutchman’s Stern Conservation Park	-32.3078	137.9703
	DUTU-B	Dutchman’s Stern Upper B		-32.3105	137.9680
	DUTU-A	Dutchman’s Stern Upper A		-32.3114	137.9680
	DUTL-E	Dutchman’s Stern Lower E		-32.318	137.9559
	DUTL-B	Dutchman’s Stern Lower B		-32.3209	137.9545
	DUTL-A	Dutchman’s Stern Lower A		-32.3222	137.9545
Mid-North	REMU-A	Mt Remarkable Upper A	Mt Remarkable National Park	-32.7486	138.1367
	REMU-C	Mt Remarkable Upper C		-32.7514	138.1370
	REMU-D	Mt Remarkable Upper D		-32.7539	138.1379
	REML-E	Mt Remarkable Lower E		-32.826	138.0306
	REML-C	Mt Remarkable Lower C		-32.8284	138.0310
	REML-A	Mt Remarkable Lower A		-32.8285	138.0334
	SPRG-A	Spring Gully A	Spring Gully Conservation Park	-33.914	138.6043
	SPRG-C	Spring Gully C		-33.9158	138.6052
	SPRG-E	Spring Gully E		-33.9188	138.6044
	TOTR-A	Tothill Range A	Tothill Range*	-34.005	138.9598
	TOTR-B	Tothill Range B		-34.0068	138.9605
	TOTR-D	Tothill Range D		-34.0076	138.9634
Fleurieu Peninsula	KAIS-C	Kaiserstuhl C	Kaiserstuhl Conservation Park	-34.5741	139.0086
	KAIS-A	Kaiserstuhl A		-34.5767	139.0071
	KAIS-E	Kaiserstuhl E		-34.5783	139.0096
	SAND-B	Sandy Creek B	Sandy Creek Conservation Park	-34.609	138.8613
	SAND-A	Sandy Creek A		-34.6094	138.8597
	SAND-D	Sandy Creek D		-34.6095	138.8576
	HALE-D	Hale D	Hale Conservation Park	-34.6818	138.9048
	HALE-B	Hale B		-34.6822	138.9074
	HALE-A	Hale A		-34.6827	138.9086
	BLCK-E	Black Hill E	Black Hill Conservation Park	-34.8792	138.7108
	BLCK -B	Black Hill B		-34.8808	138.7084
	BLCK -C	Black Hill C		-34.8823	138.7080
	MONT-B	Montacute B	Montacute Conservation Park	-34.8866	138.7885
	MONT-C	Montacute C		-34.8873	138.7873
	MONT-A	Montacute A		-34.8874	138.7908
	HORS-A	Horsnell Gully A	Giles Conservation Park	-34.9341	138.7271
	HORS-B	Horsenell Gully B		-34.9352	138.7289
	HORS-E	Horsnell Gully E		-34.9359	138.7316
	DEEP-A	Deep Creek A	Deep Creek Conservation Park	-35.6083	138.2613
	DEEP-C	Deep Creek C		-35.6092	138.2633
	DEEP-D	Deep Creek D		-35.6094	138.2644

*Private conservation reserve

Table 2: Components of ß-diversity for plant and ant assemblages.

	$ß_{SIM}$	$ß_{SNE}$	$ß_{SOR}$
Plant	0.954	0.009	0.963
Ant	0.958	0.009	0.967

$ß_{SIM}$ = species replacement, $ß_{SNE}$ = nestedness component of ß-diversity, $ß_{SOR}$ = Sørensen pairwise dissimilarity

Table 3: Correlations of environmental variables with NMDS ordinations.

Variable	**Plant assemblage**	**Ant assemblage**
Latitude	0.75^{***}	0.13^{*}
Longitude	0.28^{**}	0.03 ns
MAT	0.58^{***}	0.17^{*}
MaxT	0.87^{***}	0.18^{*}
MinT	0.41^{***}	0.14^{*}
MAP	0.85^{***}	0.28^{**}
MaxP	0.86^{***}	0.28^{**}
MinP	0.56^{***}	0.14^{*}
MAMI	0.84^{***}	0.21^{**}
MaxMI	0.71^{***}	0.20^{**}
MinMI	0.78^{***}	0.21^{**}
Elevation	0.11^{**}	0.05 ns
Aspect	0.01 ns	0.05 ns
Slope	0.10 ns	0.13^{*}
Outcrop	0.25^{***}	0.11 ns
Percentage surface strew	0.38^{***}	0.01 ns
Percentage sand	0.30^{***}	0.03 ns
NH_4	0.34^{***}	0.09 ns
NO_3	0.14^{**}	0.11 ns
P	0.13^{***}	0.06 ns
K	0.34^{***}	0.05 ns
Surface EC	0.15^{*}	0.01 ns
Surface pH	0.57^{***}	0.07 ns

Mean Annual Temperature (MAT; Bio01); Mean temperature of warmest quarter (MaxT; Bio10); Mean temperature of coldest quarter (MinT; Bio11); Mean Annual Precipitation (MAP; Bio012); Precipitation of wettest week (MaxP; Bio13) and Precipitation of driest week (MinP; Bio14) were extracted from BioClim layers of a 1960-2014 long-term average at 9 second (ca. 30m resolution).

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

Table 4: Results from stepwise model building process to establish the most parsimonious environmental variables to constrain baseline CCAs.

Variable	**Plants**	**Ants**
MAT	Yes	Yes
MaxT	Yes	Yes
MinT	Yes	Yes
MAP	Yes	Yes
MaxP	Yes	Yes
MinP	Yes	Yes
Elevation	Yes	Yes
Northness	Yes	Yes
Eastness	Yes	
Slope		Yes
Outcrop	Yes	
Surface strew	Yes	Yes
Sand		
NH_4		
NO_3	Yes	
P		
K	Yes	
Surface EC	Yes	
Surface pH	Yes	
# variables	15	10
Constrained	0.650	0.501
Variation constrained in first two components	0.337	0.422

Table 5: Comparative magnitude of determinant for average biotic response**.**

Determinant	Plant assemblage	Ant assemblage	Congruence
Model	1.28	1.95	1.93
RCP Scenario	1.18	1.83	2.03

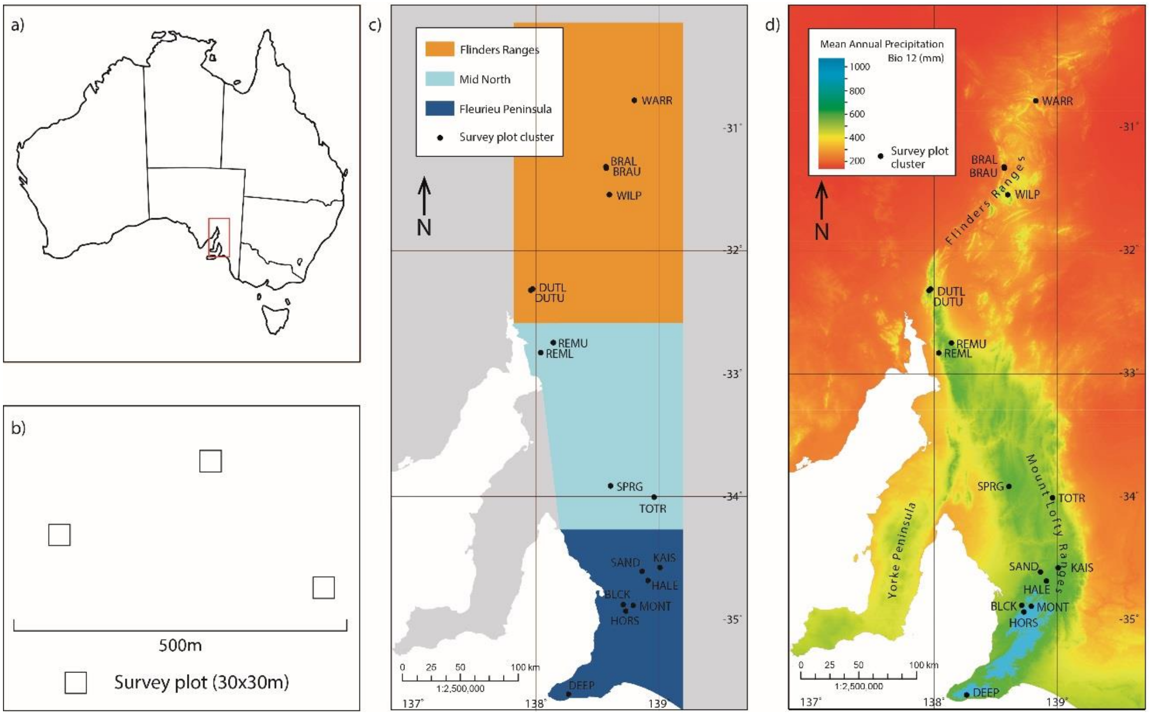


Figure 1: Map of survey plot locations with context map of Australia (a), indicative layout of three plots at each location (b), location of survey locations in a bioregional context (c) and on a precipitation gradient (d).

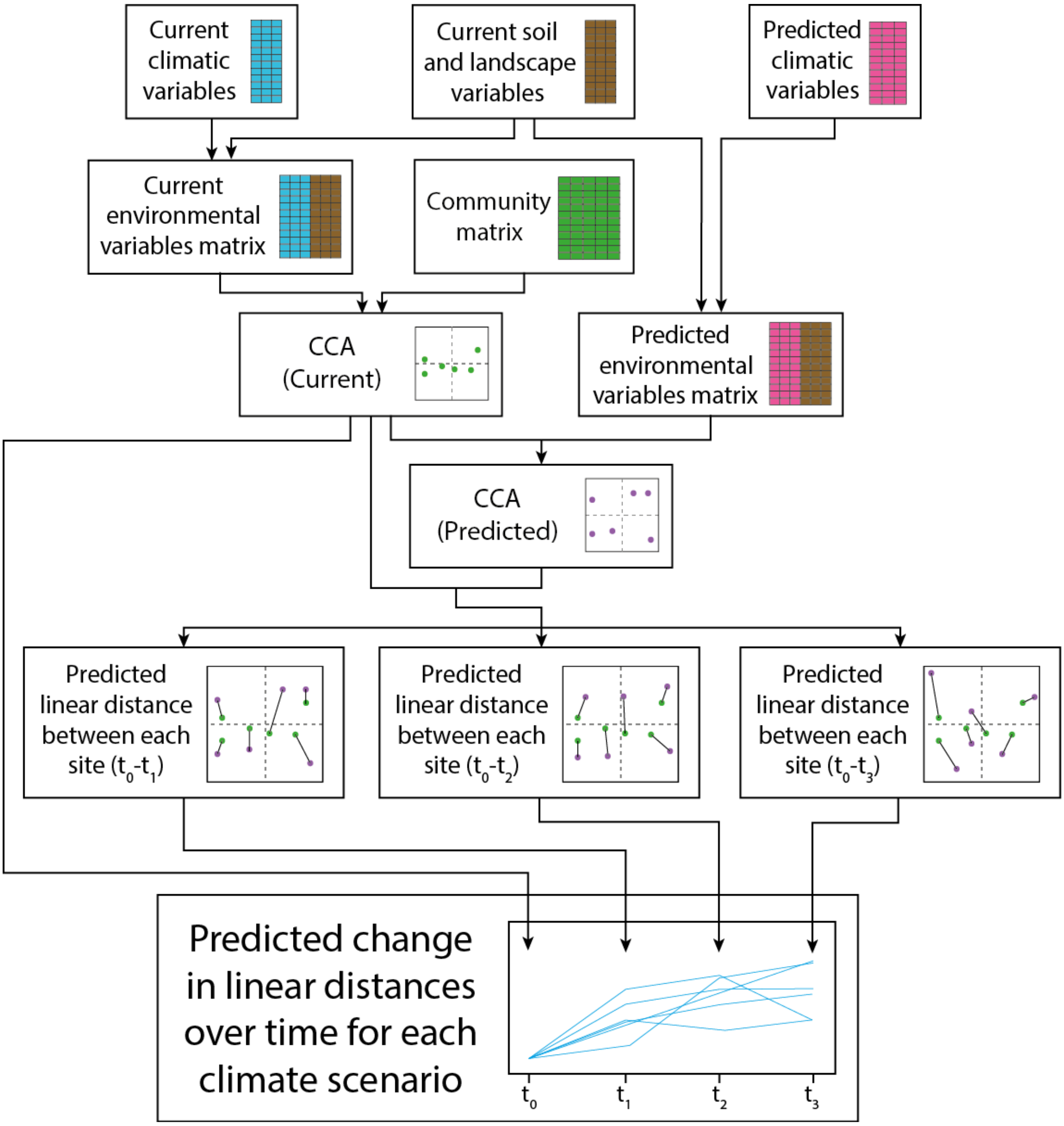


Figure 2: To predict change over time for a single ecosystem component (i.e. plants or ants), long-term climatic variables and current soil and landscape variables, together with a community matrix, were used to generate a Canonical Correspondence Analysis (CCA) ordination (green dots). A predicted CCA (pCCA; purple dots) was generated using the relationship established between the position of sites on the initial CCA and projected environmental variables (generated as a combination of current soil and landscape variables and modelled climate projections). The linear distance between each sites' coordinates on the

baseline CCA and pCCA for 2050, 2070 and 2090 was used to generate a predicted change in linear distance over time for each climate model/scenario pair. In order to project climatic shifts, the same process was undertaken using Principal Component Analysis (PCA) and predicted PCA for future scenarios.

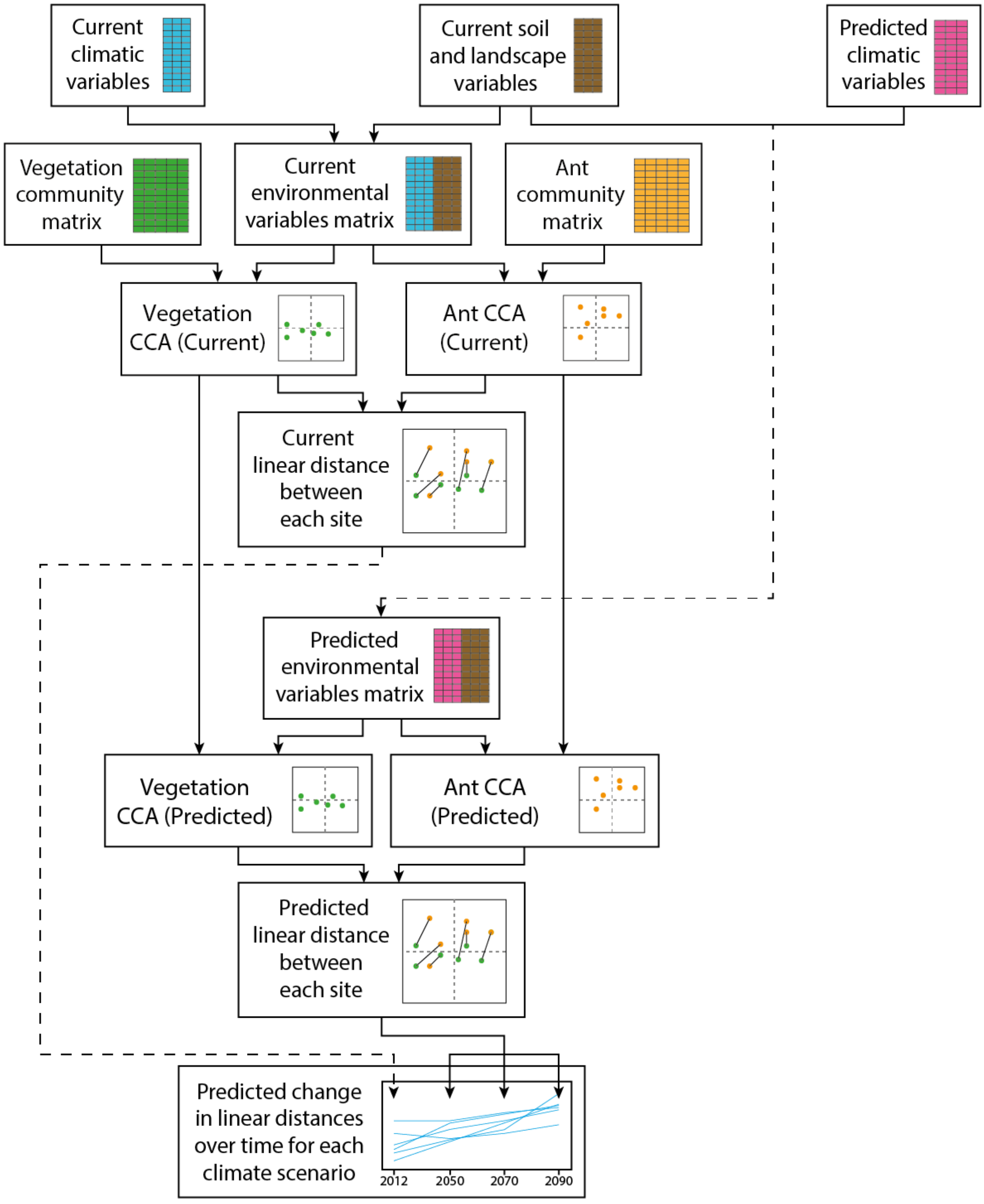


Figure 3: To project whether the network of ant and plant communities are likely to be maintained under climate change, long-term climate variables were combined with current soil and landscape variables and the ant and plant community matrices to generate CCAs of our survey sites for both the plant (green) and ant (orange) communities. The linear distance between the plant and ant communities in this initial CCA ordination space was taken as a

baseline (2012) value. pCCAs for both plant and ant communities were generated from the initial CCAs with predicted environmental variables (generated as a combination of current soil and landscape variables and modelled climate projections). The linear distance between each site's plant and ant coordinates in ordination space for pCCAs for 2050, 2070 and 2090 were subsequently recorded to show the change in relative positions between plant and ant projected ordinations over time. See Supplementary Figure S2 for more detail on the calculation of linear distances.

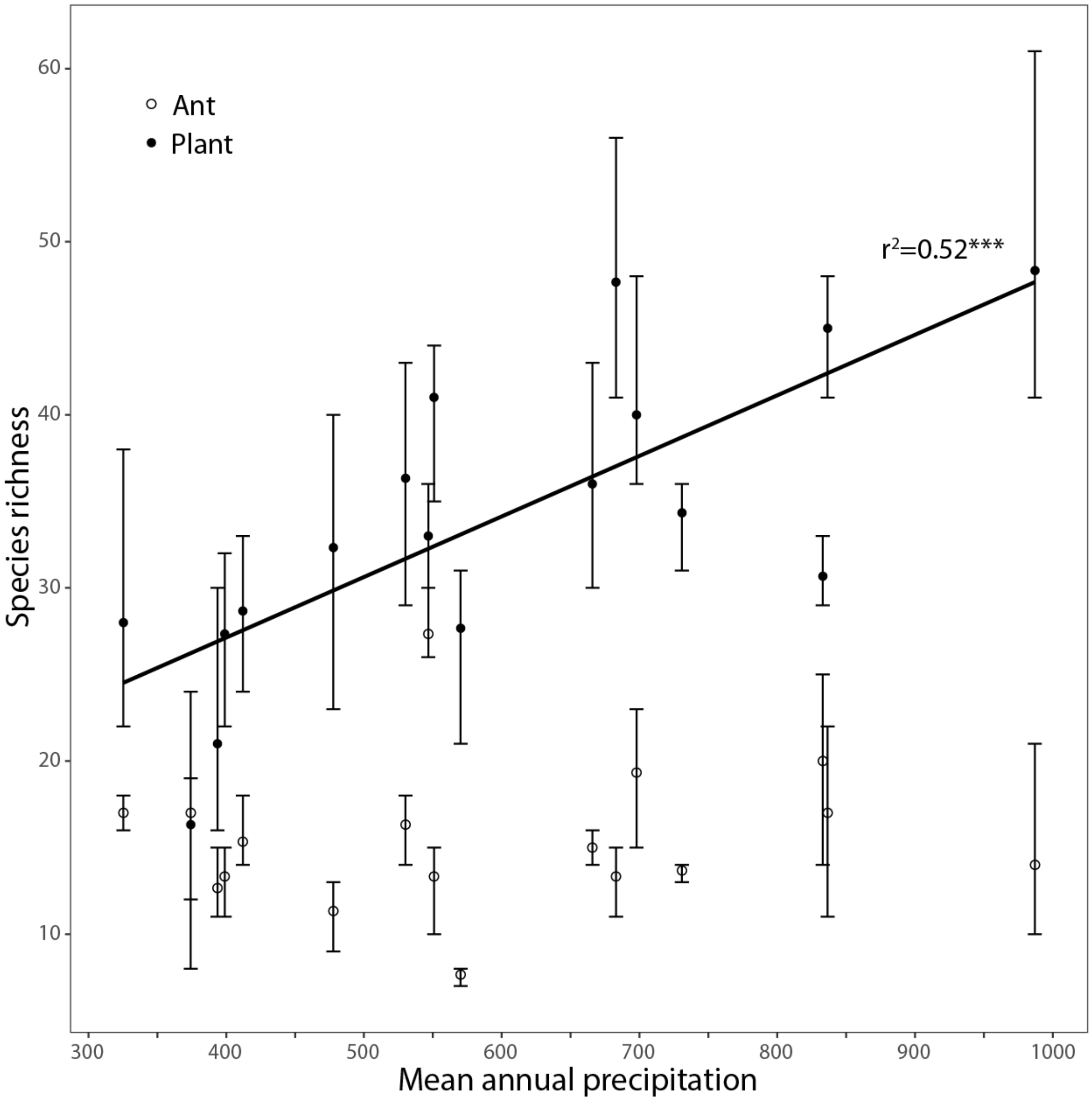


Figure 4: Plant species richness was positively correlated with mean annual precipitation ($r^2=0.52$, ***=$p<0.001$) but ant species richness was not ($r^2=-0.06$, $p>0.05$).

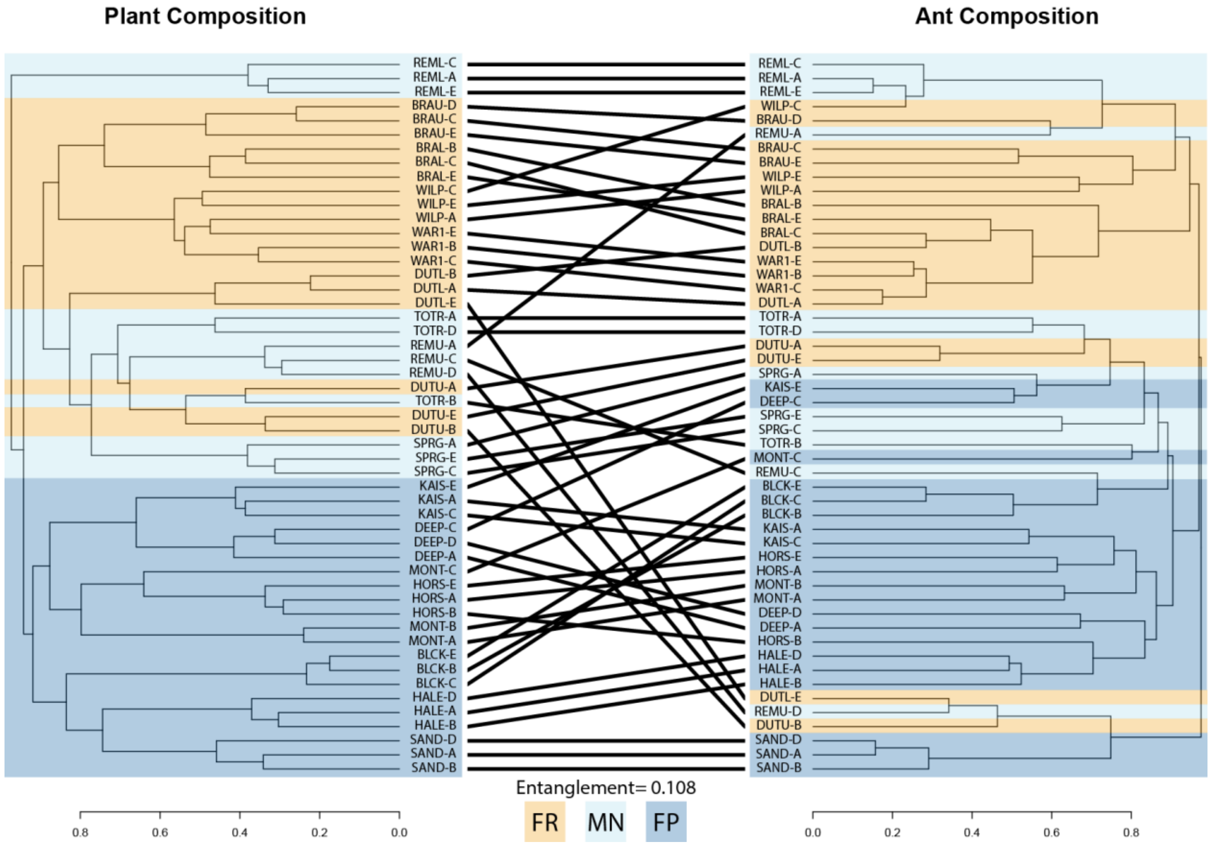


Figure 5: Tanglegram generated using stepwise disentanglement of dendrograms generated from ant and plant species composition. Following disentanglement, plots were ordered north to south. An entanglement factor of 0.108 indicates that the two dendrograms are highly aligned, with entanglement of 0 representing perfect alignment and 1 indicating no alignment of sites between trees. Colours represent bioregions (dark blue=Fleurieu Peninsula; light blue=Mid North; orange=Flinders Ranges).

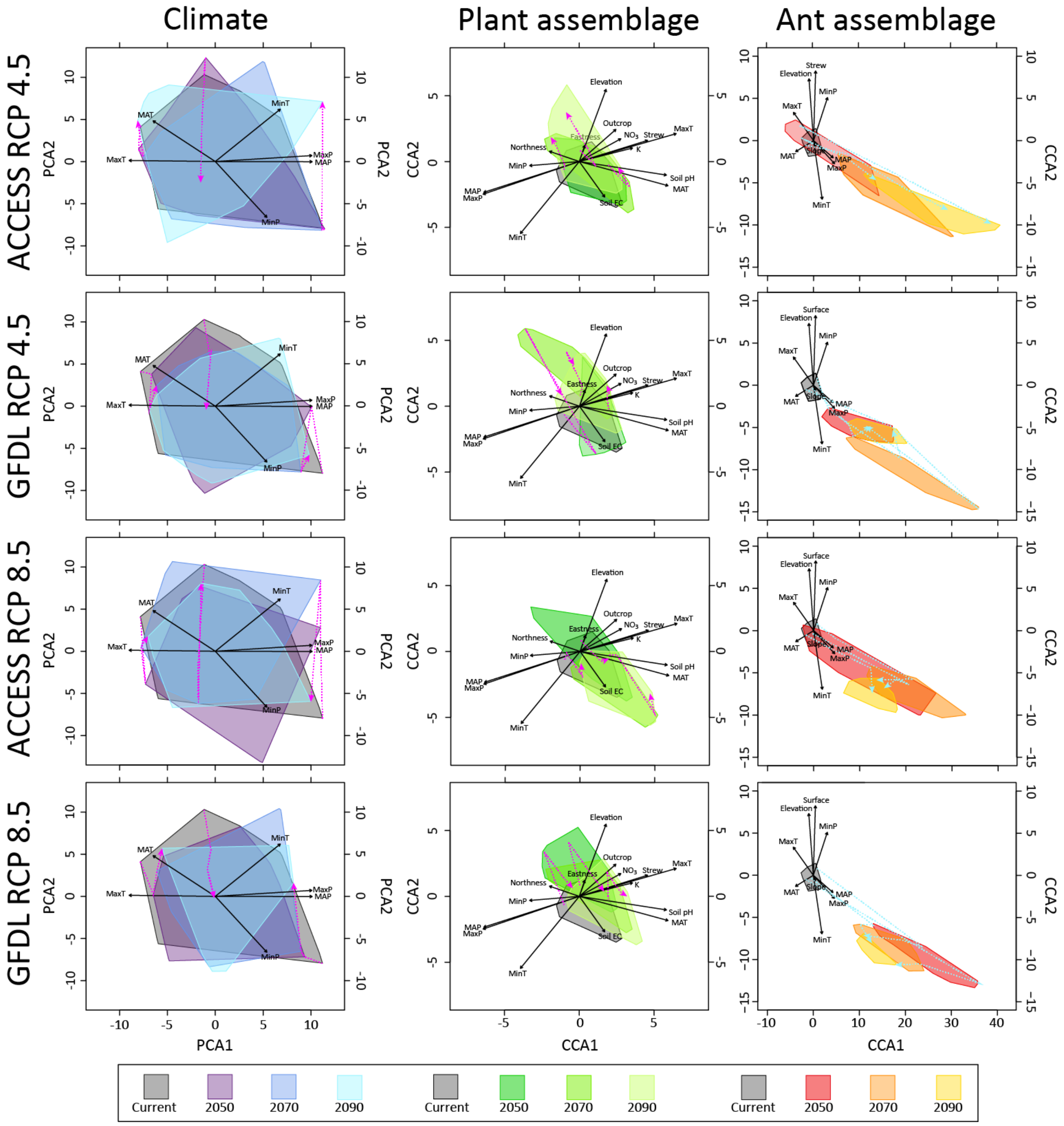


Figure 6: CCA ordinations of current environmental and species matrices (grey hulls) and predicted CCA ordinations (coloured hulls) generated using climate projections from the GFD and ACCESS models assuming RCP 4.5 (limited climate change) and RCP 8.5 (uncontrolled climate change) scenarios. Hulls are drawn from the outermost site coordinates for each ordination and thus represents the entire ordination space covered by an assemblage matrix at a single point in time. Note the different scale for ant ordinations.

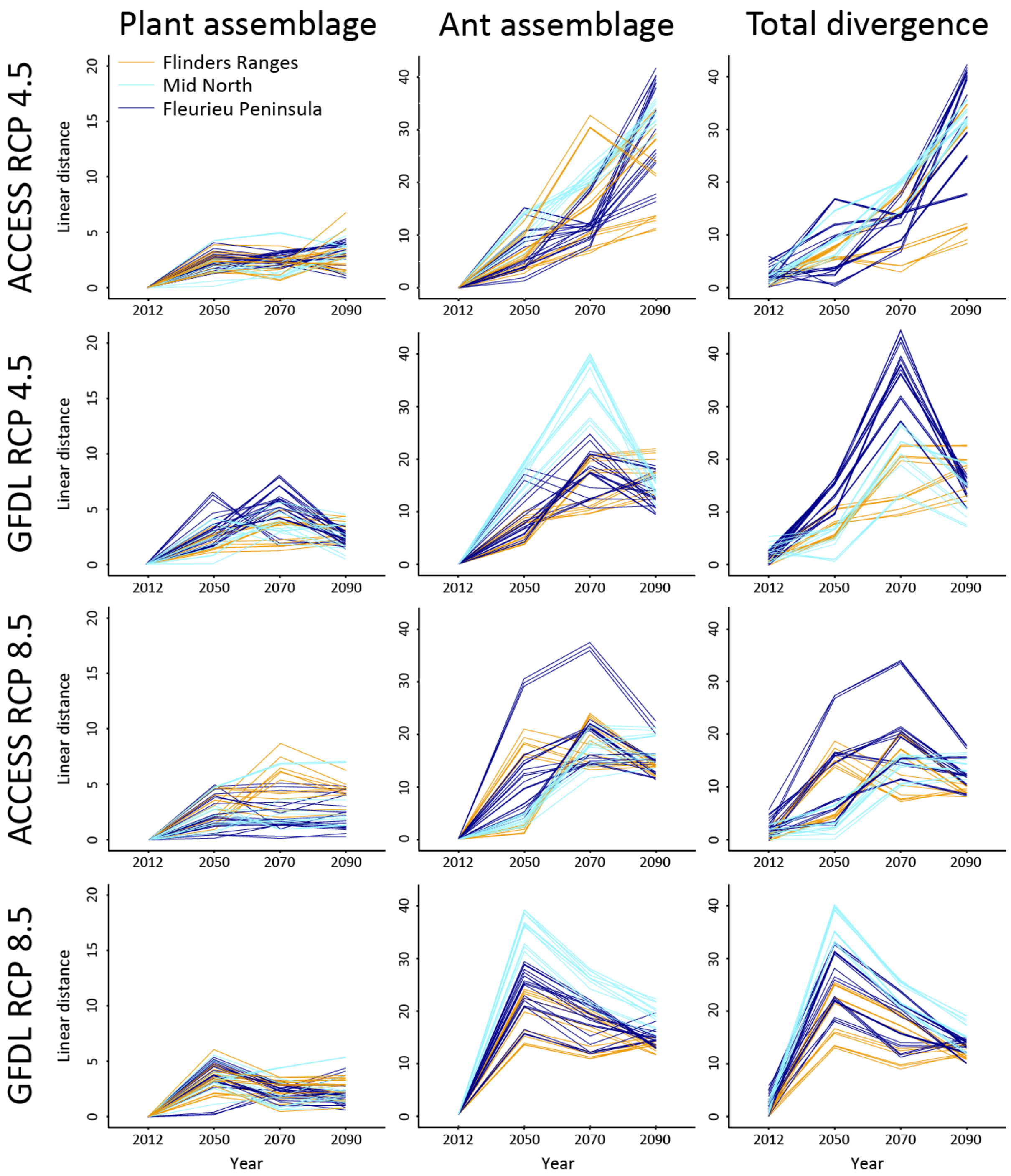


Figure 7: Linear distances between site coordinates in ordination space for vegetation communities, ant communities and between ant and plant communities (Divergence). Sites are coloured by bioregion (dark blue = Fleurieu Peninsula (most mesic, southernmost), light blue = Mid North, orange = Flinders Ranges (most arid, northernmost). Note the smaller y-axis scale for plant assemblage plots.

Supplementary Information

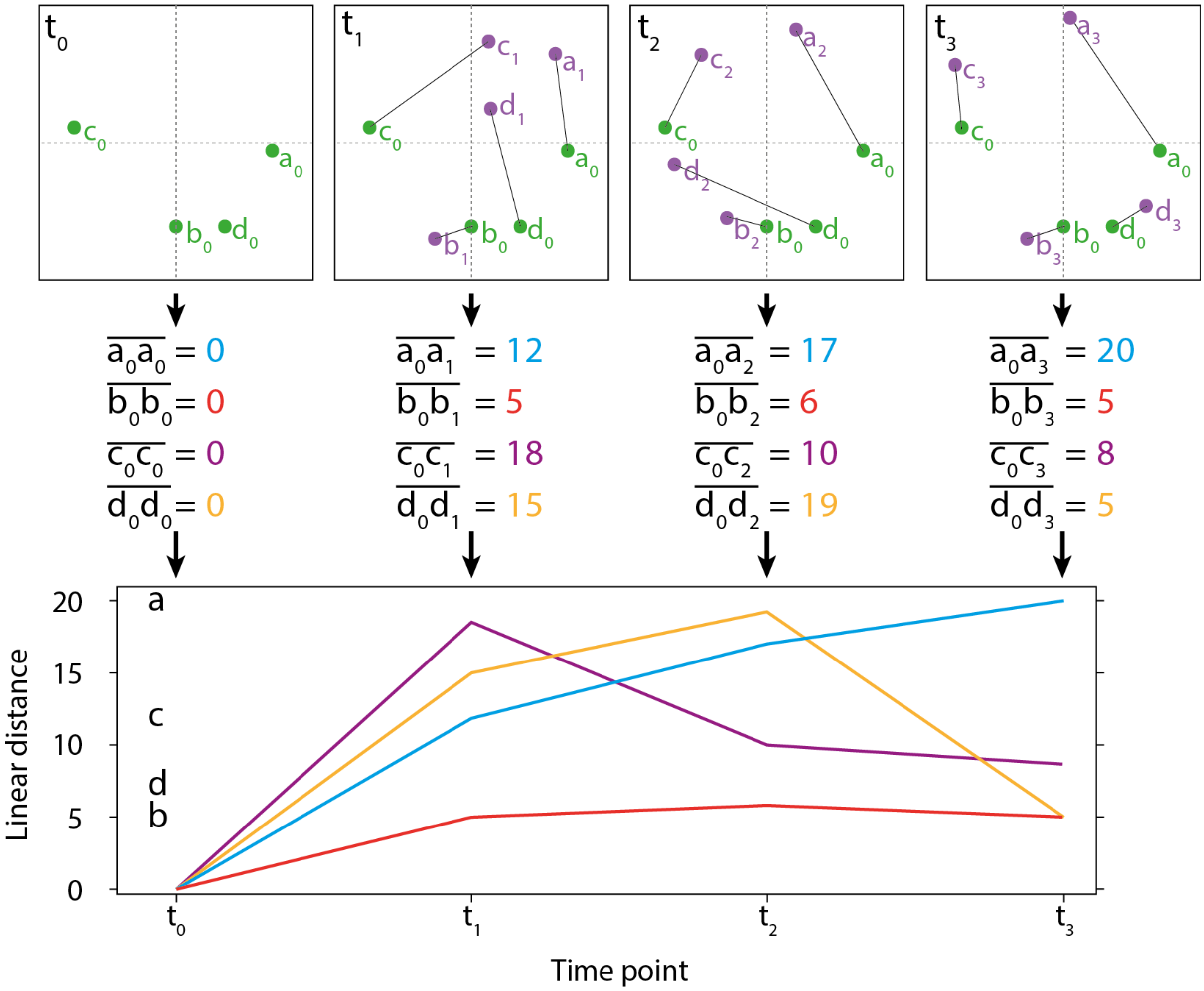


Figure S1: For each survey site (a-d), the linear distance between that site’s coordinates in the initial CCA of community matrix (green) and pCCAs for each time period. Zero is used as a baseline for the starting point (t_0). The linear distances are then mapped to show overall change in linear distance for each site. A rapid and consistent change in linear distance (a) would indicate a high level change in species assemblages. A lower magnitude response (b) correspondingly indicates a low level of linear distance change, potentially indicating a smaller assemblage change. A steep increase followed by a reduction in linear distance (c, d) may indicate an ecological recovery in species assemblages, which having been redistributed on the gradient by environmental pressures are returning to a configuration resembling their original state. Alternately, this pattern may represent species being reorganised until the environmental change exceeds a large number of species' capacities to migrate, after which time they are removed from the system and the new assemblage is more similar to the baseline (although with fewer species present).

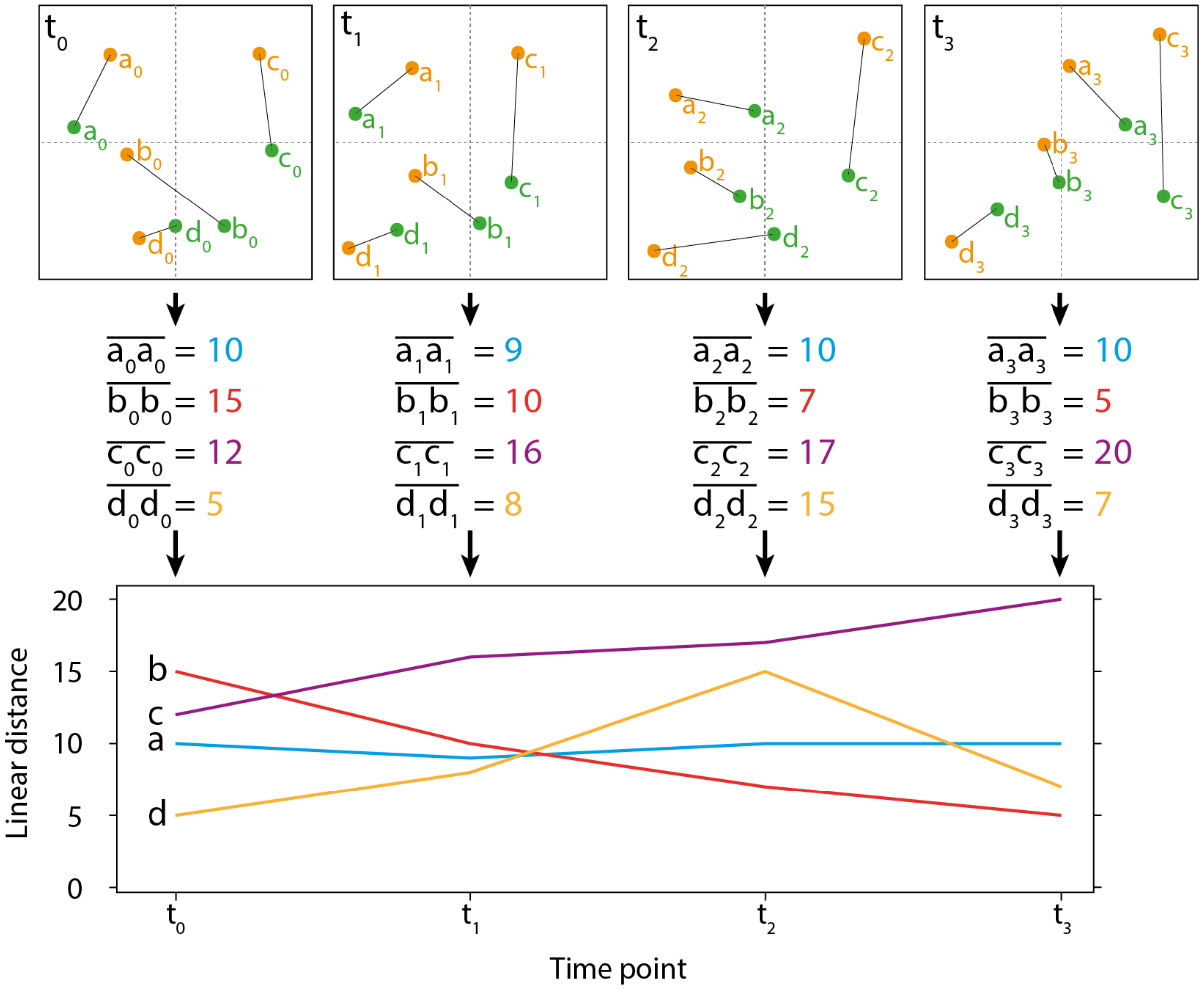


Figure S2: For each survey site (a-d), the linear distance between that site's coordinates in the initial CCA of the plant community matrix (green) and initial CCA of the ant community matrix (orange) is used as a baseline (t_0). The same process is undertaken for predicted CCAs at each time point (t_1-t_3). The linear distances are then mapped to show overall change in linear distance for each site. A maintenance of linear distance (site a) would indicate that while a site may migrate in ordination space, this is mirrored at the same scale for both plant and ant taxa, and the interactions between these groups may be maintained. A decrease (site b) or increase (site c) in linear distance indicates that plants and ants are responding unevenly, and likely represents a decoupling of these communities and a potential breakdown of ecological function. Because the origin (baseline) is the only reference point, both convergence (reducing linear distance) and divergence (increasing linear distance) are likely to both represent decoupling. However, if a site displays an increase in linear distance and then a subsequent decrease (site d) (or vice versa), this may represent an ecological recovery of that site following a climatic recovery.

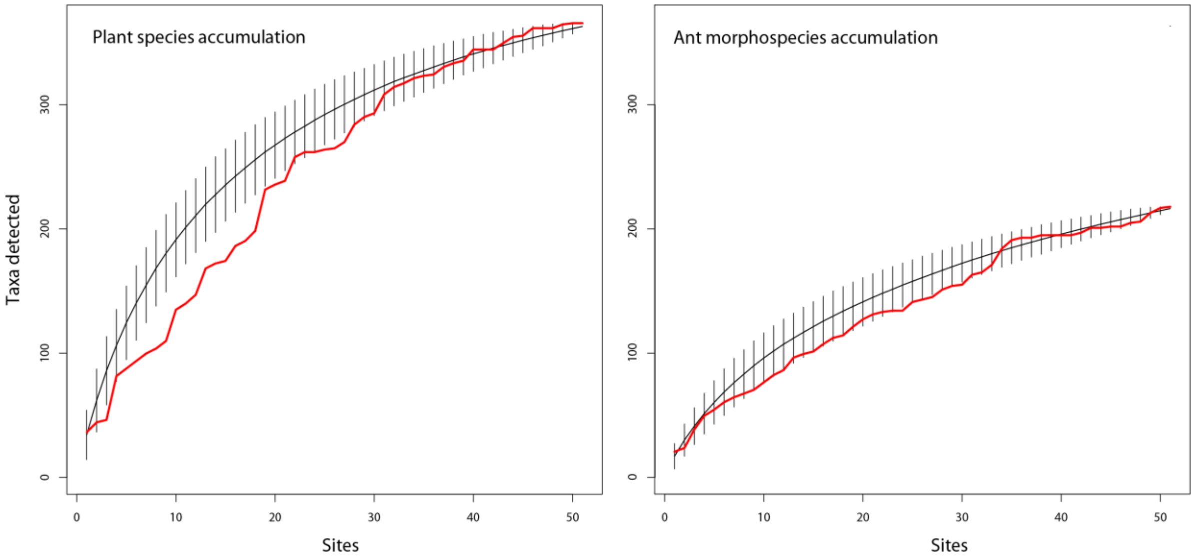


Figure S3: Accumulation curves for plant and ant species. Red lines show north (arid) to south (mesic) collector accumulation, black line indicates mean accumulation for 1000 random permutations of site sampling order with 95% confidence intervals. Flattening of both curves indicates that the majority of the regional species pool was sampled.

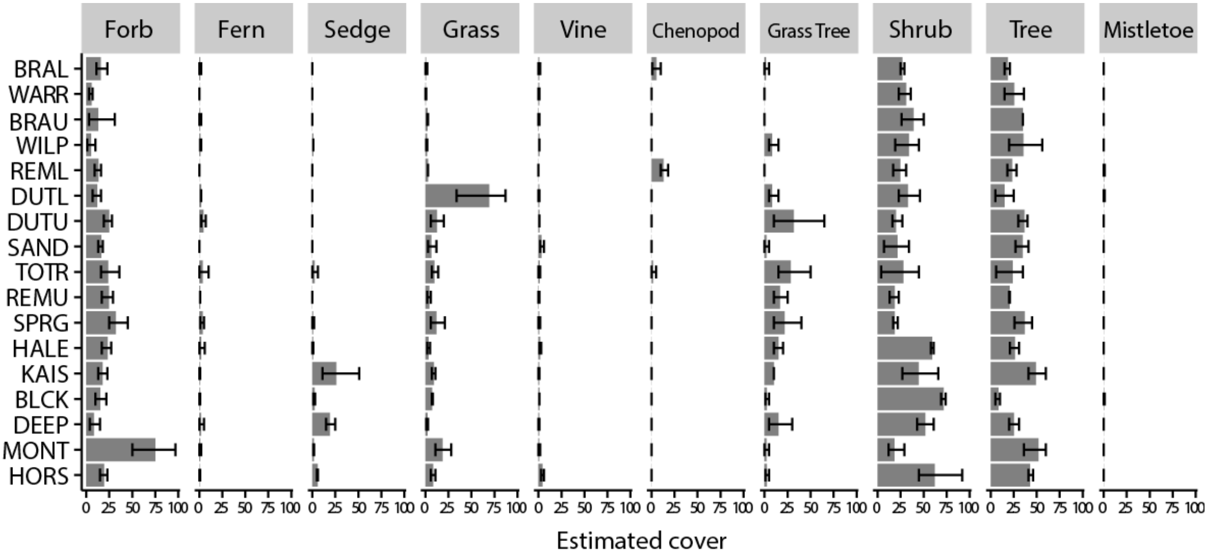


Figure S4: Mean vegetation cover by growth form for each site set ordered by mean annual precipitation (drier sites in the north). Error bars show minimum and maximum values within site set.

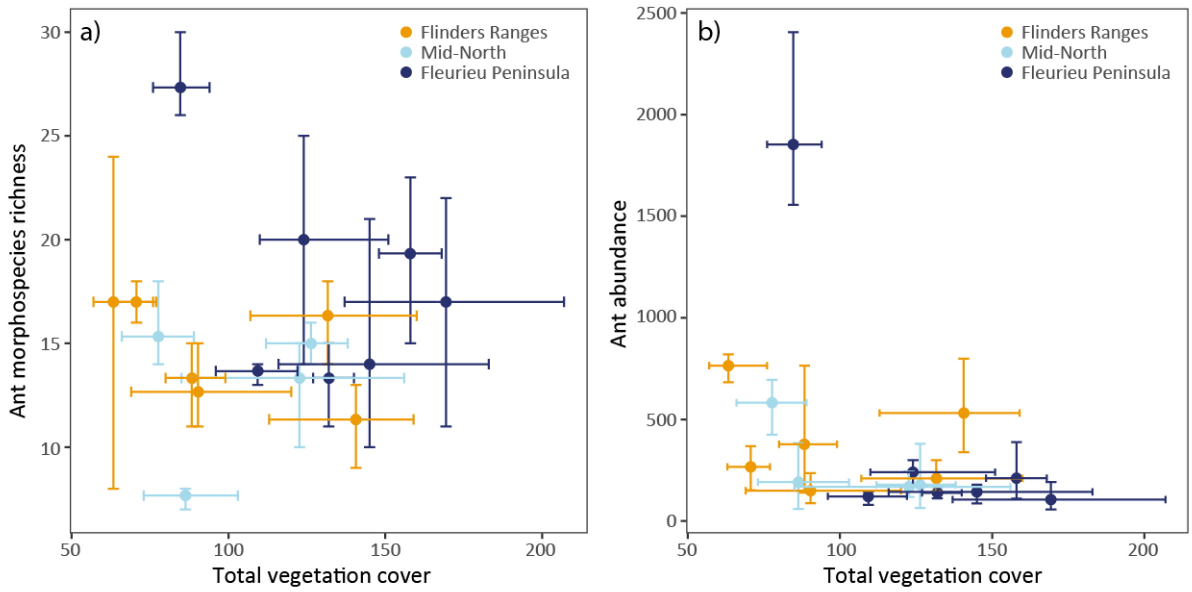
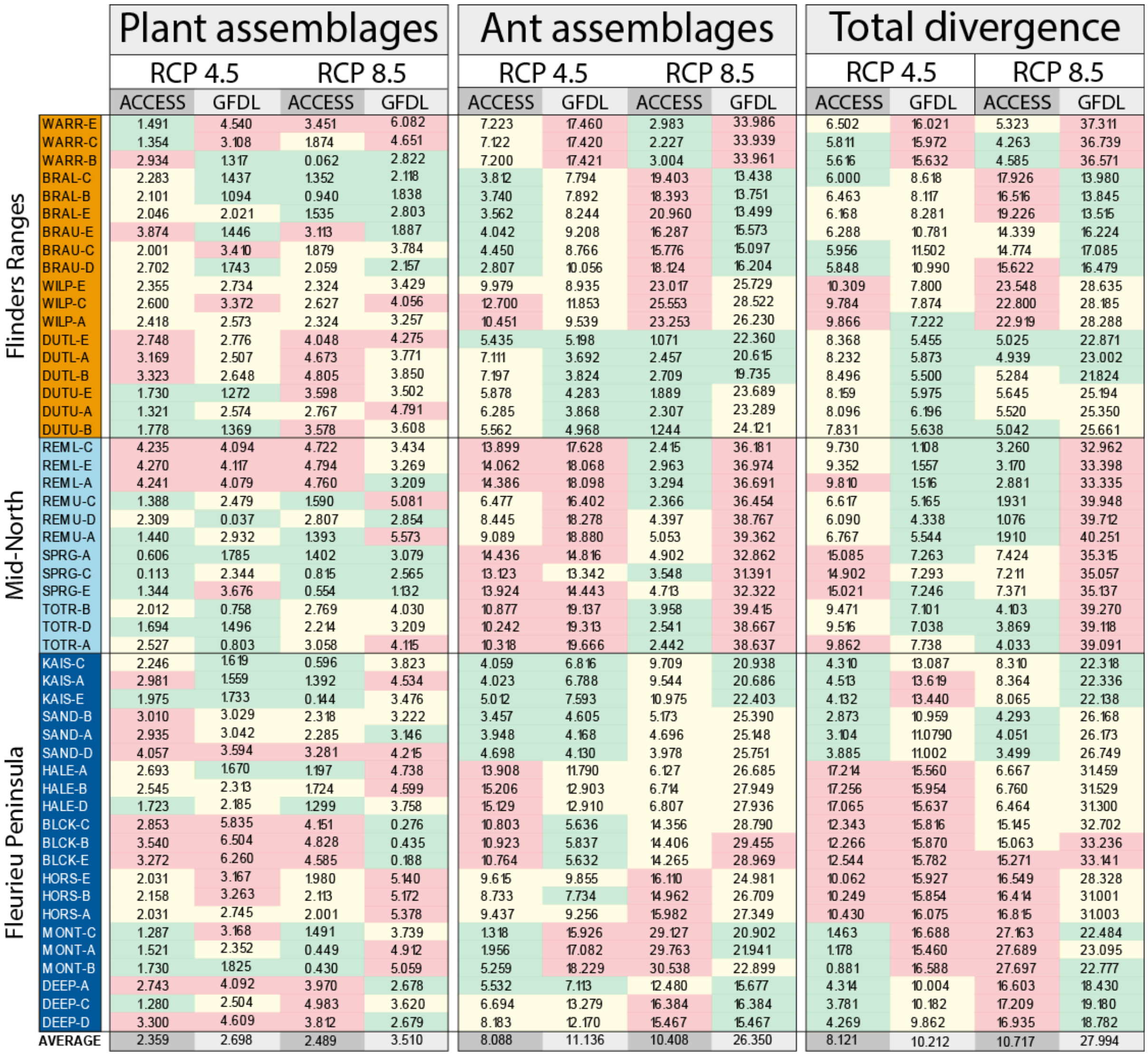


Figure S5: Total vegetation cover (summed visual cover estimates for all species at a site) was highly variable across the three sites at each location. We found no relationship between vegetation cover and ant species richness (a), or abundance (b). Error bars show the minimum, maximum and mean values for each group of three sites.



		Plant assemblages				Ant assemblages				Total divergence			
		RCP 4.5		RCP 8.5		RCP 4.5		RCP 8.5		RCP 4.5		RCP 8.5	
		ACCESS	GFDL	ACCESS	GFDL	ACCESS	GFDL	ACCESS	GFDL	ACCESS	GFDL	ACCESS	GFDL
Flinders Ranges	WARR-E	1.491	4.540	3.451	6.082	7.223	17.460	2.983	33.986	6.502	16.021	5.323	37.311
	WARR-C	1.354	3.108	1.874	4.651	7.122	17.420	2.227	33.939	5.811	15.972	4.263	36.739
	WARR-B	2.934	1.317	0.062	2.822	7.200	17.421	3.004	33.961	5.616	15.632	4.585	36.571
	BRAL-C	2.283	1.437	1.352	2.118	3.812	7.794	19.403	13.438	6.000	8.618	17.926	13.980
	BRAL-B	2.101	1.094	0.940	1.838	3.740	7.892	18.393	13.751	6.463	8.117	16.516	13.845
	BRAL-E	2.046	2.021	1.535	2.803	3.562	8.244	20.960	13.499	6.168	8.281	19.226	13.515
	BRAU-E	3.874	1.446	3.113	1.887	4.042	9.208	16.287	15.573	6.288	10.781	14.339	16.224
	BRAU-C	2.001	3.410	1.879	3.784	4.450	8.766	15.776	15.097	5.956	11.502	14.774	17.085
	BRAU-D	2.702	1.743	2.059	2.157	2.807	10.056	18.124	16.204	5.848	10.990	15.622	16.479
	WILP-E	2.355	2.734	2.324	3.429	9.979	8.935	23.017	25.729	10.309	7.800	23.548	28.635
	WILP-C	2.600	3.372	2.627	4.056	12.700	11.853	25.553	28.522	9.784	7.874	22.800	28.185
	WILP-A	2.418	2.573	2.324	3.257	10.451	9.539	23.253	26.230	9.866	7.222	22.919	28.288
	DUTL-E	2.748	2.776	4.048	4.275	5.435	5.198	1.071	22.360	8.368	5.455	5.025	22.871
	DUTL-A	3.169	2.507	4.673	3.771	7.111	3.692	2.457	20.615	8.232	5.873	4.939	23.002
	DUTL-B	3.323	2.648	4.805	3.850	7.197	3.824	2.709	19.735	8.496	5.500	5.284	21.824
	DUTU-E	1.730	1.272	3.598	3.502	5.878	4.283	1.889	23.689	8.159	5.975	5.645	25.194
	DUTU-A	1.321	2.574	2.767	4.791	6.285	3.868	2.307	23.289	8.096	6.196	5.520	25.350
	DUTU-B	1.778	1.369	3.578	3.608	5.562	4.968	1.244	24.121	7.831	5.638	5.042	25.661
Mid-North	REML-C	4.235	4.094	4.722	3.434	13.899	17.628	2.415	36.181	9.730	1.108	3.260	32.962
	REML-E	4.270	4.117	4.794	3.269	14.062	18.068	2.963	36.974	9.352	1.557	3.170	33.398
	REML-A	4.241	4.079	4.760	3.209	14.386	18.098	3.294	36.691	9.810	1.516	2.881	33.335
	REMU-C	1.388	2.479	1.590	5.081	6.477	16.402	2.366	36.454	6.617	5.165	1.931	39.948
	REMU-D	2.309	0.037	2.807	2.854	8.445	18.278	4.397	38.767	6.090	4.338	1.076	39.712
	REMU-A	1.440	2.932	1.393	5.573	9.089	18.880	5.053	39.362	6.767	5.544	1.910	40.251
	SPRG-A	0.606	1.785	1.402	3.079	14.436	14.816	4.902	32.862	15.085	7.263	7.424	35.315
	SPRG-C	0.113	2.344	0.815	2.565	13.123	13.342	3.548	31.391	14.902	7.293	7.211	35.057
	SPRG-E	1.344	3.676	0.554	1.132	13.924	14.443	4.713	32.322	15.021	7.246	7.371	35.137
	TOTR-B	2.012	0.758	2.769	4.030	10.877	19.137	3.958	39.415	9.471	7.101	4.103	39.270
	TOTR-D	1.694	1.496	2.214	3.209	10.242	19.313	2.541	38.667	9.516	7.038	3.869	39.118
	TOTR-A	2.527	0.803	3.058	4.115	10.318	19.666	2.442	38.637	9.862	7.738	4.033	39.091
Fleurieu Peninsula	KAIS-C	2.246	1.619	0.596	3.823	4.059	6.816	9.709	20.938	4.310	13.087	8.310	22.318
	KAIS-A	2.981	1.559	1.392	4.534	4.023	6.788	9.544	20.686	4.513	13.619	8.364	22.336
	KAIS-E	1.975	1.733	0.144	3.476	5.012	7.593	10.975	22.403	4.132	13.440	8.065	22.138
	SAND-B	3.010	3.029	2.318	3.222	3.457	4.605	5.173	25.390	2.873	10.959	4.293	26.168
	SAND-A	2.935	3.042	2.285	3.146	3.948	4.168	4.696	25.148	3.104	11.0790	4.051	26.173
	SAND-D	4.057	3.594	3.281	4.215	4.698	4.130	3.978	25.751	3.885	11.002	3.499	26.749
	HALE-A	2.693	1.670	1.197	4.738	13.908	11.790	6.127	26.685	17.214	15.560	6.667	31.459
	HALE-B	2.545	2.313	1.724	4.599	15.206	12.903	6.714	27.949	17.256	15.954	6.760	31.529
	HALE-D	1.723	2.185	1.299	3.758	15.129	12.910	6.807	27.936	17.065	15.637	6.464	31.300
	BLCK-C	2.853	5.835	4.151	0.276	10.803	5.636	14.356	28.790	12.343	15.816	15.145	32.702
	BLCK-B	3.540	6.504	4.828	0.435	10.923	5.837	14.406	29.455	12.266	15.870	15.063	33.236
	BLCK-E	3.272	6.260	4.585	0.188	10.764	5.632	14.265	28.969	12.544	15.782	15.271	33.141
	HORS-E	2.031	3.167	1.980	5.140	9.615	9.855	16.110	24.981	10.062	15.927	16.549	28.328
	HORS-B	2.158	3.263	2.113	5.172	8.733	7.734	14.962	26.709	10.249	15.854	16.414	31.001
	HORS-A	2.031	2.745	2.001	5.378	9.437	9.256	15.982	27.349	10.430	16.075	16.815	31.003
	MONT-C	1.287	3.168	1.491	3.739	1.318	15.926	29.127	20.902	1.463	16.688	27.163	22.484
	MONT-A	1.521	2.352	0.449	4.912	1.956	17.082	29.763	21.941	1.178	15.460	27.689	23.095
	MONT-B	1.730	1.825	0.430	5.059	5.259	18.229	30.538	22.899	0.881	16.588	27.697	22.777
	DEEP-A	2.743	4.092	3.970	2.678	5.532	7.113	12.480	15.677	4.314	10.004	16.603	18.430
	DEEP-C	1.280	2.504	4.983	3.620	6.694	13.279	16.384	16.384	3.781	10.182	17.209	19.180
	DEEP-D	3.300	4.609	3.812	2.679	8.183	12.170	15.467	15.467	4.269	9.862	16.935	18.782
	AVERAGE	2.359	2.698	2.489	3.510	8.088	11.136	10.408	26.350	8.121	10.212	10.717	27.994

Figure S6: Predicted linear distances for the year 2050 for each of the plant assemblages, ant assemblages and for total divergence between plant and ant assemblages, for each site and for the four climate change scenarios based on the two climate models (ACCESS and GFDL, and the greenhouse gas concentration scenarios (RCP4.5 earlier peak and RCP8.5 no peak in carbon emissions). Results are coloured to represent the largest projected linear distance/ecological change tertile (red), median tertile (yellow) and smallest projected linear distance/ecological change tertile (green).

Chapter 7: Thesis Discussion, Conclusions and Recommendations

This thesis has been presented as a series of manuscripts, either published, or in preparation for submission. Each chapter has its own discussion where results are considered in the context of the aims and literature associated with that chapter. Here, I present a more synthetic discussion of the overall contribution of this body of work to our understanding of how ecological communities respond to bioclimatic gradients and the implications for detecting and monitoring biotic responses to climate change.

Species and ecosystem response to environmental change, non-linear responses and ecological thresholds

One of the fundamental principles and foci of ecology is the knowledge that species and ecosystems are responsive to their environment. A growing appreciation that environment (particularly climate) is in a state of constant change and that humans have a major role in shaping this has led to an increased sense of urgency in understanding how these changes will affect biodiversity at different scales and time frames.

To study this type of response, spatial bioclimatic gradients can be used as a platform from which to investigate how a change in environment determines biodiversity response at an infra-species, species and assemblage level. Such studies provide confirmation of the importance, but also complex nature, of the response to environmental and climatic change at different levels of biological organisation.

The simplest relationships between biota and the environment are linear. Linear change, such as the relationship between plant species richness and rainfall on the TREND (Chapters 5 & 6), are easy to conceptualise and model, but given the complexity of ecosystems and their many drivers, biotic change on gradients may be non-linear, displaying curvilinear or stepped responses (Ferrier et al., 2007, Eamus et al., 2015). Given the urgent need to understand how rapid climate change is likely to impact ecosystems, non-linear ecological thresholds are of particular interest.

Thresholds have been detected in some studies but not others (Huggett, 2005, Lindenmayer and Luck, 2005). In Chapter 5, I attempted to identify one or more regions of higher than expected species turnover or structural change (which may represent an ecological threshold) on the TREND gradient, as has been described for this study region previously (Guerin et al., 2013). While I detected a threshold in community composition on the transect surveyed by Guerin et al. (2014), this threshold was not evident on the overlapping TREND-AusPlot transect, which extended further in to the arid zone. While the search for thresholds was not a focus in Chapter 4 (leaf carbon isotope responses) or the comparative sensitivity of plant and ant communities to climate change (Chapter 6), the ordination and linear regression methods used did have the potential to reveal disjunctions if present. However, no evidence for thresholds was identified in those analyses.

While ecological thresholds are unambiguously present on some environmental gradients, their detection is dependent on the analytical techniques used to search for them (Andersen et al., 2009, Francesco Ficetola and Denoël, 2009), leading to suggestions that putative thresholds should be confirmed with multiple statistical approaches (Qian and Cuffney, 2012).

Regardless of which analytical approach(es) are used, the ability to detect thresholds will be dependent on the quality of the ecological data used, meaning survey design is of critical importance. Surveys which are not representative of the study region have the potential to erroneously identify false thresholds (type I error) or fail to detect real thresholds (type II error). For this reason, threshold detection should ideally be approached using a spatially expansive, long-term data set that encompasses the known ecological variability of the study region (in both space and time). This approach is possible for regions where large-scale, systematic biodiversity surveys have taken place, but such resources are a rarity and when not available, a gradient-oriented transect design becomes an attractive and cost-effective option.

The stratification of survey times and locations is critical in considering the capacity of transects to inform on the existence of ecological thresholds. Thresholds can occur from scales ranging from metres to hundreds of kilometres. As a result, uneven survey effort may highlight minor ecotones in intensively sampled regions while poorly constraining thresholds at larger spatial scales. This is a potential issue for the TREND-AusPlots transect, which has a comparatively high concentration of sites at its northern extent, and large gaps of cleared vegetation in the mid-north region (Chapter 5). Despite this design limitation, the lack of detection of any thresholds for this transect rules out a type I error. The possibility of a type II error (i.e. false negative) in species composition cannot be overlooked, particularly given that an analysis of a much larger network of Biological Survey of South Australia plots found support for an ecotone between 400 and 600 mm mean annual precipitation (Guerin et al., 2013). It is possible that this ecotone is not readily detectable with substantially fewer sites and may highlight the high sampling intensity that can be required for ecotone detection.

Spatial and Temporal Considerations

Types of bioclimatic gradients

The primary purpose of a gradient approach is to maximise climatic variation (or more broadly, variation in some other environmental variable), while minimising spatial extent. While gradients can traverse any environmental change, the three key types of bioclimatic gradients that are commonly encountered in the scientific literature are latitudinal gradients (such as the TREND), longitudinal gradients (such as the Northeast China Transect (NECT)) and altitudinal gradients.

Latitudinal and longitudinal gradients tend to be spatially expansive, covering hundreds of kilometres and traversing broad climatic change, often from coastal to inland regions. In addition to change in climate, latitudinal gradients also encompass change in day length, which may represent a confounding influence when investigating taxa sensitive to small changes in photoperiod (e.g. mosquitoes (Urbanski et al., 2012)), and/or for very long transects traversing from (sub)polar to equatorial regions and thus massive differences in photoperiod. The <30 minute change in day length across the TREND is unlikely to have significantly impacted any of the biotic variables addressed in this thesis, but could be investigated in future work.

This thesis has largely relied on linear analyses to examine the relationship between environment and biotic response. When using linear techniques, it is important to note that the interpretation of patterns along very long transects should be approached with caution, as they may lose power to inform on the drivers of biotic change once they extend beyond a linear change in climate. For example, the TREND traverses from the high rainfall mediterranean zone in to the arid interior of the continent, and could be extended further north to increase the spatial and climatic extent. However, extension of the TREND north of ~24° latitude would encompass a transition from winter dominated to summer dominated (tropical) rainfall and increased monsoonal influence. The effect of such a long transect would be to effectively have two transitions (moving north, a temperate to arid transition, followed by an arid to tropical transition). Attributing biological change to a simple climatic change across the transect with linear statistics would therefore be problematic.

For this reason, bioclimatic transects should ideally be positioned to follow a simple and linear (i.e. monotonic) climatic transition, rather than multiple transitions. Data from multiple transects can then be integrated together (Caddy-Retalic et al., 2017). Alternately, non-linear approaches, such as Generalised Dissimilarity Modelling (Ferrier et al., 2007) may be used.

Altitudinal transects exploit the rapid change in climate associated with increased elevation (i.e. decreased temperature, increased precipitation), resulting in very compact gradients (potentially only a few hundred metres) that encompass massive environmental change. The major benefit of this approach is that the effect of geographic isolation is much lower than with spatially extensive latitudinal or longitudinal gradients, and trait or species changes across the gradient are more likely to be driven by environment than simply because they are a long way apart. However, like latitudinal transects, altitudinal transects include inherent change in other environmental variables, including air pressure and solar radiation which may confound analyses, particularly for organisms sensitive to gas pressures (e.g. plants and invertebrates).

It is possible to effectively combine multiple transect types in to a transect-network that draws on the strength of each transect type. For example, longitudinal gradients are not affected by changes in photoperiod, and therefore observation across similar climatic space on latitudinal and longitudinal transects may represent a means of isolating the impact of photoperiod on biotic change. Likewise, combining a long transect (>200km) across a mountain range with regularly positioned smaller altitudinal transects (<1km) could allow environmental and spatial distance to be disentangled and would represent a powerful approach to improving knowledge of the climatic drivers of biotic change.

Temporal considerations and space/time substitutions

Analyses of spatial gradients generally assume that the biota are temporally static. However, ecosystems change temporally, with both stochastic and directional processes altering the biodiversity present over time. It is difficult to appreciate the magnitude of these stochastic and directional processes (which are likely to affect different taxa, ecosystems and regions differently) without repeated measurements, which are beyond the scope of most studies. This is true for any ecological investigation, but holds particular relevance for gradient studies for which there is an interest in using space as a proxy for time (Pickett, 1989, Blois et al., 2013).

In the absence of temporal data for most systems, the concept of measuring biotic change over space to inform on biotic change at a location over time is attractive, particularly given the ongoing effort to understand the impacts of climate change on ecosystems. There are two major limitations to this approach. The first is our ability to constrain biotic variability as a response to environment, and the second is establishing equivalence between the environmental change that occurs over space and over time.

Associating any biotic response with the accompanying environmental change is a reasonably straightforward process. In Chapter 6, I was able to explain nearly two thirds of variation in plant assemblages and over half of variation in ant assemblages with a small number of climate and soil variables. However, while this biotic response may indeed be driven by those environmental variables, there may be a raft of other drivers and environmental filters which have shaped the biota to coincide with an environmental gradient. Such drivers could include ancient or recent processes include fire, glaciation, species introductions, landscape fragmentation, or unaccounted for environmental change (e.g. grazing pressure), as well as the effects of biotic interactions (Wisz et al., 2013, Ovaskainen et al., 2017). It is also unknown to what degree biotic change is related to the variation in a single environmental variable (e.g. mean annual precipitation) or a potentially complex combination of variables (e.g. mean annual precipitation; maximum temperature of the warmest month, soil nitrate content and aspect). Therefore, an unknown degree of uncertainty is introduced to the biodiversity response model that cannot be readily constrained.

The degree to which spatial environmental change parallels temporal change is also difficult to determine. For much of South Australia, we expect a general climatic trend of increased temperatures and increased rainfall variability (together having an aridifying effect) (CSIRO and Bureau of Meteorology, 2015). This pattern does occur across the TREND spatial gradient, but the degree to which the future climate of a location on the TREND can be matched by a simple move north is uncertain. This uncertainty is related to the inherent uncertainty of climate models, both in terms of their ability to represent global circulation models, and our projections of atmospheric greenhouse gas concentrations. While we expect climate to shift, landscape and soil variables are not expected to change rapidly. The movement of climate but not soil will result in novel environmental conditions for most locations, making it difficult to predict the biodiversity response.

Above, I have considered the degree to which space may provide a proxy for predicting future change. Predicting, or hindcasting, past change is much more straightforward, and it is certainly more feasible to conduct a time-scale study and then relate it to a spatial analogue. While this process would be interesting, there is less of an imperative to explain environmental change in the recent past, which may explain the lack of interest in exploring this question to date.

In summary, while it is possible to draw some parallels between change in space and time, incomplete knowledge of how biota respond to a complex mix of environmental variables and the degree to which future environmental change will mirror current spatial change, makes anything further than generalised predictions fraught with uncertainty.

Key findings

This study sought to "investigate the utility of spatial bioclimatic gradients in understanding the environmental drivers of within-species, species and community level biotic change" (Chapter 1). In Chapter 2, I argued that transect-based research can be strengthened through replication of observations on multiple transects, and the observation of multiple taxa on single transects. The subsequent chapters demonstrate this approach through a) the replication of the same observations on spatially isolated transects (Chapter 4), b) surveying spatially overlapping gradients with different methodologies (Chapter 5) and c) surveying multiple taxa on the same gradient to investigate concordance/discordance amongst different groups in response to environmental change (Chapter 6).

Chapter 4 provided a case study which used three subcontinental scale transects to empirically test the universality of the relationship between precipitation and leaf carbon isotope ratio. The study indicated that while there is a general pattern of increasing carbon isotope discrimination with moisture availability (Farquhar et al., 1989, Cernusak et al., 2013), the magnitude of this change varied substantially between different species and gradients, and was not consistent between growth forms. Therefore, Chapter 4 demonstrated that the "universal scaling relationship" proposed by Prentice et al. (2011) lacked empirical support. The study also demonstrated that by observing a trait (leaf carbon isotope ratio) that can be measured irrespective of species identity, it is possible to expand from a model of change established on a single transect (e.g. Prentice et al. (2011) and Dong et al. (2017)), those models can then be tested on other gradients to allow the model to be validated or falsified (Cadotte et al., 2011).

In Chapter 5, I examined vegetation change on the same environmental gradient using two overlapping transects. I determined that the ability to detect a previously identified plant community ecotone is dependent on the scale of the gradient, with a disjunction apparent in the spatially restricted transect, but not when the transect was resurveyed with a different methodology and extended a relatively short distance (ca. 100 km). This result highlights the importance of conducting analyses of environmental change at a scale appropriate to the environmental change present in the study system of interest. Determining an appropriate spatial/environmental scale is likely heavily dependent on the study system and may only be determinable through empirical investigation. However, work presented in this thesis and analyses of other Australian transects (e.g. the South West Australian Transitional Transect (Gibson et al., 2017) and North Australian Tropical Transect (Williams et al., 1996) indicates that several degrees of mean annual temperature and/or several hundred mm of mean annual precipitation (equating to hundreds of kilometres) is appropriate for the detection of major vegetation ecotones.

In testing the consistency of climate sensitivity between ant and plant assemblages on the TREND (Chapter 6), I demonstrated that despite similarity in current compositional patterns, ant assemblages are likely to have far greater sensitivity to future environmental change. This finding suggests that we are likely to observe an ecological “decoupling” of plant and ant assemblages under climate change, with the potential for a significant loss of ecosystem function through lost biotic interactions. The uneven sensitivity between flora and ant fauna demonstrates the value in testing environmental responses of multiple taxa to investigate the potential impacts of climate change on ecosystem function through altered species associations.

Each of these four case studies (Chapters 2, and 4–6) demonstrated the potential for single-transect studies to be augmented to improve the veracity and relevance of gradient-derived models beyond one specific study system. Together, these studies provide a range of analytical approaches in which effective use of the survey effort can be achieved. The major innovation of this work was to demonstrate that with careful design, traditional gradient-based approaches can be improved to bolster their inferential power and effectiveness in detecting and explaining change within species and ecological communities. These approaches can be united to provide a coherent and efficient platform from which to explore ecosystem responses to climate change.

Considerations for future work

Methodological considerations

Survey methodology is, as always, an important consideration for any ecological study (Kent, 2011). Species are detected by searching or sampling each site, and the percentage of species detected will be a function of the thoroughness of the search/sampling effort. For plants, this is a straightforward process, and over small areas, few species are likely to be overlooked. A comparison between the two vegetation survey methods used (AusPlots and the TREND-Guerin method) is discussed in Chapter 5, but differences in plot size and nestedness may alter pattern detection. Smaller plots tend to produce less stable ordinations than larger plots, primarily due to small-scale differences in vegetation patterning overriding a larger environmental signal (Otypková et al., 2006). Such effects are particularly strong with very small plots (i.e. <100 m^2) and in areas with low β-diversity (Otypková et al., 2006). Plot size was comparatively large for both TREND-Guerin (900 m^2) and AusPlots (1 ha), and β-diversity was high (>0.95; Chapter 5), indicating plot size is unlikely to have had a substantial influence on pattern detection for most of the transect. It is possible that plot size may have had an impact in the drier Stony Plains sites, where vegetation was relatively sparse (i.e. <40% total cover; Chapter 5), but only AusPlots surveys were undertaken at these locations.

Survey methodology is likely to have had a larger impact on the characterisation of ant assemblages. There are several methods available for sampling ants, including pitfall trapping, hand trapping, Winkler litter extraction and baited traps (Delabie et al., 2000), and the efficacy of each method for providing a sample representative of the local ant fauna is dependent on the habitat type and type of ant fauna present (Lopes and Vasconcelos, 2008). To thoroughly survey the species present in even a small plot, it is likely that a variety of methods and extended period of time will be required (Agosti and Alonso, 2000). For this reason, pitfall trapping is commonly used as a “general purpose” approach that allows rapid detection of many species, facilitating assessment of diversity and biogeographical analysis, and comparison of results across the scientific literature. Nevertheless, differences in trap size, baits, preservative and trap arrangement can bias the taxa collected (Schirmel et al., 2010, Hancock and Legg, 2012).

The surveys in Chapter 6 were undertaken with unbaited, uncovered traps left in the field for 48 hours. While the ant species accumulation curve indicates that we were able to sample the majority of the ant fauna present, this may only represent a fraction of species readily caught in pitfall traps over a short period. Species detection is related to trapping period, with one study finding that most species are detected within 48 hours, but some species may only be captured after several days (Borgelt and New, 2006). Additionally, some species are very recalcitrant to standard pitfall trapping and may only be detected through specialist traps designed to detect cryptic species (Schmidt and Solar, 2010).

Finally, all plant and ant surveys were undertaken in a single visit, which certainly prevented our detection of some species. In addition to providing additional search time, repeated surveys would have increased the potential of less dominant ants and ephemeral plant species (i.e. annual herbaceous species; such as orchids).

Ultimately, resource constraints make elaborate or prolonged field campaigns difficult, particularly over large study regions, but it is reasonable to assume that our sampling primarily represents the most dominant and/or easily trapped species, and that including other survey techniques and/or leaving traps in the field for longer would have yielded more complete sampling of the regional ant fauna. Ongoing work on the TREND (including DNA metabarcoding of soils) may facilitate surveys with different methodologies in the future which may yield a more complete picture of both plant and ant biodiversity.

Multiple drivers of change

Most of the work in this thesis addresses the degree to which biota respond to changes in climate, but there are several factors that might limit the detection of a climate signal. One of the primary factors is non-climatic environmental change, including edaphic (soil chemistry and structure, lithology, etc.) and land use change (including fire regime, grazing pressure, ecosystem fragmentation and altered hydrology).

A suite of biotic drivers can also drive ecological change. Introduced species including plants (e.g. buffel grass (*Cenchrus ciliaris*) and olives (*Olea europa*)) herbivores (e.g. rabbits (*Oryctolagus cuniculus*) and goats (*Capra aegagrus*)) predators (e.g. cane toads (*Rhinella marina*) and cats (*Felis catus*)) and pathogens (e.g. chytrid fungus

(*Batrachochytrium dendrobatidis*) and myrtle rust (*Uredo rangelii*)) can alter species composition through competition, herbivory, predation or pathogenesis. Such impacts can occur in concert with climate and land use impacts to lead to greater cumulative impacts or “threat syndromes” (Burgman et al., 2007). The cumulative impacts of climate change, altered hydrology and fire regimes and introduced pest species has led to the southwest mediterranean zone being listed as one of Australia’s most vulnerable ecosystems (Laurance et al., 2011).

Finally, there may be a biotic lag in the response of many species to extrinsic change (Chapter 6). Populations might not be able to adapt quickly enough to maintain phenotypic fitness, particularly in situations where there is low standing genetic diversity or barriers to gene flow (e.g. fragmented populations). This “adaptation lag” could eventually lead to extirpation, particularly if combined with other extrinsic threats such as increased competition from invasive species, new pathogens or changed fire regime (Aitken et al., 2008). Additionally, some long lived species can persist for decades despite their environment becoming hostile enough to prevent ongoing recruitment (Jackson and Sax, 2010, Talluto et al., 2017). As with adaptation lags, such “extinction debts” might not be immediately obvious, and if not detected, the magnitude of biotic response to environmental change is likely to be underestimated.

Understanding that climatic and non-climatic drivers can independently or cumulatively impact on species composition and ecosystem function in space and time is critical to modelling biotic responses to climate change. If the impact of non-climatic drivers is not effectively isolated from change models, responses may be hard to constrain in the context of the environmental gradient of interest.

The power of using bioclimatic gradients

Climate is understood to be a main driver of species distribution and influences the composition of species within communities and their dynamics and function within ecosystems.

Understanding how biodiversity changes in time is a vexed issue. Governments, together with research institutions have made major investments in ecological surveys to answer the question “what biodiversity do we have?”. In Australia, this investment has come through programs such as the Biological Survey of South Australia (BSSA) (Armstrong et al., 2003) and Bush Blitz (Preece et al., 2015) for terrestrial systems, with similar investments such as the Marine Biodiversity Hub for marine systems (Butler et al., 2010). These programs necessitate extensive field campaigns and resources, requiring substantial investment from governments. This investment is justified by the dual ambitions of facilitating exploration and providing a baseline against which future change can be measured.

Establishment of a baseline condition (i.e. the state from which any change is measured) is critical to drawing any kind of inference on the “trajectory” of one or more ecosystems. Unfortunately, interest in a particular region or species often peaks in response to some type of perturbation, at which point it is impossible to go back in time and collect baseline data from a pre-impact state (Pickett, 1989). Once a baseline has been collected, however, its value is limited without ongoing time-series (monitoring) data. It is this post-baseline data that is most often lacking. Once the original “discovery” phase has been completed, there appears to be reluctance to invest in ongoing monitoring. The reasons for this are complex and relate to cost, competing priorities and political interest. The need for this data is strong, however, and has been underlined by a string of publications over the last three decades (e.g. Hinds, 1984, Lindenmayer et al., 2012b).

Despite continued predictions of biodiversity shifts due to climate and land use change in southern Australia (Brereton et al., 1995, Williams et al., 2009, Pecl et al., 2017), there has been no systematic resurvey of the BSSA plot network since its establishment. The need for monitoring data is clear: a lack of coordinated, long term ecological data has been regularly cited as one of the primary limitations in an ability to track the condition of Australia’s ecosystems (e.g. Jackson et al., 2017). An inability to measure the trajectories of important ecosystems limits our understanding of the impacts of climate and other stressors are having on these systems, and thus stymies our ability to effectively intervene or manage those systems.

Ecological monitoring is time consuming and expensive and must be targeted in order to be cost effective (Nichols and Williams, 2006, Lindenmayer et al., 2012a). The survey methodology used for monitoring is also likely to be different to that for biodiversity discovery, as the former should include considerations to minimise observer bias (Milberg et al., 2008) and be sensitive to relatively small ecological change (Reynolds et al., 2011).

The regular and ongoing resurvey of large plot networks such as the BSSA (which comprises 15, 615 sites), would require a substantial ongoing commitment, which may be beyond the capacity of the management agencies involved. An alternative approach would be to identify key gradients within the survey plots, which could be used to identify a small subset to be targeted for ongoing monitoring. For example, an analysis of 3,567 BSSA vegetation plots on the Adelaide Geosyncline identified a putative arid-mesic ecotone that was predicted to be sensitive to future climate shifts (Guerin et al., 2013), and was a key result in justifying the ongoing development of the TREND. Identifying a small number of similar gradients across the BSSA network through analysis of existing baseline data, together with likely ecological stressors (e.g. fragmentation, dryland salinity, desertification, change in fire regime, etc.) would effectively allow a subset of the BSSA network to be prioritised to focus on those areas most likely to change. If combined with the development of an appropriate monitoring methodology that would allow comparison with the existing baseline but focus on robust change detection, a strong monitoring program could be achieved with a much smaller ongoing investment and would be suitable for any large plot network.

Implications for monitoring and management

Ecological management is generally focused on maintaining biodiversity and ecosystem function within a region. The standard approach for this is to conduct baseline surveys and establish ecological values that are to be conserved (e.g. the presence of one or more species or communities, or the maintenance of ecosystem services such as carbon sequestration (forests) or protection from storms (reefs and mangroves). Resources to undertake baseline surveys are usually limited, prohibiting the widespread, intensive field surveys that would be required to thoroughly document the biodiversity of a large area. The gradsect approach of positioning survey transects across one or more environmental gradients allows the greatest environmental range to be covered with a given number of plots, which represents the most efficient survey design when time and/or money resources are limited (Chapter 2; Austin and Heyligers, 1991).

Gradsects used for biodiversity discovery can be repurposed as transects which allow the rate of biotic change to be associated with the gradient as discussed earlier. Models of change can be developed for taxa of interest at a trait- species- or assemblage- level. Repeated measurement of the transect/s over time can then form the basis of a cost-effective monitoring strategy.

The value of transects as research infrastructure and future priorities

The Australian Transect Network (ATN) was established to facilitate:

> *"the study of ecological structure and processes over major biophysical gradients to document ecological change and adaptation in relation to climate variation across Australia's major terrestrial biomes. The ATN's primary focus is observations and monitoring of natural and semi-natural terrestrial ecosystem gradients, generating data and products to enable researchers to predict how species and ecosystems will change in the future."*
>
> (Rodrigo and Andersen, 2016).

In order to maximise the value of the ATN (or other investments in transect infrastructure) in furthering these aims, it is useful to review the strengths of the transect approach and identify areas of potential future investment which may further bolster transect platforms for advancing our understanding of biotic responses to environmental change.

By providing a spatially explicit platform on which to examine the effect of environmental drivers on the biota, transects can be used to test biological hypotheses in the real world. For example, the universal scaling hypothesis of leaf carbon isotope response to aridity (Chapter 4) and the leaf nitrogen coordination hypothesis (Dong et al., 2017). Co-locating many such investigations on one or a small number of gradients allows those systems to become better characterised over time, increasing their usefulness for new research as well as providing opportunities for time-series studies. Existing surveys of the flora and ant fauna on the TREND (Chapters 5 and 6) and other ATN transects have provided impetus to characterise the bacteria, fungi, archaea and eukaryotes present in the soil microbiome (Bissett et al., 2016) to determine if they display similar biogeographic patterning to other taxa and improve our capability to accurately forecast biological responses to climate change.

Ideally, common measurements conducted on multiple transects should be undertaken that can be independent of species identity. Such common measurements could include observations of species composition for different groups (e.g. plant, invertebrate surveys), as well as infra-species traits such as leaf traits (δ^{13}C, specific leaf area, etc. which can be undertaken on any vegetation community).

The utility of bioclimatic transects to predict change in ecosystems is limited by the types of transitions that are studied. For example, the TREND traverses the mediterranean to arid zones and is therefore potentially useful for studying the aridification of mediterranean systems. To be able to generalise across many biome transitions, observations repeated on multiple gradients are required (e.g. tropical to arid, temperate to subarctic, etc.), and to be most useful, a network of transects should encompass as many different large-scale transitions as possible.

It is possible to use transects across environmental gradients to associate putative environmental (e.g. climatic) drivers with a biotic response and create a model of predicted change. Because many environmental factors co-vary on gradients, it is not possible to gauge the accuracy of models built solely from observational data, particularly when they are extended in to novel conditions (such as those already occurring under climate change). The strength of biotic change models can be improved through manipulative experiments, which allows the disentanglement of single environmental drivers (Chapter 2). The predictions made from these models can then be validated through ongoing monitoring.

The Australian transect infrastructure used for the studies presented in this thesis have been developed by the ATN, which is part of the Terrestrial Ecosystem Research Network

(TERN) (Thurgate et al., 2017). Future investment in Australian transect infrastructure, either through TERN or other means, could profitably focus on: 1) improving common observations on several transects (e.g. plants and ants with the same methodology) to facilitate comparisons between regions and taxa; 2) increasing the spatial coverage of the network to encompass areas of Australia's geographic and climatic space currently unrepresented in the network, particularly in cooler regions such as the Australian alps and Tasmanian highlands that are expected to undergo rapid climatic shifts in the future (Chapter 2, Figure 2); 3) the augmentation of the network with manipulative experiments such as common gardens and reciprocal transplants; and 4) ongoing monitoring of survey sites (e.g. repeated surveys every 1-5 years) to validate and further refine predictions under climate change.

In conclusion, bioclimatic transects represent an efficient and powerful methodological approach to improving our understanding the environmental drivers of biodiversity change in space and time. While gradient-based studies are inherently observational, through careful design, replication and augmentation with embedded experiments, methodological weaknesses can be overcome. Such an approach provides an opportunity for robust scientific study to help build our understanding of future biodiversity change.

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Chapter 8: Appendices

During my PhD candidature, I have contributed to a number of publications which have not been included in the main body of this thesis because they are of peripheral relevance and/or I was not a lead author. These publications are attached in the following appendices.

1. Lowe, A. J. & **Caddy-Retalic, S.** (2014) Will the climate debate end up being fought in court? *The Conversation*
2. Campbell, C. A., Lefroy, E. C., **Caddy-Retalic, S.**, Bax, N., Doherty, P. J., Douglas, M. M., Johnson, D., Possingham, H. P., Specht, A., Tarte, D. & West, J. (2015) Designing environmental research for impact. *Science of the Total Environment* 534 pp. 4-13. DOI: 10.1016/j.scitotenv.2015.11.089
3. Baruch, Z., Christmas, M. J., Guerin, G. R., **Caddy-Retalic, S.**, McDonald, J., Jardine, D. I., Leitch, E., Gellie, N., 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* 42(5) pp. 553-561. DOI: 10.1111/aec.12474
4. Bissett, A., Fitzgerald, A., Meintjes, T., Mele, P. M., Reith, F., Dennis, P. G., Breed, M. F., Brown, B., Brown, M. V., Brugger, J., Byrne, M., **Caddy-Retalic, S.** *et al*. Introducing BASE: the Biomes of Australian Soil Environments soil microbial diversity database. *GigaScience* 5(21). DOI: 10.1186/s13742-016-0126-5
5. Dong, N., Prentice, I. C., Evans, B. J., **Caddy-Retalic, S.**, Lowe, A. J. & Wright, I. J. (2016) Leaf nitrogen from first principles: field evidence for adaptive variation with climate. *Biogeosciences* 14 pp. 481-495. DOI: 10.5194/bg-2016-89
6. Guerin, G., Sweeney, S., Pisanu, P., **Caddy-Retalic, S.** & Lowe, A. (2016) Establishment of an ecosystem transect to address climate change policy questions for natural resource management. South Australian Department of Environment, Water and Natural Resources Technical Report 2016/14.
7. Nielson, K. E., McInerney, F. A. & **Caddy-Retalic, S.** (2017) SWATT Foliar Carbon Isotope Pilot Study. A report for the Western Australian Department of Biodiversity, Conservation and Attractions.
8. *Baruch, Z., **Caddy-Retalic, S.**, Guerin, G. R., Sparrow, B., Leitch, E., Tokmakoff, A. & Lowe, A. J. (in review) Floristic and structural assessment of Australian rangeland vegetation with standardized plot-based survey. *PLOS ONE.*
9. *Howard, S., McInerney, F. A., **Caddy-Retalic, S.** & Hall, P. A. (in review) Modelling leaf wax *n*-alkane inputs to soils along a latitudinal transect across Australia. *Organic Geochemistry.*
10. *Sparrow, B. D., Foulkes, J. N., White, I. A., Wardle, G. M., Leitch, E. J., **Caddy-Retalic, S.**, van Leeuwen, S. J., Tokmakoff, A., Thurgate, N. Y., Guerin, G. R. & Lowe, A. J. (submitted) A vegetation survey method for surveillance monitoring of rangeland environments. *Journal of Applied Vegetation Science*.

*Manuscript not included here as it will form part of another PhD thesis.

THE CONVERSATION
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Will the climate debate end up being fought in court?

July 4, 2014 6.32am AEST

Could politicians and scientists in the future be charged with "climate negligence"? Julie G/Flickr, CC BY-ND

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Society generally has a clear idea of what constitutes a crime, and those in positions of power are usually held to very high standards. Politicians charged with making decisions on the needs of society are held accountable for unprofessional behaviour.

New South Wales Premier Barry O'Farrell, for example, chose to resign in April over a "massive memory fail", after initially denying he had received an expensive bottle of wine from an Australian Water Holdings executive.

Neglecting to take action can also be considered criminal. In the same way that doctors who fail to diagnose an illness may be charged with malpractice, politicians can face similar charges for failing to adequately do their jobs.

These crimes may seem more clear-cut – but what happens when it comes to accountability for environmental issues, and more specifically, climate change?

Predicting disasters and legal risk

When government action or inaction leads to the direct harm of citizens due to environmental risks and natural hazards, they should be held to account.

This logic saw residents of New Orleans sue the United States government for damages caused by flooding associated with Hurricane Katrina, after a federal judge ruled the US Army Corps of Engineers displayed gross negligence by failing to maintain a shipping channel next to a levee protecting the city.

In another case in 2009, seven scientists and civil servants were convicted of manslaughter after failing to give adequate warning of an impending earthquake in L'Aquila, Italy, that killed 309 people.

We are yet to see if and how politicians and scientists will be held accountable for increased greenhouse gas emissions leading to climate change. But a recent area of legal development is arising in this area, known as climate legal risk, defined as the risk of liability or adverse legal outcomes arising when the impacts of climate change (such as flooding, bushfire and coastal hazards) affect an organisation's operations.

"Unacceptable impacts from predicted climate change" has been used to reject planning applications. In 2010 the Victorian Civil and Administrative Tribunal rejected a proposal to subdivide a coastal property for development due to predictions that the land would be inundated within a century. The case marked a critical point in planning law and sent an important message to coastal planning decision makers about the increasing relevance of climate-related flooding.

In another case brought to the courtrooms by environmentalist Pete Gray, the Land and Environment Court of New South Wales found that the approved expansion of the Anvil Hill Coal Mine had failed to properly assess the greenhouse gas pollution impacts of the future use of mined coal.

The most recent Intergovernmental Panel on Climate Change report paints a bleak picture of what will happen if we continue to pump greenhouse gas emissions into the atmosphere. The risks of extreme weather, droughts, floods, cyclones and marine inundations are all significantly increased.

Currently, governments and mainstream politicians that openly dispute human-caused climate change are rare. What is far more prevalent is a lack of meaningful action in government to combat it.

But with the IPCC so clearly stating the need for action, there is now the very real risk that politicians, media outlets and scientists could face legal prosecution for their role in delaying action that could have saved properties, livelihoods and lives.

A broader international criminal framework identifying destruction of ecosystems, including through increasing greenhouse gas emissions, has been developed and termed "ecocide", though it has yet to be legislated.

Should scientists be held accountable for inaction?

As the number of climate change related extreme events increase, we need to ask who should be held accountable for them. As we saw in L'Aquila, some believe that at least some of the responsibility falls on scientists. Perhaps it is the role of scientists to ensure that climate change warnings (such as those made by the IPCC) lead to actions like evacuation of natural disaster areas and meaningful policy change.

Scientists don't have the power to make decisions in government or society. They are funded as researchers and experts, to advance knowledge and advise our elected officials. Scientists can only control what they say, and the urgency that they attach to it; not what is done with that advice.

But, like other people, scientists can be prone to hyperbole. Scientists have been criticised for overdramatising the consequences of inaction with regards to climate change, which can be overwhelming and may lead to a paralysis of action - a situation termed "climate fatigue".

Transforming scientific research into policy is a messy process. It requires a range of scientific, communication and change management skills, the combination of which most scientists do not possess, and perhaps should not be expected to. However as we have seen, individuals and groups can be held accountable for inaction that leads to disastrous outcomes, and neither climate scientists or policy makers are likely to get a free pass.

Avoiding lawyers at 50 paces

In most situations, legal action comes only as a last resort when all other avenues of communication have broken down. And so in the climate debate, lawyers at 50 paces may only further inflame and entrench positions.

The climate issue needs leadership, not recrimination. We need leadership from scientists who can move from proclaiming the problem into practical uptake of solutions.

Likewise, leadership is needed from elected officials, who need to start working with the scientific community they have supported to develop evidence-based policy.

We need leadership from industry, to start engaging with the climate debate. And in the run up to the United Nations Climate Summit set for September 2014 in New York and further talks in Paris next year, we need global leaders to step up to help move society to the next phase of climate action.

In the future, it will not have been enough of a defence to say that climate change inaction was a result of lack of evidence. We have the evidence and we know that we should act. If we do nothing now, future generations may take a legal perspective on our actions, or lack of them, bringing to The Hague a retrospective crime against humanity – climate negligence.

The authors would like to acknowledge the valuable contribution of Tim Vines in discussion of the ideas behind this piece.

Climate change Law IPCC Planning United Nations Barry OFarrell IPCC Fifth Assessment Report international climate action Hurricane Katrina climate risk Climate change mitigation Climate talks liability

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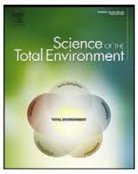

Designing environmental research for impact

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HIGHLIGHTS

- This paper explores how environmental research can be more influential.
- Transdisciplinary research means researchers working with end users.
- Funders, researchers and end users have a shared stake in successful outcomes.
- Research is most likely to be influential when all three groups have shared goals.
- Mutual trust, continuity of personnel and adaptive capacity are key success factors.

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ABSTRACT

Transdisciplinary research, involving close collaboration between researchers and the users of research, has been a feature of environmental problem solving for several decades, often spurred by the need to find negotiated outcomes to intractable problems. In 2005, the Australian government allocated funding to its environment portfolio for public good research, which resulted in consecutive four-year programmes (Commonwealth Environmental Research Facilities, National Environmental Research Program). In April 2014, representatives of the funders, researchers and research users associated with these programmes met to reflect on eight years of experience with these collaborative research models.

This structured reflection concluded that successful multi-institutional transdisciplinary research is necessarily a joint enterprise between funding agencies, researchers and the end users of research. The design and governance of research programmes need to explicitly recognise shared accountabilities among the participants, while respecting the different perspectives of each group. Experience shows that traditional incentive systems for academic researchers, current trends in public sector management, and loose organisation of many end users, work against sustained transdisciplinary research on intractable problems, which require continuity and adaptive learning by all three parties. The likelihood of research influencing and improving environmental policy and management is maximised when researchers, funders and research users have shared goals; there is sufficient continuity of personnel to build trust and sustain dialogue throughout the research process from issue scoping to application of findings; and there is sufficient flexibility in the funding, structure and operation of transdisciplinary research initiatives to enable the enterprise to assimilate and respond to new knowledge and situations.

1. Introduction

Human society faces a number of 'grand challenges', several of which arise from the relationship between people and the environment. These include climate change adaptation and mitigation, food security,

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energy and water security, habitat loss and species extinctions, pollution, and the spread of weeds, pests and diseases.

These and other 'wicked problems' (Brown et al., 2010) are characterised by technical complexity and often uncertainty, large scales in space and time, a mix of social, economic and biophysical drivers, abundant but disparate and heterogeneous data, and contested issues among diverse stakeholders. The nature of such contest is itself important: it may be rooted in conflict over values and norms, and/or uncertainty in the data. Notwithstanding complexity, uncertainty, risk and conflict, on such issues there is nevertheless typically a need for governments, industries and communities to make a choice, reflected in decisions and actions. Such choices are often negotiated, often messy rather than clear-cut, and for most environmental issues the choice to do nothing (whether made actively or by default) also has environmental consequences.

A key response to such environmental challenges is to invest in applied research, which the Australian Bureau of Statistics (1998) defines as '*work undertaken primarily to acquire new knowledge with a specific application in view*'. The nature of these challenges is such that they can rarely be comprehended satisfactorily within a single scientific discipline, or indeed by science alone. There is a significant literature on the conceptual challenges associated with multi-, inter- and trans-disciplinary research (Fry, 2001; Klein, 2008; Gibbons et al., 2008; Bammer, 2013), and on the imperative for new ways of organising research — e.g. 'Mode 2' research and 'Post-normal science' (Funtowicz and Ravetz, 1993). Less has been published about the practice of working with end users to design and organise multi-institutional environmental research to tackle large scale, long-term environmental problems, based on analyses of current and past experience (Campbell and Schofield, 2007; Tress et al., 2005a, 2005b).

Australia has invested significantly over the last twenty years in organising applied research collaborations at national scale, including the Cooperative Research Centres programme (Allens, 2012), Rural Research and Development Corporations (Productivity Commission, 2011), and Centres of Excellence funded by the Australian Research Council and the National Climate Change Adaptation Research Facility (NCCARF, 2014).

This paper briefly reviews what we mean by transdisciplinary research, then discusses the findings of a participative, 'structured reflection' involving researchers, funders and end users of successive national environmental research initiatives in Australia, adapting an analytical framework developed by Roux et al. (2010).

Table 1
A framework to guide co-reflection on progress in transdisciplinary research programmes that incorporates the accountabilities of funders, researchers and end users (after Roux et al., 2010).

Functional domain	Accountability indicators
Funders of research	Strategic planning and leadership Continuity and scientific competency Discourse between funders, providers and users to ensure effective programme goals and model Flexibility to adjust programme model and goals to meet research provider and user needs Adaptive learning
Providers of research	Professionalism Knowledge sharing Relevance to end-user needs Capacity building Research excellence
Users of research	Capacity for adoption Adaptive decision-making and policy revision Continuity of personnel Co-location of personnel Capacity to build upon emerging research

2. Transdisciplinary research

Roux et al. (2010) propose a "framework for participative reflection on the accomplishment of transdisciplinary research programs". They distinguish between post-normal science (Funtowicz and Ravetz, 1993; Francis and Goodman, 2010), sustainability science (Clark and Dickson, 2003; Burns and Weaver, 2008), and interdisciplinary studies (Newell, 2001; Repko, 2008), while noting 'considerable overlaps of purpose' between these approaches and the key point that all purport to complement, rather than replace traditional disciplinary research. Transdisciplinary studies incorporate elements of all these approaches in applying insights and tools from different disciplines, explicitly embracing complexity and uncertainty, acknowledging multi-stakeholder perceptions and values, in addressing problems that are 'user inspired and context driven' (Roux et al., 2010). A key feature of transdisciplinary research thus defined is the engagement of non-scientist stakeholders — in particular the end users of research — in the research enterprise (Roux et al., 2010):

> *"A key characteristic of transdisciplinary research is that the domains of science, management, planning, policy and practice are interactively involved in issue framing, knowledge production and knowledge application."*

Accordingly, Roux et al. (2010) suggest that there are three key groups of stakeholders in transdisciplinary research: researchers, end users of research, and funders of research. While all three groups may have shared broad goals to *acquire new knowledge with a specific application in view* they are likely to have different perspectives on those goals and how to achieve them, and to define success in different ways. Roux et al. (2010) propose a framework that sets out different accountabilities for the three 'functional domains' of funders, researchers and end users, as in Table 1 below.

More detail explaining each of these accountabilities is set out in Roux et al. (2010) who caution that these are not proposed as definitive or comprehensive, but to serve as a departure point from which this framework could be modified in the context of a specific research initiative.

3. Australia's national environmental research programmes

The Roux et al. (2010) framework was seen to be ideally suited for use as an analytical lens to distill lessons for the design and management of collaborative, multi-institutional applied environmental research from the experience of national environmental research programmes sponsored by the Australian government.

The key process in the application of the Roux et al. (2010) framework was a 'structured reflection' workshop such as the one involving the authors of this paper in April 2014. The workshop participants between them had well over one hundred person years of experience in leading and/or funding multi-institutional, transdisciplinary research programmes, with total investment exceeding $500 m. The workshop was further informed by an on-line survey of 500 participants with experience in the programmes. Each respondent was asked to self-identify as a researcher, research funder or end-user/stakeholder. A response rate of around 9% was obtained, of whom 57% claimed to be researchers, 11% research funders, and 32% were end-users and/or stakeholders. Several respondents identified with more than one role.

The two research programmes analysed in depth at the workshop were the Commonwealth Environmental Research Facilities (CERF) programme, which was initiated by the Australian government environment ministry in 2006, and subsequently evolved into the National Environmental Research Program (NERP) from 2010. The $160 m CERF programme was evaluated by Urbis (2010). The $154 m NERP programme is described by DEWHA (2010) and was evaluated by Spencer et al. (2014). Both programmes were designed to meet the perceived knowledge needs of the environment portfolio, and to tackle issues that were not being adequately addressed by research investments through other government programmes.

The CERF programme commenced with a national call for research proposals against a programme prospectus. Well over one hundred proposals were evaluated on merit by an independent, expert reference group that recommended a suite of investments to the Minister for the Environment, including individual research projects, 'hubs' (clusters of research projects focused on particular problems/themes/ecosystems) and fellowships. The NERP programme drew on the experience and the evaluation of the CERF programme (Urbis, 2010) in having a competitive national Expression of Interest process against broad research priorities, but then focusing its investment primarily around five research hubs, all of which evolved out of successful antecedents in the CERF programme (Appendix A).

As of March 2014, almost 560 researchers from 53 organisations and many more end users had participated in NERP projects, many of whom were also involved in the preceding CERF programme. Unfortunately, there was not a seamless transition from CERF to NERP, but rather a significant hiatus in funding during which some researchers who had been funded through CERF moved on to other roles. In the transition from CERF to NERP, the federal environment department sharpened its focus to concentrate on biodiversity conservation and management, and framed itself more explicitly as the key client and end-user of the outputs of the programme. The NERP programme was thus expected to inform policy development and programme implementation within the federal environment department first and foremost. However, the programme was supported by an equivalent level of co-investment from other research users and partners, including other departments, governments (at state and local levels), industries and communities, who also expected useful outputs from the research relevant to their interests.

The ability of the five NERP hubs (Appendix A) to respond to the needs and interests of their research users meant that they evolved subtly different structures and *modus operandi*. Three had a strong and extensive geographic focus: the Tropical Ecosystems hub focused on the Great Barrier Reef, its rainforest hinterland and the Torres Strait; the Marine Biodiversity Hub focused on Australia's marine territory; and the Northern Australian Biodiversity hub focused on Northern Australian aquatic and terrestrial systems. These foci largely determined their research users and stakeholder groups, and resulted in a combination of bottom up self-organisation around specific research issues and top down coordination to resource and deliver large, complex research programmes. The Environmental Decisions hub worked in partnership with a wide range of research users in the public and private sectors across the country, identifying discrete research topics through focused workshops after which small teams worked with end users on projects of varying duration from several months to several years. The Landscapes and Policy hub identified several regions as case studies, with biophysical and social researchers working in interdependent teams on questions defined by the management agencies in each region.

Aligned with a general trend over the past twenty years for increased participation across all sectors in environmental management (Holley, 2010), the environment department outlined five key design parameters for strengthening links between researchers and policy makers (Box 1).

The five current NERP hubs now constitute a considerable body of experience and expertise in multi-institutional, transdisciplinary research collaborations focused on contemporary challenges in environmental science, policy and management. All NERP hub directors, plus senior representatives of funders and end users, participated in the ACEAS workshop.

Lessons emerging from each of the hubs and the insights of their directors are elaborated further below. While the NERP hubs were all selected against the same national prospectus and funded by the same government agency against the same overall objectives, guidelines and accountability measures, it is notable that each developed in quite different ways. All now have distinct and markedly different identities and *modus operandi*, yet the recent evaluation found each to be effective against both hub and programme level objectives. This suggests that there is no single 'magic bullet' formula for designing a successful collaborative applied environmental research programme. Rather, programme design, management structure and research practice should respond to the specific ecosystem/issue, mix of stakeholders and end users and the nature of their knowledge needs, cognizant of the history of research investment in that context.

Box 1
Design parameters for the NERP programme to improve linkages between research and policy.
Excerpt from DIISRTE (2012).

NERP builds on the Commonwealth's experience in implementing and evaluating the previous Commonwealth Environment Research Facilities programme, and includes increased focus on mechanisms to ensure improved delivery to the end-users of funded research, particularly in government for evidence-based policy.In support of this objective, the programme reflects best practice principles for strengthening the links and alignment between research and the needs of policy makers:

- *involving policy makers in the framing of research questions*: NERP programme guidelines and research priorities are based upon consultation across the department, with a selection panel involving both researchers and departmental representatives then working through a two-stage process to allow for the further refinement of proposals.
- *specific focus on knowledge brokering and translation*: programme guidelines require that 10% of the funding for each hub must be devoted to communication and knowledge brokering activities – the programme also acknowledges that effective translation requires integration – across research disciplines and of new and existing knowledge.
- *facilitating access to research*: in addition to other communication efforts, all NERP-funded research outputs must be made freely and publicly available to allow their use by a broader range of decision-makers.
- *enhancing mutual understanding*: the programme also supports enhanced two-way engagement through mechanisms such as the identification of departmental end-users and contact officers for each hub, short-term secondments for researchers into the department and the 'pairing' of researchers and policy staff.
- *innovation in evaluation*: the NERP monitoring and evaluation strategy requires regular reporting on the usefulness of research in policy, with a mix of quantitative and qualitative measures employed.

Common challenges of linking research and policy remain, such as differing timelines and time pressures, and particularly the reward structures within which research and policy staff work, which often do not explicitly value the types of activity outlined above.

Acknowledging the importance of context in shaping local responses, we nevertheless contend that principles of good applied environmental research practice emerge across all hubs. The following section attempts to elucidate these using the framework proposed by Roux et al. (2010), focusing on the five NERP hubs that originated in the CERF programme, summarised in Appendix A.

4. The relative accountabilities of researchers, funders and end users in transdisciplinary research programmes

In using the Roux et al. (2010) accountabilities as a lens through which to reflect on the experiences and achievements of the five hubs, we involved a mix of researchers, funders and end users, both in the survey and the workshop. As suggested by Roux et al., we also monitored the utility of the framework during this reflection, and identified potential improvements.

Roux et al. (2010) cluster the accountabilities according to the functional domain (funders, researchers, end users) primarily responsible for their realisation. This implies that there could be shared accountabilities across domains, but this is not the impression conveyed (Table 1). We contend that multi-institutional, transdisciplinary research is a shared enterprise across funders, researchers and end users. All three domains have important roles to play, and most of these are shared responsibilities. The ultimate performance measure for such research is the generation of useful and relevant new knowledge that is applied by end users, resulting in a net environmental benefit that exceeds the cost of the research. It is very difficult for this to be realised, and it is not genuinely transdisciplinary research if any of the three domains is disengaged or discharges their responsibilities poorly.

Reflecting the conceptual framework of a shared enterprise, at the workshop we assigned a simple 3, 2 or 1 score to the degree of responsibility a given domain has for a given accountability (with 3 being most important), and we also modified the Roux et al. (2010) accountabilities slightly to better fit the NERP context, splitting some, combining others and deleting 'co-location'. The consensus view of the researchers, funders and end users involved in the April 2014 workshop produced a modified version of the Roux et al. (2010) framework.

These weighted accountabilities are illustrated in Fig. 1, enabling a visual comparison across the three domains.

The accountabilities seen as important for all three groups were leadership, engagement and discourse. All participants in collaborative transdisciplinary research need to demonstrate leadership and to remain engaged and actively communicating throughout the research process. Successful leadership and engagement require that each domain is able to understand and explain its own needs and potentials in ways that can be related to the needs and/or potentials of other domains.

The leaders of NERP-funded research hubs felt that it is important that funding agencies maintain sufficient continuity in staffing to be intelligent purchasers, able to 'take the long view' and undertake high quality strategic planning and adaptive management at a research programme level — responding to changing circumstances and priorities as necessary, but no more than necessary. Research funders need competent project management systems, extending to management of data, information and the knowledge 'legacy' from concluding research programmes. They need sufficient scientific capacity to be able to evaluate research proposals and to compare the track records of competing research providers, but not to the extent of second-guessing researchers once programmes and projects are contracted.

Researchers' accountabilities emphasise scientific competence, relevance, willingness to engage in two-way knowledge sharing and to respond to the needs of end users, competent project management and underpinning the quality of their research through publishing in strong journals, in addition to communication designed to be meaningful for end users.

The accountabilities for research end users underscore their willingness to engage in the research process to the extent necessary to maximise the chances of research outputs being fit for purpose, meeting research user knowledge needs and able to be implemented in their real world in industry, government or the community. This requires end users to have sufficient organisational research capacity and scientific competence to be able to engage effectively with researchers in problem definition and/or co-design of the research, which in turn requires continuity in personnel engaged in the research process.

The ultimate performance measure for such research investments is the extent to which programme outputs are adopted, and the resulting environmental benefit. The capacity to interrogate, adapt and utilise research outputs, and their ability to engage in adaptive learning and decision-making as new knowledge emerges, are crucial accountabilities for end users.

5. Discussion

The experience of the NERP hubs confirms that in successful transdisciplinary research programmes, research end users are not passive recipients of knowledge products arising from a linear process conceived by researchers and/or funders and implemented by researchers. Rather, it is essential that they work collaboratively with funders and researchers to define the problem and scope knowledge needs, work out approaches to tackle that problem, and then interact with researchers during the active inquiry phase of the programme so that researchers develop as deep an understanding as possible of the end users' context, why their research is important, and how their results will be used. Some problems will require more effort from the end user in defining questions, than from researchers in responding to them.

The shared experiences spanning the implementation of both the CERF and NERP models suggests that all participants' understanding of knowledge gaps evolves as collaborative applied research programmes unfold, which is why accountabilities such as engagement and discourse are important and continuity is critical for all three groups.

A design feature of the CERF programme that was seen as very successful and consequently built into the NERP programme (Box 1), was the requirement that each hub invest at least 10% of its budget in knowledge brokering and communication activities. Knowledge brokers are professional intermediaries (people or organisations) who facilitate knowledge exchange and sharing between researchers and practitioners. Knowledge brokering emerged in the public health sector (CHSRF, 2003) and is now applied in diverse ways in multiple sectors (Bielak et al., 2008; Michaels, 2009). Some NERP hubs have knowledge brokers embedded with end users, others with researchers, but all have explicit and significant investments in people and processes designed to ensure that end users are engaged in the research, and that research outputs are tailored to meet the needs of end users. While transaction costs may be high, the CERF and NERP experience is that direct, face-to-face interaction between researchers and end users is the most effective.

Knowledge brokering is situated along a spectrum of knowledge processes from conventional, linear dissemination of information (science communication) on the left hand side, through intermediary and brokering strategies in the middle, to co-production of knowledge, social learning and more systemic innovation (Fig. 2). A characteristic of knowledge brokering is that knowledge is provided at the time and in the form required by the end user rather than those most convenient to the researcher.

In some contexts, these knowledge intermediary processes may begin where the research stops, to improve uptake of research results and amplify research impact. In other contexts however — for example the complex, multi-dimensional and multi-stakeholder problems being addressed by the CERF and NERP hubs — brokering processes between the producers and users of knowledge (who may overlap to a significant degree) are seen to greatly enhance programme efficacy, particularly if undertaken *before* research is initiated, to refine research questions, influence methodologies, determine an appropriate form of delivery, and ensure that intended end-users have a degree of ownership of research outputs. In the context of the Australian environment, this is particularly relevant to respectful engagement with Indigenous Traditional Owners of Country. In such contexts, scientific inquiry may not be the only or even the most appropriate mode of knowledge production. Local, tacit, experiential and other forms of knowledge can emerge through various types of inquiry.

(a) funders

(b) researchers

(c) end users

Fig. 1. Weighted accountabilities of (a) funders, (b) researchers and (c) end users in transdisciplinary research programmes.

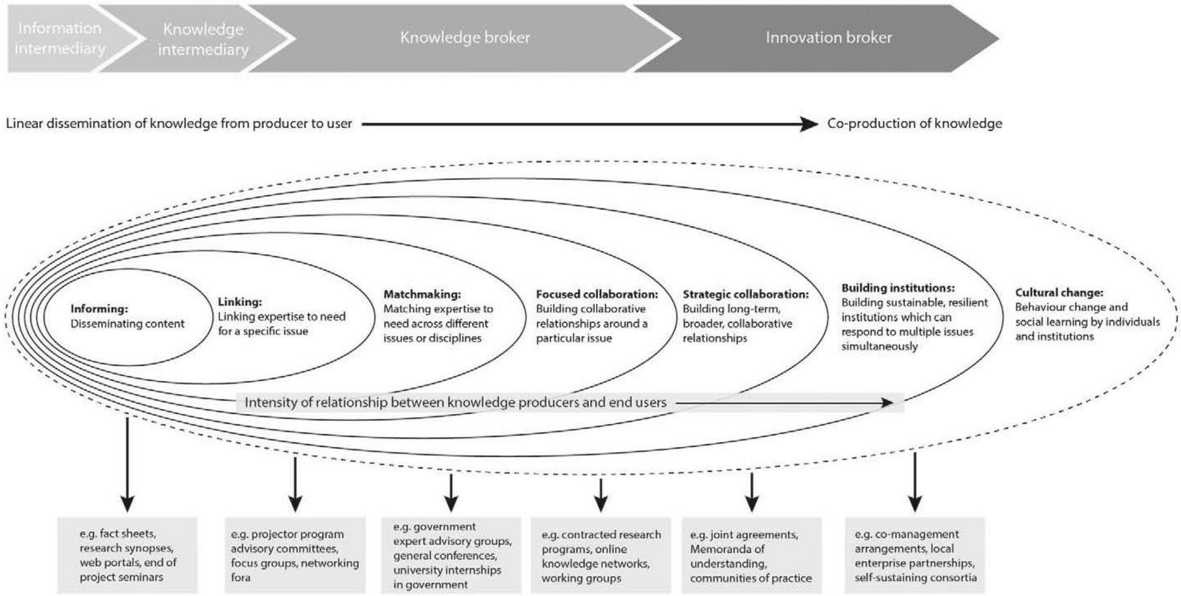

Fig. 2. Knowledge roles and functions from information dissemination to social learning. After Michaels (2009).

Of course useful research outcomes can and do occur without knowledge brokering, but they involve a greater element of chance which can and should be avoided, especially in times of constrained research funding and greater emphasis on accountability. It is doubtful that an organisation or research programme can jump to sophisticated knowledge intermediary processes (the right hand side of Fig. 2) without being competent at the basics of science communication: the ability to pick up research highlights early and present them well; good web interface and search capabilities; effective media and event strategies; and the ability to synthesize research outputs in attractive ways targeted to the knowledge needs of intended audiences. This requires dedicated resources, recognised in the CERF–NERP requirement to allocate at least 10% of budget to communication and knowledge brokering processes.

In designing transdisciplinary, multi-institutional environmental research programmes for impact, we need to understand the knowledge system we are seeking to influence. This means more than researchers' understanding their market, which is weakest with the Indigenous sector. Our key point, exemplified by the experience of the CERF and NERP hubs, is that such research is a shared enterprise between researchers, funders and end users, built on a platform of shared goals and social capital across these three functional domains.

Fig. 1 illustrates that continuity is an important attribute for all three groups. With sufficient continuity of personnel across the collaboration, elements of social capital such as trust and reciprocity become increasingly valuable as collaborations evolve and mature. Extended interaction over a number of years bridges the cultural differences between the different worlds of researchers and end users, it helps researchers to understand the needs of end users, it makes it easier for end users to challenge researchers and to interrogate research findings more freely, and it gives funders more confidence to invest in possibly riskier, less well-defined or more adaptive projects in a spirit of co-learning. The latter is facilitated when the funding body is also an end user, as the Australian Department of the Environment was with respect to the CERF and NERP programmes.

It is now all too common in Australia for research programmes to be funded for four years or less, which makes it difficult to sustain continuity of personnel and to build social capital (familiarity, respect, trust, reciprocity) between funders, researchers and end users. So the fact that five CERF hubs were successful in a national competitive funding round and hence became NERP hubs was very important in the evolution — and we would argue the success — of this overall investment.

The scale and complexity of 'wicked' environmental problems require both a transdisciplinary approach and sustained effort. Within the Tropical Ecosystems' NERP Hub, several research projects required at least ten years of sustained work to be useful, for example: (a) problems that require temporal data to track the response of an ecosystem after a management intervention such as rezoning or an extreme weather event; and (b) complex problems such as coastal water quality that have been attacked in bite-size (i.e. fundable) portions.

However it is important to note that continuity of funding for five hubs from CERF to NERP was by no means deliberate or guaranteed. In fact there was a funding gap between CERF and NERP, during which many CERF-funded researchers on short-term contracts moved on to other roles, thus undermining staff continuity and hub cohesion in the transition to NERP. Both the CERF and NERP programmes began with competitive funding processes, subject to normal Commonwealth procurement rules around contestability and competitive neutrality (DoF, 2014). Under such rules, against a background of three-year electoral cycles and budget processes, designing and sustaining long-term transdisciplinary research investments are inherently difficult. Two CERF hubs that were seen by the Department as being highly relevant and effective (focused on taxonomy and marine mammals), were not funded under NERP, due to revised government priorities for the programme and alternative funding sources.

The reviews of the CERF (Urbis, 2010) and NERP (Spencer et al., 2014) programmes revealed that the hubs' flexibility and responsiveness to identify research topics in detail with their research users enabled them to address environmental issues in their specific contexts, at the appropriate scales and with objectives relevant to research users. Importantly, funding contracts with most of the NERP hubs were signed before all research projects were designed and specified in detail. Whether deliberate or not, the flexibility allowed to these NERP hubs in terms of refining research methods and detailed research programmes and projects in response to end user needs, turned out to be one of the strengths of the programme. Stakeholders and research users had a meaningful opportunity to influence the research direction and allocation of funds once the hubs became real and people were seriously engaged, rather than 'joining in' to established research projects after they had already been designed and funds already committed. As well as improving the relevance and impact of research outputs for users, in the opinion of the manager of the CERF and NERP programmes within the Department of the Environment, this ability to be flexible and responsive '*contributed to a positive cultural change to problem solving between researchers and the Environment Portfolio*'.

Where research programmes were specified in detail and contracted as such from the outset, subsequent lack of flexibility became a problem as it constrained meaningful consultation with end users, which was especially problematic for Indigenous interests.

Political scientist Brian Head (2008) argues that in modern pluralist democracies, the response to any given policy problem is ultimately informed by the interplay between three distinctly different types of knowledge and evidence, as illustrated in Fig. 3.

In this formulation, scientific research is one 'lens' through which Ministers and their advisers seek to understand an issue, weighed up against political judgement and the organisational knowledge, corporate memory and professional practices of relevant agencies. Each lens has a distinctive epistemology — in effect polarized by its own context and experience. Evidence that may seem compelling viewed through one lens may be virtually invisible, unconvincing or rejected through another. For example, research and independent inquiries might produce evidence that pricing instruments (e.g. carbon pricing) are economically efficient means of achieving a desired policy outcome (e.g. reductions in net greenhouse gas emissions), but such evidence may be ignored, contested or rejected through an ideological political lens if election commitments have explicitly and vociferously ruled out pricing carbon.

However if researchers, funders and end users are working closely together in a joint enterprise with shared goals and a high level of social capital, and if programme design pays close attention to the accountabilities in Fig. 1, then over time the overall programme is more likely to be seen as useful and hence influential through all three lenses. Ministers and their officers seek feedback from clients and end users in making political judgements, and active engagement of civil servants with research programmes is likely to accelerate osmosis from research findings into organisational knowledge. A well-designed and managed transdisciplinary research programme is more likely to position itself in the 'sweet spot' in the centre of Head's Venn diagram than more conventional approaches wherein scientists carry out research in isolation, then publish their findings in academic journals, then lament the lack of uptake in policy. An anonymous reviewer of this paper put it well: "*engagement, dialogue, planning etc. all help to shift the polarities so that everyone can see the sweet spot.*"

The Australian science ministry examined the use of science in policy development in the Australian public service (DIISRTE, 2012) and concluded that the five key challenges to the use of science in policy development in the Australian public service are 'timeliness, cultural differences, relationships, timeframes and access to data and information'. A senior environmental policy maker at the workshop noted that the CERF–NERP programmes "*have been significant in building strong relationships between environment portfolio staff and researchers. But maintaining enduring relationships, particularly in the face of churn and changing priorities, remains a challenge.*"

As noted at the bottom of Box 1, and consistent with DIISRTE (2012), reward systems for researchers and policy makers differ markedly. The timeframes within which policy decisions need to be made are usually much shorter than a typical research project. Consistent with the doctrine of New Public Management (Hood, 1991), the Australian public sector is characterised by 'churn' or frequent turnover of personnel, a suspicion of deep subject matter expertise, preference for generic process skills and a default tendency to assume that any services can simply be purchased through competitive tendering processes. Consequently it is difficult and rare for staff inside government agencies to build sufficient domain expertise and/or researcher contacts to be able to understand, articulate or interrogate research needs, or to wish to be involved in iterative development of research programmes through negotiation with researchers and end users.

In our experience, these factors are prevalent across the modern public sector in Australia at all levels of government. They work against effective transdisciplinary research to inform policy.

Paradoxically, they also make investment in such research more essential.

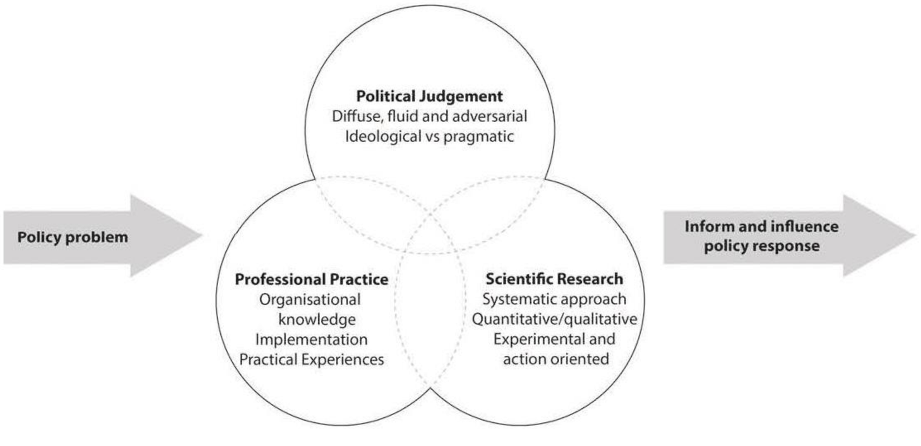

Fig. 3. Three lenses of knowledge and evidence through which public policy is informed. After Head (2008).

We found the framework developed by Roux et al. (2010) to be a useful starting point for framing a structured reflection among experienced research leaders to elicit lessons learned from the collective experience of five national research hubs over eight years.

There is a high level of consensus among the leaders of multi-institutional, transdisciplinary environmental research programmes in Australia that the chances of such research influencing and improving policy are maximised when research investments are designed such that funders, end users and researchers have *shared goals*, sufficient *continuity* of personnel to build *trust* and sustain *dialogue* throughout the research process from issue scoping to application of findings, and sufficient *flexibility* to be able to adjust and respond to new knowledge, changing circumstances and priorities. These design criteria are important for all three functional domains of researchers, end users and funders. Other accountabilities proposed by Roux et al. (2010) were also important for one or two functional domains as outlined in Fig. 1.

As this paper was being finalised, the Australian government was evaluating proposals for research hubs against six national environmental research priorities, for a new six-year $125 m National Environmental Science Programme (NESP) from 2015. In a two-stage process, the detail of hub research plans is to be worked out through negotiation between the Department of the Environment and successful proponents in consultation with end users, with the Department acting as both a funder and end user. Hopefully that process will be characterised by shared goals, dialogue, trust, continuity and flexibility across researchers, funders and end users, extending from the planning phase over the six years of the Programme. It is encouraging that many of the lessons from CERF and NERP distilled in this paper appear to have informed the design of the NESP.

The diverse operating models of research hubs in the CERF and NERP prove that there is no single magic formula for the design and governance of multi-institutional, transdisciplinary environmental research programmes. In spite of this, there are important design criteria that all players – researchers, funders and end users – need to keep in clear focus as research investments are planned and implemented in order to realise an environmental benefit that exceeds the cost of the research.

Acknowledgements

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Appendix A. Research hubs of the National Environmental Research Program (NERP) and their antecedent hubs of the Commonwealth Environmental Research Facilities (CERF) programme

Hub	Research focus	Scientific disciplines	# research providers*	# researchers		Funding		
				CERF 2007–2011	NERP 2011–14	CERF 2007–2011	NERP 2011–14	Total 2007–14
NERP environmental decisions hub (CERF applied environmental decision analysis)	Understanding major biodiversity drivers to maintain ecosystems and maximise their resilience against human impacts	• Climate science • Ecology • Economics • Public policy	9 Core partners: • ANU • CSIRO • NSWOEH • PV • RMIT • UMelb • UQ • UWA • VDEPI	108	118	CERF: $7.81 M Co-contributions: $9.9 m Total: $27.71 m	NERP: $11 m Co-contributions: $6.4 m Total: $17.4 M	Australian government: $18.81 mCo-contributions: $16.3 m Total: $35.11 m
NERP landscape and policy hub (CERF landscape logic)	Retrospective evaluation of the impact of public environmental funding. Regional scale assessment of biodiversity including social and institutional drivers and functional attributes.	• Geography • Climate science • Ecology • Economics • Hydrology • Public policy • Social science	7 • ACE • ANU • CSIRO • CSU • GU • MU • UTAS	58	37	CERF: $8.75 Co-contributions: $12.32 m Total: $21.07 m	NERP: $6 M Co-contributions: $9.2 m Total: $15.2 m	Australian government: $14.75 m Co-contributions: $21.52 m Total: $36.27 m
NERP marine biodiversity hub (CERF marine biodiversity hub)	Provision of biodiversity and baseline data to underpin marine decision making, particularly in reference to marine bioregional planning, protected areas and natural resource management	• Earth science • Fisheries • Marine biology • Oceanography • Public policy • Remote sensing	7 • AIMS • CDU • CSIRO • GA • MVic • UTAS • UWA	45	102	CERF: $6.6 M Co-contributions: $12.64 Total: $19.24	NERP: $11 m Co-contributions: $18.6 m Total: $29.6 M	Australian government: $17.6 m Co-contributions: $31.24 m Total: $48.84 m

NERP Northern Australia hub (CERF tropical rivers and coastal knowledge)	Improvement of biodiversity outcomes in northern Australian terrestrial, freshwater and estuarine systems. Combining biodiversity monitoring and reporting with adaptive planning and community based natural resource management to improve biodiversity outcomes and Indigenous livelihoods	• Agricultural science • Ecology • Limnology • Marine biology • Natural resource management • Planning • Public policy • Traditional knowledge	16 • AIMS • ANU • AWC • BCYDC • CDU • CSIRO • Djelk • GU • JCU • NAILSMA • NTLRM • USyd • UWA • WLML • WGAC • UTS	113	59	CERF: $8.8 M Co-contributions: $11 m Total: $19.8 m	NERP: $14.7 m Co-contributions: $15.8 m Total: $30.5 M	Australian government: $23.5 m Co-contributions: $26.8 m Total: $50.3 m
NERP tropical ecosystems hub (CERF marine and tropical sciences research facility)	Improvement of scientific understanding and environmental decision making in far north Queensland with particular reference to the Great Barrier Reef, rainforests of the Wet Tropics and Torres Strait	• Climate change • Ecology • Fisheries • Limnology • Marine biology • Natural resource management • Public policy • Traditional knowledge	13 • AIMS • ANU • AR • CSIRO • GAC • GBRMPA • GU • JCU • LLR • RAPA • TSRA • UQ • WTMA	267	242	CERF: $37.2 m Co-contributions: $45 m Total: $82.2 m	NERP: $28.5 M Co-contributions: $33.4 m Total: $61.9 m	Australian government: $65.7 m Co-contributions: $78.4 m Total: $144.1 m
Australian government						$69.16 m	$71.2 m	$140.36 m
Partners						$90.86 m	$83.4 m	$174.26 m
Total			52	591	558	$160.02 m	$154.6 m	$314.62 m

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*ACE: Antarctic Climate and Ecosystems Cooperative Research Centre, AIMS: Australian Institute of Marine Science, ANU: Australian National University, AR: Apudthama Rangers, AWC: Australian Wildlife Conservancy, BCYDC: Balkanu Cape York Development Corporation, CDU: Charles Darwin University, CSIRO: Commonwealth Scientific and Industrial Research Organisation, CSU: Charles Sturt University, Djelk: Djelk Rangers (Bawinanga Aboriginal Corporation), JCU: James Cook University, GA: Geoscience Australia, GAC: Girringun Aboriginal Corporation, GBRMPA: Great Barrier Reef Marine Park Authority, GU: Griffith University, LLR: Lama Lama Rangers, MU: Macquarie University, MVic: Museum of Victoria, NAILSMA: Northern Australian Indigenous Land and Sea Management Alliance, NSWOEH: New South Wales Office of Environment and Heritage, NTLRM: Northern Territory Department of Land Resource Management, PV: Parks Victoria, RAPA: Rainforest Peoples Aboriginal Alliance, RMIT: Royal Melbourne Institute of Technology, TSRA: Torres Strait Regional Authority, UMelb: University of Melbourne, UQ: University of Queensland, USyd: University of Sydney, UTAS: University of Tasmania, UTS: University of Technology Sydney, UWA: University of Western Australia, VDEPI: Victorian Department of Environment and Primary Industries, WLML: Warrdeken Land Management Limited, WGAG: Wunambal–Gaambera Aboriginal Corporation, WTMA: Wet Tropics Management Authority.

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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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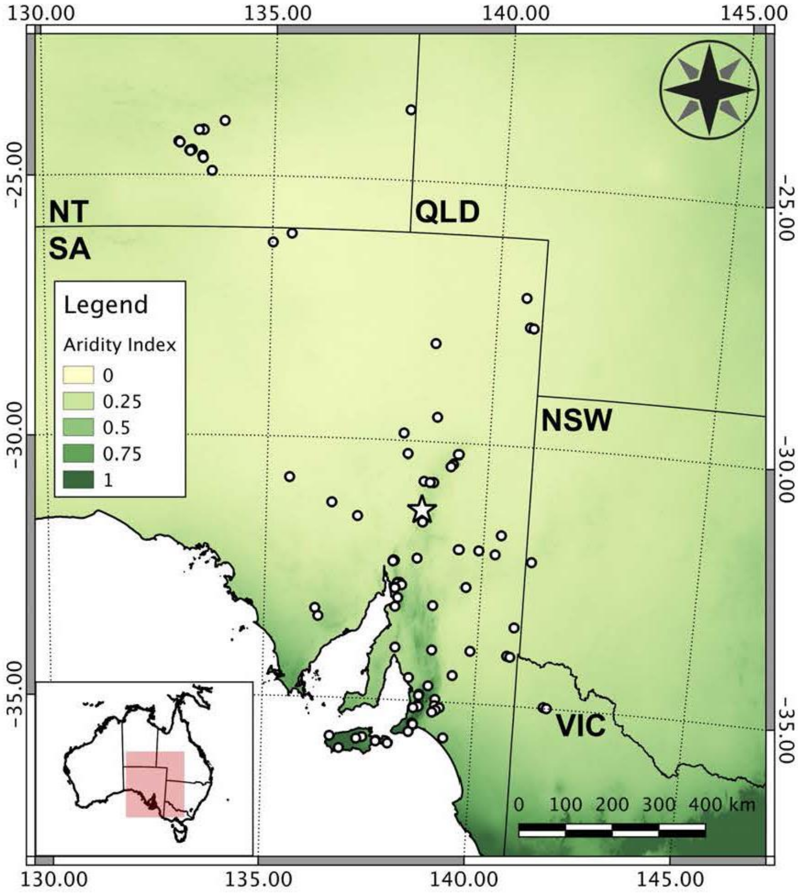

Fig. 1. Location of the sampled populations of *Dodonaea viscosa* subsp. *angustissima*. Shades represent the aridity index (range: 0–1; more to less arid). The star symbol indicates the elevation gradient site. Insolation, temperature range and aridity increase northwards, whereas rainfall and soil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

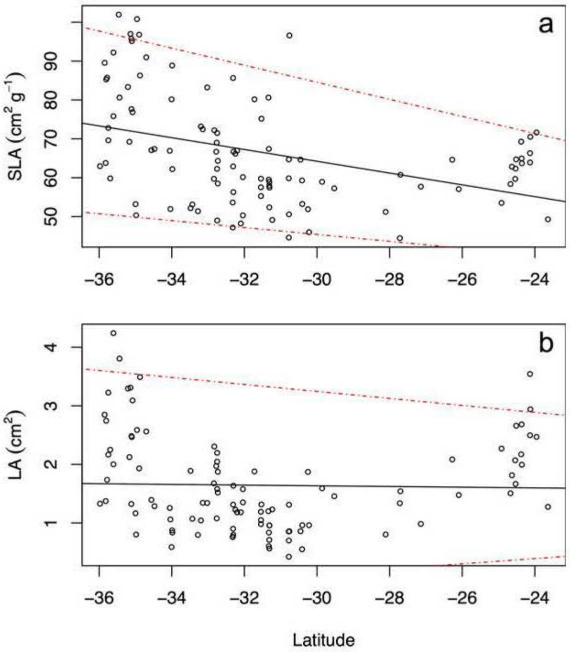

Fig. 2. Leaf trait responses of *Dodonaea viscosa* subsp. *angustissima* to the latitudinal gradient. Linear regressions between (a) SLA and (b) LA and latitude. Coefficients and statistical significance are shown in Table 1. Symbols represent the average for each population. In all panels, broken lines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

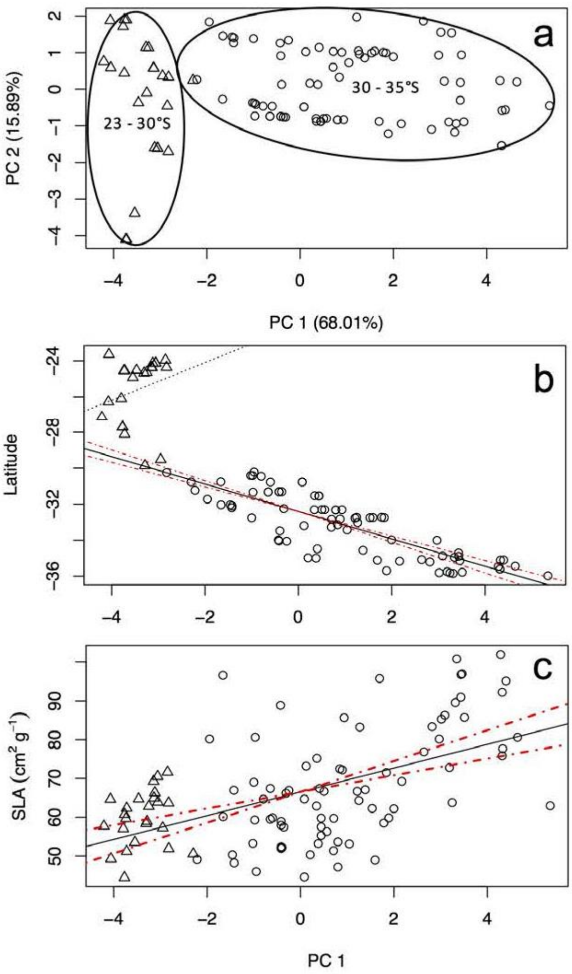

Fig. 3. (a) Principal component analysis (PCA) of the environmental variables shown in Table 2. In parentheses is the percentage of total variation explained by each PC axis. (b) Linear regression between latitude and the coordinates along Axis 1 of PCA. Solid line indicates regression for samples below –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.

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

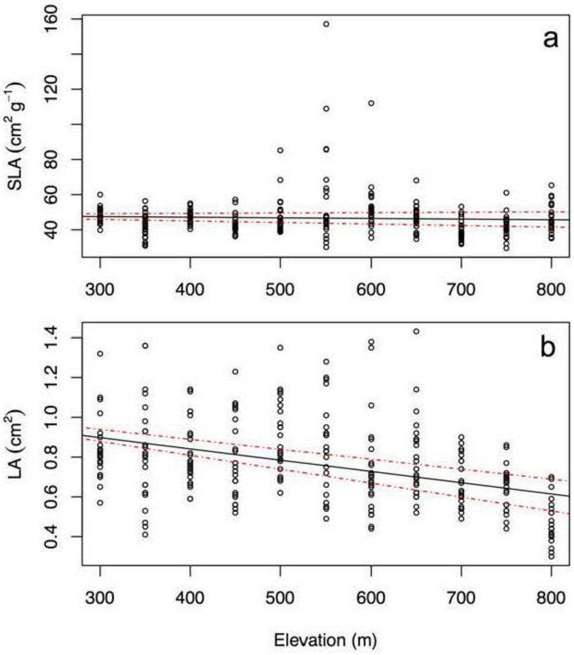

Fig. 4. Linear regression between *Dodonaea viscosa* subsp. *angustissima* (a) SLA and (b) elevation. Symbols represent values for individual leaves. In all panels, the broken lines indicate 95% confidence limits and the coefficients and statistical significance are shown in Table 1.

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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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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GigaScience

DATA NOTE Open Access

Introducing BASE: the Biomes of Australian Soil Environments soil microbial diversity database

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Abstract

Background: Microbial inhabitants of soils are important to ecosystem and planetary functions, yet there are large gaps in our knowledge of their diversity and ecology. The 'Biomes of Australian Soil Environments' (BASE) project has generated a database of microbial diversity with associated metadata across extensive environmental gradients at continental scale. As the characterisation of microbes rapidly expands, the BASE database provides an evolving platform for interrogating and integrating microbial diversity and function.

Findings: BASE currently provides amplicon sequences and associated contextual data for over 900 sites encompassing all Australian states and territories, a wide variety of bioregions, vegetation and land-use types. Amplicons target bacteria, archaea and general and fungal-specific eukaryotes. The growing database will soon include metagenomics data. Data are provided in both raw sequence (FASTQ) and analysed OTU table formats and are accessed via the project's data portal, which provides a user-friendly search tool to quickly identify samples of interest. Processed data can be visually interrogated and intersected with other Australian diversity and environmental data using tools developed by the 'Atlas of Living Australia'.

Conclusions: Developed within an open data framework, the BASE project is the first Australian soil microbial diversity database. The database will grow and link to other global efforts to explore microbial, plant, animal, and marine biodiversity. Its design and open access nature ensures that BASE will evolve as a valuable tool for documenting an often overlooked component of biodiversity and the many microbe-driven processes that are essential to sustain soil function and ecosystem services.

Keywords: Microbiology, Microbial ecology, Soil biology, Australia, Database, Microbial diversity, Metagenomics

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Data description

Human society is dependent on the ecosystem goods and services mediated by soil organisms [1]. Soils filter water, provide the growth medium for vegetation and crops, mediate global carbon and nutrient cycles, degrade xenobiotics, and are habitats for many organisms. Soils are a valuable source of biologically active industrial and medical compounds, are a storage and remediation medium for waste, and are sources for mineral exploration. The resident microbial communities mediate most soil processes, yet we know comparatively little about their diversity, biogeography, community assembly and evolutionary processes, symbiotic networks, adaptation to environmental gradients, temporal stability or responses to perturbation [2, 3]. Critically, the relationship between microbial identity and abundance (community composition), species interactions (community structure) and biogeochemical rate transformations (bioactivity) in natural and domesticated soils are largely unknown, which limits our influence on these factors to maximise desirable outcomes. This knowledge gap is at odds with observations that microbial communities make substantial contributions to ecosystem processes, as demonstrated in simple microcosms [4, 5] and in natural ecosystems [6–9]. Better understanding of soil-related microbial communities and processes is required to ensure continued (or improved) provision of the soil-moderated ecosystem services that promote environmental and human health, food security, mineral wealth and climate stability.

Most soil microorganisms cannot be cultured using standard microbial growth media [10]. Many were unknown until the 1990s when phylogenetic marker gene sequencing (meta-barcoding) revealed that they constitute the most diverse microbial communities on Earth [11]. DNA shotgun sequencing of environmental samples (metagenomics) soon revealed that microbial taxonomic diversity was also reflected in the richness of functional genes and pathways encoded in their genomes [12]. Only recently, however, have advances in high-throughput sequencing and bioinformatics made it possible to obtain data sets that are commensurate with the complexity of microbial communities. Nonetheless, to do this on a scale enabling generalised conceptual advances in ecological understanding, rather than in a smaller, piecemeal manner, requires targeted, coordinated and highly collaborative efforts. The Biomes of Australian Soil Environments (BASE) project (http://www.Bioplatforms.Com/soil-biodiversity/) is one such effort. BASE now provides a database of amplicon data (with metagenomic data currently being generated), complete with rich contextual information on edaphic, aboveground diversity and climate. These data were collected according to stringent guidelines across the Australian continent and extending into Antarctica (Fig. 1, Table 1). This database provides researchers with a national framework data set of microbial biodiversity encompassing much of the soil, vegetation and climate variation within Australia, and is set in the context of a cultural progression in science towards open access to data [13]. The BASE database represents infrastructure that can, among other things, be used to investigate the evolution of Australian soil microbes; biogeographic patterns of microbial community change and their environmental drivers; effects of land management on genes, functions, species or community assemblages; use as indicators for underlying mineral deposits and restoring degraded environments. With many soils in Australia (and globally) considered severely degraded, efforts to restore the soil physical and chemical properties of soil must be complemented with restoring biological function. BASE data will support efforts to manage soil microbes for improved ecological and agricultural outcomes, just as microbial medicine has developed into a potent tool to promote human health.

Selection and characteristics of soil samples

As of August 2015 the BASE data set represents >1400 samples taken from 902 locations across Australia (Fig. 1). These samples represent a wide variety of Australian bioregions and land-uses, and were collected from the soil inhabited by a diverse array of plant communities. Samples span a continental scale (>7.7 million km^2).

To investigate microbial diversity in soils, each sample was subjected to phylogenetic marker (amplicon) sequencing to characterise the diversity of bacterial (16S rRNA gene), archaeal (16S rRNA gene) and eukaryotic (18S rRNA gene) community assemblages. Fungal diversity was captured to a certain extent by the 18S rRNA gene amplicon; however, because fungi are such an important component of soils, and because the internal transcribed spacer (ITS) region is more informative than 18S rRNA for many fungal groups, we also included a fungal-specific ITS region amplicon to characterise fungal community assemblages. These amplicons cover the diverse range of microbes resident in soils.

Methods

Data collection followed the conceptual outline given in Fig. 2.

Soil sampling

Soil samples were collected from 902 sites across Australia (Fig. 1) according to the methods described at the BASE data portal (Http://www.Bioplatforms.Com/sample-collection-procedure). These sites covered 27 IBRA 7 regions (Interim Biogeographic Regionalisation for Australia (https://www.Environment.Gov.Au/land/nrs/science/ibra#ibra). Many land-use categories were covered,

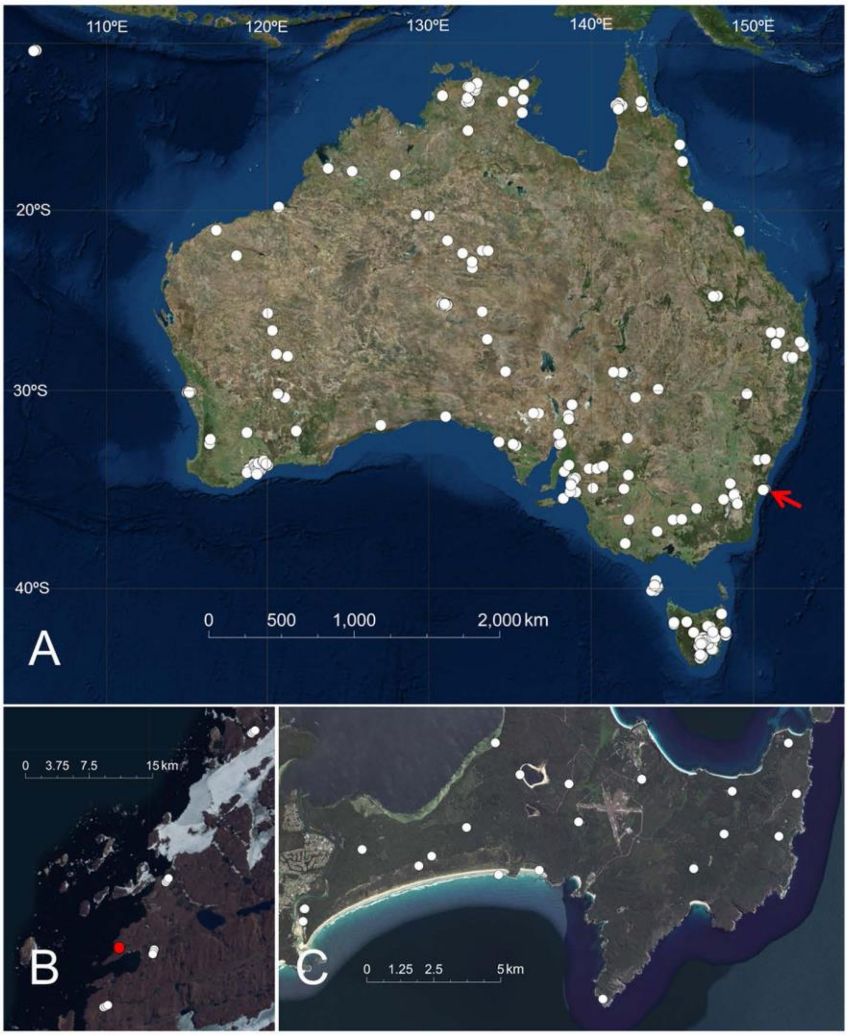

Fig. 1 Position of BASE sample sites (August 2015). **a** Australian mainland and Christmas Island samples; **b** location of Antarctic sampling locations (white), with Davis station indicated in red; and **c** finer detail of sampling position indicated by red arrow in (**a**)

representing most key vegetation types, and about 50 % of samples came from conservation reserves. Native restoration sites and production landscapes, including orchards and cereal croplands, were also sampled. Briefly, each mainland Australian soil sample comprised nine discrete soil samples from a 25 × 25 m quadrat sampled at two depth ranges (0–0.1 and 0.2–0.3 m), while Antarctic samples comprised the 0–0.1 m horizon only. Two discontinuous depths (0–0.1 m and 0.2–0.3 m) were sampled to ensure independent samples from both surface and shallow subsurface. Eight samples were taken at the corners and midpoints of the 25 × 25 m sides of the quadrat, and one from the centre. The quadrat size was chosen to represent the smallest pixel size of Australian soil mapping efforts [14] and to ensure enough soil for sequencing, chemical/physical analyses and sample archiving. While the 25 × 25 m sample unit size does not allow questions of finer scale (<25 m) heterogeneity to be addressed, it does allow high level integration with current Australian soil [15] and aboveground diversity mapping efforts [16], and facilitates meaningful temporal sampling (single point sampling is destructive and so not amenable to temporal sampling efforts). The nine subsamples were combined for each depth, to return a single surface and deeper soil sample per quadrat. Samples for molecular analysis were stored on ice until they could be frozen and transported to either the Adelaide node of the Australian Genome Research Facility (AGRF) laboratories (Australian samples) or, for the Antarctic samples, the Australian Antarctic Division (AAD), for DNA extraction. Australian samples for chemical and physical

Table 1 Contextual data collected from each soil sample

Soil chemical properties		
moisture	Total Carbon	Zinc
Ammonium	Organic Carbon	Exchangeable Aluminium
Nitrate	Conductivity	Exchangeable Calcium
Total Nitrogen	pH	Exchangeable Magnesium
Phosphorus	Copper	Exchangeable Potassium
Potassium	Iron	Sodium
Sulphur	Manganese	Boron
Soil physical properties		
Texture	Color	Particle size distribution
Soil/site descriptors		
Overlying vegetation identity	Aspect	Elevation
Slope	Landscape position	Land-use history
Land-use Management		

analysis were air-dried and transported to CSBP Laboratories (Perth, Western Australia) (https://www.Environment.Gov.Au/land/nrs/science/ibra#ibra), while edaphic properties of Antarctic samples were determined by the AAD. To minimise operator bias DNA extraction was carried out at AGRF or AAD (Antarctic samples only). At the time of sampling all other contextual data were collected including: sample location (coordinates taken at the centre point of the sampling quadrat), overlying plant cover (coverage and composition), slope, elevation above sea level, position in landscape (upper, mid, lower slope, valley, ridge) and land-use history.

Contextual data

Soil chemical and physical attributes were usually determined at CSBP Laboratories. Soil moisture (% GWC) was measured gravimetrically [17], and ammonium and nitrate levels were determined colorometrically, following extraction with 1 M potassium chloride (25 °C) [18, 19]. Available phosphorus and potassium were measured using the Colwell method [17]. Sulphur levels were determined by the Blair/Lefroy Extractable Sulphur method [20]. Organic carbon was determined using the Walkley-Black method [21]. For pH analysis, CaCl pH and electrical conductivity ($EC_{1:5}$), soils were extracted in deionised water for 1 h to achieve a soil:solution ratio of 1:5. The water pH and $EC_{1:5}$ of the extract were subsequently measured using a combination pH electrode; calcium chloride solution was then added to the soil solution and, after thorough mixing, the calcium chloride pH determined [17]. Diethylene-triamine-pentaacetic acid (DTPA) extractable trace elements (Cu, Fe, Mn, Zn) were determined by atomic absorption spectroscopy following extraction with (DPTA) for 2 h [17]. Soils were extracted with a 0.01 M calcium chloride solution and analysed for extractable aluminium using inductively coupled plasma spectroscopy (ICP) [22]. Boron was measured by ICP after hot $CaCl_2$ extraction [17]. Soil exchangeable cations (Mg, K, Na, Ca) were determined using a 1:5 soil:water extraction. This test was used in

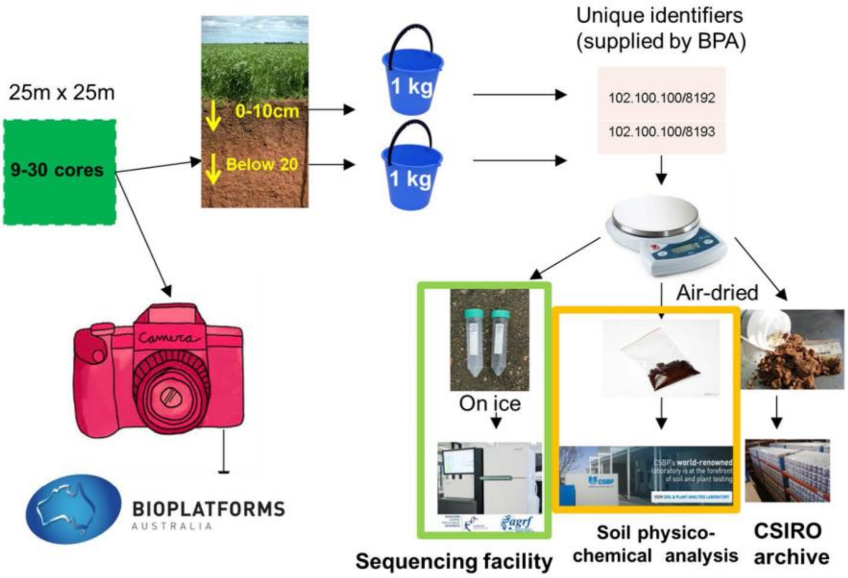

Fig. 2 Sampling strategy. Approximately 1 kg of soil was taken, at two soil depths, by bulking 9 – 30 soil cores a 25 × 25 m quadrat. Each sample was assigned a unique identifier and subdivided for DNA extraction and sequencing, soil physico-chemical analyses and soil and DNA sample archiving for future use. A photograph of each site was also taken

combination with the $NH_4Cl_2/BaCl_2$ extractable exchangeable cations test, where the value for water soluble exchangeable cations is subtracted from the value for $NH_4Cl_2/BaCl_2$ extractable exchangeable cations [17].

Soil particle size distribution was also measured. Soils were sieved to 2 mm (particles greater than 2 mm were considered gravel), treated with hydrogen peroxide to remove organic matter, and then treated with a 1:1 calgon–sodium hydroxide mixture to disperse particles. Using a standardised table of particle sedimentation times, 25 ml aliquots were removed from the shaken sample and the remaining sample sieved. The samples were evaporated, oven-dried and weighed to determine the sand, silt and clay contents [23].

DNA extraction

All soil DNA was extracted in triplicate according to the methods employed by the Earth Microbiome Project (Http://www.Earthmicrobiome.Org/emp-standard-protocols/dna-extraction-protocol/).

Sequencing

Sequencing was carried out using an Illumina MiSEQ, as described in detail both on the BASE protocols webpage (Https://ccgapps.Com.Au/bpa-metadata/base/information) and in the sequencing_methods_readme.txt on the data portal. Briefly, amplicons targeting the bacterial 16S rRNA gene (27 F–519R; [24, 25]), archaeal 16S rRNA gene (A2F–519R; [25, 26]), fungal ITS region (ITS1F–ITS4 [27, 28]) and eukaryotic 18S rRNA gene (Euk_1391f–EukBr, (http://www.Earthmicrobiome.Org/emp-standard-protocols/18s/) were prepared and sequenced for each sample at the Australian Genome Research Facility (Melbourne, Australia) and the Ramaciotti Centre for Genomics (Sydney, Australia). The 16S and ITS amplicons were sequenced using 300 bp paired end sequencing, while 18S amplicon reads were generated using 150 bp paired end sequencing.

Amplicon sequence analysis

16S rRNA genes

The quality of all Illumina R1 and R2 reads was assessed visually using FastQC [29]. Generally, a significant drop in read quality was observed in the last 50–100 bp of R2 and the last 10 bp of R1. As many base pairs as possible were trimmed, while still leaving an overlap to allow reliable merging of R1 and R2 reads, as assessed manually after merging with FLASH [30]. The 5' end of each R1 sequence was trimmed by 10 bp, and each R2 by 70 bp. Sequences were merged using FLASH [30]. Several hundred sequences were merged manually and the results compared to the FLASH merges to ensure merging efficacy. Once efficacy was confirmed, merged sequences were passed to the open reference Operational Taxonomic Unit (OTU) picking and assigning workflow.

Following merging, FASTA format sequences were extracted from FASTQ files. Sequences < 400 bp, or containing N or homopolymer runs of > 8 bp, were removed using MOTHUR (v1.34.1) [31]. The remaining sequences were passed to the open reference OTU picking and assigning workflow (described below).

18S rRNA genes

Illumina R1 and R2 reads were both trimmed by 30 bp to remove primers and adaptors. The reads were merged using FLASH [30] as described for 16S rRNA above, and results compared to a random subsample of sequences merged by hand. Following merging, FASTA-formatted sequences were extracted from FASTQ files. Sequences < 100 bp, or containing N or homopolymer runs of $>$ 8 bp, were removed as described above. The remaining sequences were then passed to the open reference OTU picking and assigning workflow.

ITS regions of rRNA operons

Only R1 sequences were used for ITS regions. R1 included the ITS1 region, upon which our current workflow is based. ITS2 region reads (from R2 reads) are available on request. FASTA files were extracted from FASTQ files, and complete ITS1 regions were extracted using ITSx [32]. Partial ITS1 sequences and those not containing ITS1 were discarded. Sequences comprising full ITS1 regions were passed to the OTU picking and assigning workflow.

Open OTU picking and assignment

Each of the four amplicons was submitted to the same workflow, separately, to pick OTUs and assign read abundance to a Sample-by-OTU matrix. This workflow followed a similar conceptual outline to that advocated in the QIIME open reference OTU picking pipeline [33], with the following differences: a) USEARCH 64 bit v8.0.1517 was employed directly; b) reference OTUs were not initially assigned via a round of closed reference picking, instead *de novo* OTUs were picked (OTUs were classified later); c) in order make compute time manageable for *de novo* picking, OTUs were initially picked on the numerically dominant sequences only (sequences with > 6 representatives across the full dataset); d) instead of randomly picking sequences that failed to be recruited to OTUs for subsequent clustering, all sequences with >2 representatives were used. USEARCH was primarily used for analysis, but other programs could be equally efficacious. The workflow can be summarised as follows:

1. Dereplicate sequences.
2. Sort sequences by abundance and keep sequences with > 6 representatives.
3. Cluster sequences into OTUs of ≥ 97 % similarity using UPARSE [34] and check for chimeras (outputs

comprised both a representative OTU sequence file and a UPARSE file).

4. Cluster chimeric sequences to produce a representative sequences file for each OTU cluster (97 % similarity) [35] using the UPARSE output from (3) to obtain chimeric reads. The USEARCH "fast cluster" algorithm [34, 35] was used.
5. Concatenate de novo OTUs from (3) and chimeric OTUs from (4) into a single OTU FASTA mapping file.
6. Map reads in the original dataset of quality-checked sequences (1) against the output from (5) using the "usearch_global" function in USEARCH [34].
7. Split mapped reads (hits) from (6) into chimeric and non-chimeric output files.
8. Retrieve non-mapped reads (misses) from (6) from the original data to create a data set of non-mapped and non-chimeric reads, forming the basis of a second round of OTU picking.
9. Repeat the process from (2) with the non-mapped sequences from (8), with the number of required representatives per sequence at (3) reduced appropriately (e.g. from 6 to 2).
10. Concatenate the resultant USEARCH cluster files to create a final mapping file.
11. Convert the final mapping file to an OTU table.
12. Concatenate all representative OTU sequence files to produce final OTU representative set.
13. Identify OTUs using Green Genes (13-5) for bacteria and archaea; UNITE (v7.0) for fungi and SILVA (123) for eukaryotes. Classify MOTHUR's implementation of the Wang classifier [36] at 60 % sequence similarity cut-off.
14. Create a final sample-by-OTU data matrix and taxonomy file by discarding sequences not identified as belonging to the correct lineage (i.e., bacteria, archaea, fungi, eukaryotes), unidentified at the phylum level, or having < 50 sequences across all samples in the database.

These final curation steps were guided by the inclusion of mock community samples (data not included) and reduced the number of OTUs considerably (e.g., bacterial OTUs from > 400,000 to < 90,000), while only removing < 1 % of the total sequences. It should be noted that these curation steps were performed for OTU table generation; raw FASTQ files of sequences (i.e. all sequences generated) are also available from the database.

Database description

BASE objectives and data usage

BASE is being developed to:

- Generate a comprehensive audit of Australian soil biodiversity;
- Assist bio-discovery to add to the known global diversity of key ecological groups;
- Model relationships between environmental parameters and microbial diversity;
- Examine the importance of microbes in generating ecological complexity, stability and resilience;
- Test broad biogeographical and evolutionary hypotheses regarding microbial evolution and plant–microbe co-evolution;
- Inform the restoration of soil communities as part of on-going broad-scale re-vegetation;
- Provide a baseline reference data set to examine the effects of land management;
- Inform the role of microbes in plant productivity, mineralogy and general soil health.

The BASE database [37] provides a rich source of microbial sequences and associated metadata for Australian soil ecosystems that can be used to further understanding of soil microbiological processes critical to ecosystem function and environmental health. The BASE project has sampled 902 sites and is continually expanding as new data become available. Although the number of potential biases that might influence data utility in any metagenomics/amplicon-based analysis (e.g. DNA extraction [38], PCR primer choice [39, 40], reagent contamination [41] etc.) is large, all samples were treated with the same protocols and therefore should all have the same biases. For microbiome characterisation we used the same protocols as those employed by the Earth Microbiome Project (EMP) [42] to ensure maximum compatibility with global data. To this end, the BASE project has also taken precautions to ensure that all procedural and analytical variables have been recorded, all samples were collected and transported according to the same method, and all DNA extractions and soil analyses were conducted by one of two facilities (Australian and Antarctic samples).

Many methods are available to analyse amplicon data; each having advantages and disadvantages. Indeed, it is often necessary to tailor the analysis to the specific question being addressed. The rationale behind amplicon data analysis for the BASE project was to provide a searchable framework for data exploration via our data portal, with sample-by-OTU matrices for most applications, and to ensure that raw data sources can be identified to allow future reanalysis if required.

All data collected by the project is publically available via the BASE data portal (Https://ccgapps.Com.Au/bpa-metadata/base/) which provides a searchable interface to explore BASE data, identify samples of interest and download data. The database contains biological, edaphic and other site-related data for each sample collected. The data may be interrogated for all data types (biological or non-biological), together or separately. For non-biological data comprising a single matrix of site-wise contextual data, empty cells indicate that no data is available for that sampling point, while a 'sentry' value of 0.0001 indicates values below the detection threshold for a particular assay. Actual detection limit values for each assay are displayed via a link on the contextual data page (Https://ccgapps.Com.Au/bpa-metadata/base/contextual/samplematrix). Columns on this page may be sorted numerically or alphabetically.

We aim to include a minimum of 20,000 sequences in the BASE database for each sample and amplicon. While previous work has shown that around 2000 sequences are enough to preserve between sample (treatment) differences [43], this number of sequences does not saturate coverage curves in most environments. We have therefore sought to produce as many sequences as resources allow. Most samples sequenced thus far exceed this number, and those falling below this threshold are being re-sequenced to increase the number of sequences per sample to > 20,000. Details of sequencing outputs for each amplicon are contained in Table 2 and diversity for each land-use category is presented in Fig. 3. Biological data are available as both processed and raw sequence data for all samples or subsets, as defined by database searches. Processed data comprises sample-by-OTU tables for the samples/taxonomies of interest, and a FASTA-formatted sequence file containing representative sequences for all OTUs. These are provided separately for each amplicon. Data are also provided as raw Illumina paired end sequence files for each sample. These data can be searched and downloaded via the database (Https://ccgapps.Com.Au/bpa-metadata/base/search). This search facility allows users to identify samples of interest based on amplicon taxonomy and/or site contextual data.

The database portal also contains a sample distribution map showing sample sites and providing site-specific information in the context of site geographic position (Https://ccgapps.Com.Au/bpa-metadata/base/contextual/sites), contextual data tables for all sites (https://ccgapps.Com.Au/bpa-metadata/base/contextual/samplematrix), all BASE project related methods, and lists of all currently available amplicon and metagenomic samples.

Sampling design

The sampling protocols for the BASE project were developed with several constraints in mind:

1. For every physical sample sequenced, soil contextual data are required.
2. The more contextual data variables collected, the greater the requirement for physical sample.
3. A soil sample at any size/scale appropriate for both sequence and contextual data generation is necessarily a composite sample. The sample may be as small as possible to give the required amount of soil for sequencing and contextual data generation, but the sample is nonetheless required to be well mixed/homogeneous.
4. Single point samples are destructive and do not easily facilitate temporal monitoring.

The sampling scheme as described above (nine samples over a 25 m × 25 m quadrat, homogenised into a single sample) was chosen because it generated sufficient physical sample material for sequencing (i.e. enough DNA for amplicon and shotgun library generation), chemical and physical analyses, and sample archiving; easily facilitated temporal sampling points, allowed integration of microbial data with landscape elements and other biological data collected at similar scales; and is easily implemented by unskilled practitioners. This sampling scheme provides broad benefits for increasing our knowledge of soil biomes at a continental, regional and local scale, although is not suitable to answer questions relating to scales less than 25 × 25 m. Indeed, the sampling scheme is a compromise between available resources and the competing uses for which data are generated.

Data visualisation

The current visualisation tools available via BASE are being developed in an on-going collaboration with the Atlas of Living Australia (Http://www.Ala.Org.Au) and provide a platform to visualise BASE-derived microbial diversity data in the context of other Australian diversity and environmental data [44]. Currently, analysed BASE OTU and contextual data are available via a persistent

Table 2 Details of sequencing outputs for each amplicon

Amplicon	Bacteria	Archaea	Eukaryote	Fungi
Total reads[a]	67578131	99533527	65086341	86322772
Mean per sample	74837 ± 59400	97009 ± 56696	74153 ± 58634	103504 ± 131838
OTU Richness	85596	5421	21552	43708
% classified[b]	72 %	22 %	40 %	69 %

[a] Total number of sequences after all QC and processing

[b] % classified to family level (>60 % probability) against Green Genes for Bacteria and Archaea, UNITE for Fungi and SILVA for Eukaryotes

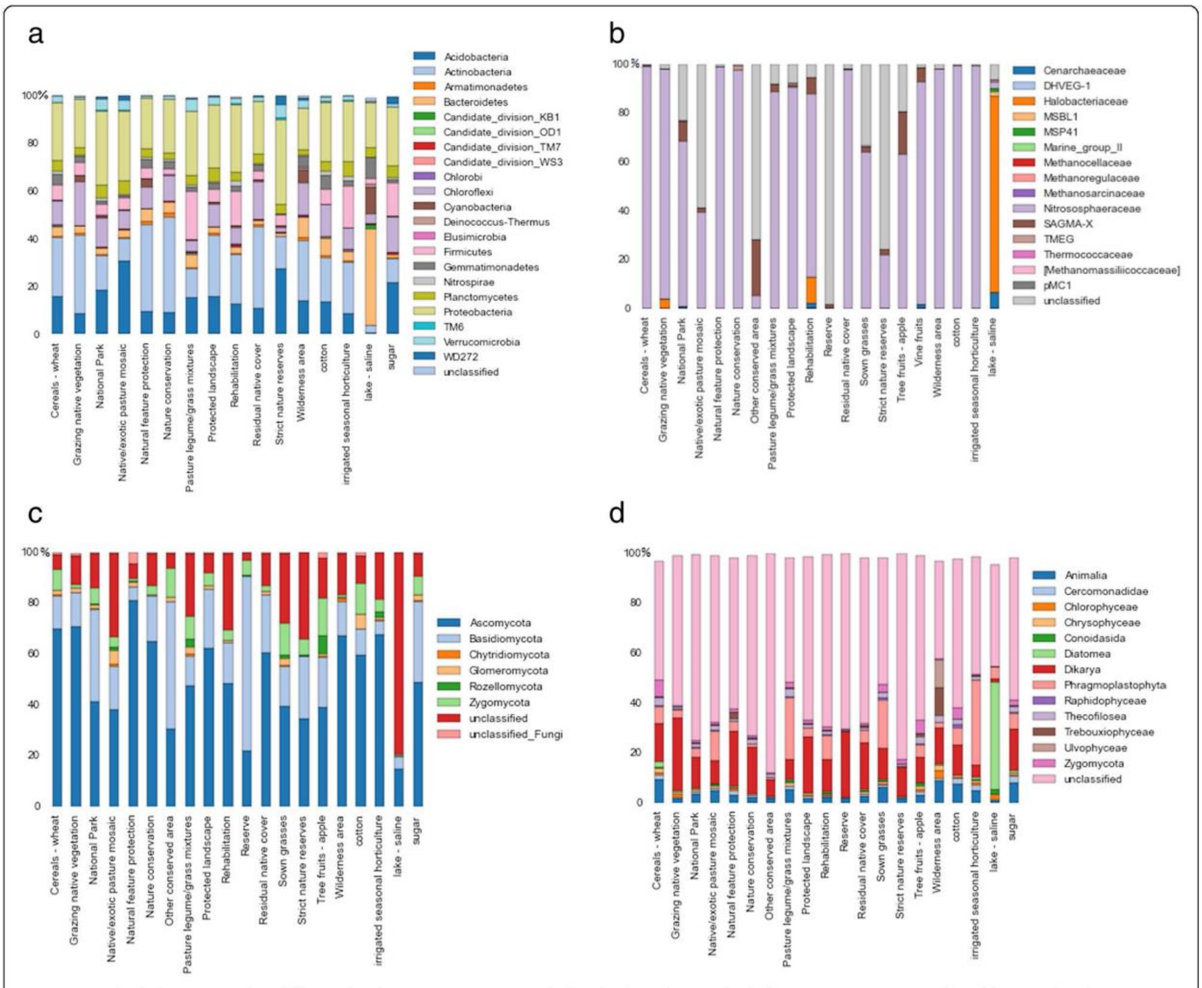

Fig. 3 Microbial diversity under different land-use categories sampled in BASE. **a** Bacterial phyla comprising > 1 % of total bacterial 16S rRNA gene amplicons; **b** archaeal families comprising > 1 % of total archaeal 16S rRNA gene amplicons; **c** fungal phyla comprising > 1 % of total fungal ITS1 region amplicons; and **d** eukaryotic phyla comprising > 1 % of 18S rRNA gene amplicons. All abundances are expressed in % of the total read number for each group, and land-use categories refer to land-use categories as described in the Australian land use and management classification (http://www.agriculture.gov.au/abares/aclump/land-use/alum-classification-version-7-may-2010)

instance of ALA's sandbox tool (Http://base.Ala.Org.Au/datacheck/datasets). This resource is linked from the BASE data portal and the BASE project description pages, and allows users to both visualise BASE site-related data on geographic maps, as text records, plot charts showing sample attribute distributions, and to intersect BASE collected data with ALA provided environmental, occurrence, diversity and climate data. Five datasets are currently available (site contextual data and data for the four BASE amplicons targeting bacteria, archaea, fungi and eukaryotes).

Current uses

Data from the project has helped to address questions about the impacts of agricultural management practices; for example, the use of nitrogen fertilizer on soil microbiomes in sugar cane production in coastal Queensland. Previous work demonstrated that nitrogen applied to soils is diminished within 2–3 months, although the crop requires nitrogen from soil for at least 6 months. Soil microbes convert fertilizer into leachable and gaseous forms of nitrogen, including the greenhouse gas nitrous oxide, which results in considerable inefficiencies and environmental penalties [45]. Metagenomic data confirmed elevated abundances of genes involved in nitrification and denitrification following fertilizer application, corroborating the inference that agricultural soil microbiomes are attuned to scavenging nitrogen for their own energy metabolism [46]. The study demonstrated that low rates of nitrogen fertilizer application over several years did not increase the abundance of diazotrophic microbes and Nif genes in soil or in association with sugarcane roots, indicating that active manipulation of microbial communities may be required to boost biological nitrogen fixation [35]. Amplicon data also indicated a small yet significant effect of fertilizer application on bacterial [46] and fungal community composition [47]. This approach also identified the microbes that were enriched in the rhizosphere and roots, allowing subsequent tests as to whether beneficial or detrimental microbes are prevalent, and which microbes are potential candidates for formulating bioinocula with plant-growth-enhancing rhizobacteria [48].

In other applications, BASE data are used to model microbial community spatial turnover, the effect of edaphic and climate factors on microbial community structure, to elucidate microbial community assembly and maintenance drivers at the continental scale, and to inform the most efficacious target sites for future sampling efforts. For example, at various points in the development of the database survey gap analysis methods [49, 50] were used to identify Australian soils that may contain diversity not yet captured in the database [51, 52].

BASE: future outlook

The BASE database is an evolving, continuously improving resource, both in terms of the number of samples included in the database, and the way in which the database may be utilised. We will provide updates on advances and tool development on the project's online documentation pages.

Despite providing useful data exploration resources, the present BASE visualisation tools available via ALA are limited to presence/occurrence of organisms (rather than abundance). Furthermore, they are linked to current taxonomy/classifications and cannot directly compare two or more sites. Through on-going collaboration with the ALA, BASE is developing methods to address these shortcomings, including incorporating abundance data. BASE data will make use of the ALA phylogeny-based interrogative visualisation tools (Http://phylolink.Ala.Org.Au) [53]. ALA Phylolink will allow users to view Australian soil microbial diversity in terms of phylogeny, in addition to taxonomy, through the incorporation of collapsible phylogenetic trees. These trees will interact with Australian diversity map layers to allow users to build powerful visualisations of soil microbial and other soil/diversity data, bringing the BASE data set into context with other Australian biodiversity data (e.g., mapped soil edaphic properties, plant and animal diversity etc.). We are developing the capability to compare and graph differences between two or more samples. Finally, we anticipate that the current segregation of species occurrence data by domain/kingdom and environment (e.g., soil, aquatic, marine) will not persist, and that all biodiversity and site contextual data will be combined into an integrated system. This will allow integrative ecological approaches to be pursued. Incorporation of the BASE data set into wider Australian ecological data sets, as used by ALA, for example, will be an important step in achieving in this.

The priorities for additional sampling include the incorporation of a temporal aspect by re-sampling sites, the inclusion of more examples/replicates of each land-use and management strategy within land-use, particularly for agricultural samples, and samples identified from survey gap analysis as likely harbouring uncaptured diversity. As well as directly generating further samples through this initiative, we aim to accommodate independently generated Australian microbial diversity data within the database.

Finally, the BASE database currently comprises primarily amplicon-derived data from all three domains of microbial life. However, this will be expanded to include amplicon-free metagenomic sequencing from approximately 500 sites (0–0.1 m depth) (Https://ccgapps.Com.Au/bpa-metadata/base/information). These sites have been chosen to maximise geographic spread, and diversity of land-use, soil type and aboveground ecosystem. Initially, metagenomics data have been made available via the European Bioinformatics Institute (EBI) metagenomics portal (Https://www.Ebi.Ac.Uk/metagenomics/) and can be found by searching "BASE" in EBI metagenomics projects. Data are uploaded to EBI as they become available (12 sites available so far). Once the ~500 samples have been sequenced (expected by May 2016), a trait-by-sample table will be added to the BASE data portal search facility, where "trait" refers a functional gene metabolic pathway.

Summary

The BASE project represents the first database of Australian soil microbial diversity that has been developed in the context of an open data/open access framework. It will continue to grow as more samples are sequenced and added, and as the community of users grows. As the BASE data set expands it will become further linked with other biodiversity exploration efforts (global microbial, plant, animal, marine, etc.) and environmental data sets. Immediate priorities include additional sampling to improve the representation of Australia's climate, soil, ecological and land-use diversity, and to incorporate a temporal dimension by repeat sampling of selected sites. Database design elements, combined with these additional priorities, will allow the BASE project to evolve as a valuable tool to document an often overlooked component of biodiversity and address pressing questions regarding microbially mediated processes essential to sustained soil function and associated ecosystem services.

Availability of supporting data

The dataset supporting this article is available in the BioPlatforms Australia project's data portal (Https://ccgapps.Com.Au/bpa-metadata/base/), DOI 10.4227/71/561c9bc670099 [37]. All raw data has been deposited in the Sequence Read Archive (SRA) under the Bioproject ID PRJNA317932. Information on all SRA accessions related to this dataset can also be found at (Https://downloads.Bioplatforms.Com/metadata/base/amplicon/amplicons). All OUT pipelines can be found at (http://www.Bioplatforms.Com/soil-biodiversity/) under "BASE protocols and Procedures".

Abbreviations

AAD: Australian Antarctic Division; AGRF: Australian Genome Research Facility; ALA: Atlas of Living Australia; BASE: Biomes of Australian Soil Environments; OTU: Operational Taxonomic Unit.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

AB, AF, AY and PM designed the project. AB, AY, PMM, FR, PGD, BB, MFB, MVB, JB, MB, SCR, BC, DJC, BCF, WSRG, KH, PH, MK, AJL, SM, MM, EP, CP-L, LP, MAR, SS, NS, IS, YK and CZ collected and prepared samples and contextual data. TM implemented and maintained the BASE data portal. RP, DM and AB, designed and implemented visualisation tools. LM prepared DNA. AB, AF, CCC, AH, JK, KIN, JRS and MT designed and performed next-generation sequencing. AB performed sequence/bioinformatics analysis. JRP and KW analysed data. All authors have read and approved the manuscript.

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Leaf nitrogen from first principles: field evidence for adaptive variation with climate

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Abstract. Nitrogen content per unit leaf area (N_{area}) is a key variable in plant functional ecology and biogeochemistry. N_{area} comprises a structural component, which scales with leaf mass per area (LMA), and a metabolic component, which scales with Rubisco capacity. The co-ordination hypothesis, as implemented in LPJ and related global vegetation models, predicts that Rubisco capacity should be directly proportional to irradiance but should decrease with increases in $c_i : c_a$ and temperature because the amount of Rubisco required to achieve a given assimilation rate declines with increases in both. We tested these predictions using LMA, leaf $\delta^{13}C$, and leaf N measurements on complete species assemblages sampled at sites on a north–south transect from tropical to temperate Australia. Partial effects of mean canopy irradiance, mean annual temperature, and $c_i : c_a$ (from $\delta^{13}C$) on N_{area} were all significant and their directions and magnitudes were in line with predictions. Over 80 % of the variance in community-mean (ln) N_{area} was accounted for by these predictors plus LMA. Moreover, N_{area} could be decomposed into two components, one proportional to LMA (slightly steeper in N-fixers), and the other to Rubisco capacity as predicted by the co-ordination hypothesis. Trait gradient analysis revealed $c_i : c_a$ to be perfectly plastic, while species turnover contributed about half the variation in LMA and N_{area}.

Interest has surged in methods to predict continuous leaf-trait variation from environmental factors, in order to improve ecosystem models. Coupled carbon–nitrogen models require a method to predict N_{area} that is more realistic than the widespread assumptions that N_{area} is proportional to photosynthetic capacity, and/or that N_{area} (and photosynthetic capacity) are determined by N supply from the soil. Our results indicate that N_{area} has a useful degree of predictability, from a *combination* of LMA and $c_i : c_a$ – themselves in part environmentally determined – with Rubisco activity, as predicted from local growing conditions. This finding is consistent with a "plant-centred" approach to modelling, emphasizing the adaptive regulation of traits. Models that account for biodiversity will also need to partition community-level trait variation into components due to phenotypic plasticity and/or genotypic differentiation within species vs. progressive species replacement, along environmental gradients. Our analysis suggests that variation in N_{area} is about evenly split between these two modes.

1 Introduction

Nitrogen (N) is an essential nutrient for primary production and plant growth, and nitrogen content per unit leaf area (N_{area}) is a key variable in plant functional ecology and biogeochemistry. A strong correlation between leaf N and photosynthetic capacity has been observed, and is to be expected because typically almost half of the N in leaves is invested in the photosynthetic apparatus (Field and Mooney, 1986; Evans and Seemann, 1989; Evans, 1989). This component of N_{area} is approximately proportional to the maximum rate of carboxylation (V_{cmax}) at standard temperature, also expressed per unit area (Wohlfahrt et al., 1999; Takashima et al., 2004; Kattge et al., 2009). Cell walls account for a further significant fraction of leaf N (Lamport and Northcote, 1960; Niinemets and Tenhunen, 1997; Onoda et al., 2004). Leaf mass per area (LMA) is positively correlated with cell-wall N (Onoda et al., 2004) and is used as an index of plant investment in cell-wall biomass (Reich et al., 1991; Wright and Cannon, 2001). Thus, N_{area} can usefully be considered as the sum of a "metabolic" component related to V_{cmax} and a "structural" component proportional to LMA. Leaves with high V_{cmax} usually have high LMA, and so these two quantities can be at least partially correlated, as seen clearly (for example) in parallel vertical gradients of V_{cmax} and LMA within canopies of one species (e.g. Niinemets and Tenhunen, 1997). Across different species and environments, however, there is scope for considerable independent variation in V_{cmax} and LMA, implying the need to consider them separately.

Dynamic global vegetation models (DGVMs) are being extended to include interactive carbon (C) and N cycles (Thornton et al., 2007; Xu-Ri and Prentice, 2008; Zaehle and Friend, 2010), but there remain many open questions about the implementation of C–N coupling (Prentice and Cowling, 2013), including the control of leaf N content, which is treated quite differently by different models. For example, one common modelling approach predicts photosynthetic capacity from N_{area}, and N_{area} in turn from soil inorganic N supply (e.g. Luo et al., 2004). This implies an assumption that the soil environment, and soil microbial activity in particular, are the primary controls of N_{area} and photosynthetic capacity at the leaf level. An alternative assumption is that photosynthetic capacity is optimized as a function of irradiance, leaf-internal CO_2 concentration (c_i), and temperature (Haxeltine and Prentice, 1996; Dewar, 1996) – implicit in the widely used LPJ DGVM (Sitch et al., 2003) and other models derived from it, including LPJ-GUESS (Smith et al., 2001) and LPX (Prentice et al., 2011a; Stocker et al., 2013). This "plant-centred" approach embodies the idea that plant allocation processes (and thus, not soil microbial processes) determine leaf-level traits. Limited N supply, by this reasoning, should lead to the production of fewer leaves, rather than leaves with suboptimal capacity. More specifically, it is derived from a long-standing concept, the "coordination hypothesis", which states that the Rubisco- and electron transport-limited rates of photosynthesis tend to be co-limiting under average daytime conditions (Chen et al., 1993; Haxeltine and Prentice, 1996; Maire et al., 2012). Co-limitation is optimal – even though mechanistically, it may be an inevitable outcome of leaf metabolism (Chen et al., 1993) – in the sense that it provides the right balance of investments in the biochemical machineries for carboxylation and electron transport. It implies that enzyme activities adjust, over relatively long periods (weeks or longer), so that co-limitation holds. An important consequence is that the predicted responses of photosynthetic traits and rates to environmental variables observed in the field (whether temporally, comparing different seasons, or spatially, comparing different environments) are substantially different from those seen in short-term laboratory experiments. Specifically, V_{cmax} (and thus the metabolic component of N_{area}) is predicted to be directly proportional to irradiance, to decrease with increasing $c_i : c_a$, and to decrease with increasing temperature. These predictions are supported in general terms by an observed positive relationship between N_{area} and irradiance (Field, 1983; Wright et al., 2005), a negative relationship between N_{area} and $c_i : c_a$ (Wright et al., 2003; Prentice et al., 2011b, 2014), and (in woody evergreens at least) a negative relationship between N_{area} and temperature (845 species: data from Wright et al., 2004). But there has been no systematic attempt to quantitatively assess the relationship of leaf N with environmental and structural predictors across environmental gradients. Such empirical work is needed to assess and underpin methods of C–N cycle coupling in DGVMs.

Here we set out to test the predictability of N_{area} using measurements carried out on dried plant material collected by the Terrestrial Ecosystem Research Network (TERN) AusPlots and Australian Transect Network facilities, at 27 sites on a north–south transect across the Australian continent. The transect extended from the wet–dry (monsoonal) tropics to the dry–wet (mediterranean) temperate zone via the arid interior, and encompassed substantial variation in all of the hypothesized controls of N_{area} (Fig. 1). The AusPlots protocol involves sampling all species within a 100×100 m plot (White et al., 2012). We measured N_{area}, $\delta^{13}C$, and LMA on all species at each site, and tested and quantified the effects of irradiance, $c_i : c_a$ ratio (from $\delta^{13}C$), temperature, LMA, and N-fixation ability (26 % of the species sampled were N-fixers) on variation in N_{area}. The sampling design also allowed us to implement the trait gradient analysis method introduced by Ackerly and Cornwell (2007), which has been surprisingly little used to date. A growing body of field mea-

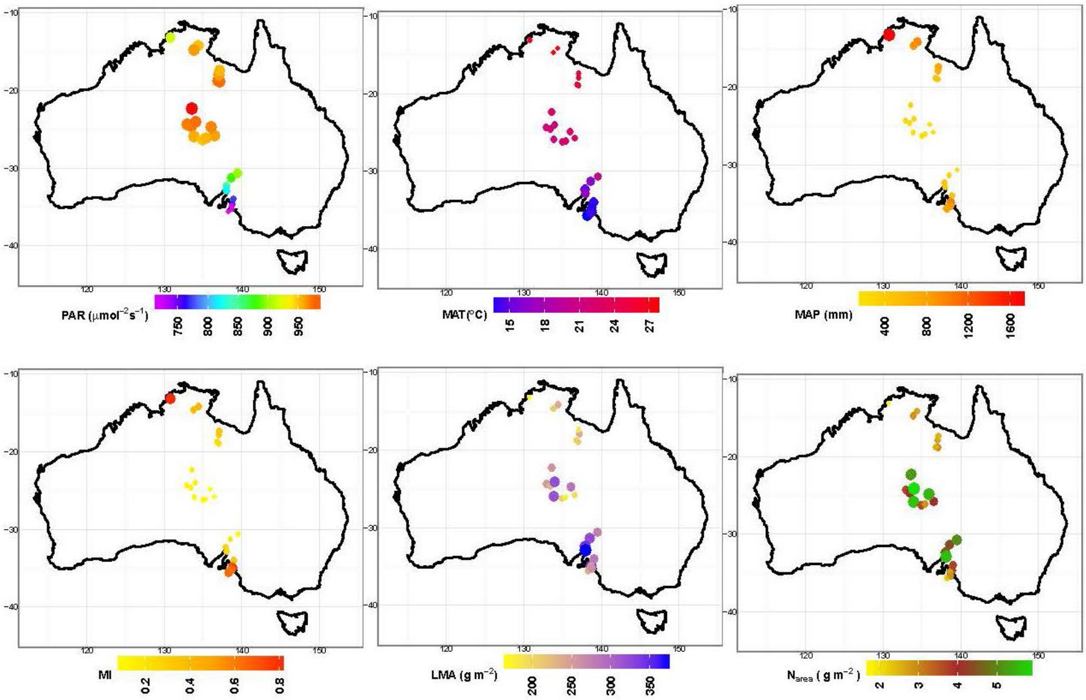

Figure 1. Site locations, climate, and leaf-trait distributions: mean annual precipitation (MAP, mm), mean annual temperature (MAT, °), mean incident daytime photosynthetically active radiation (PAR, μmol m^{-2} s^{-1}), moisture index (MI). Site mean N_{area} ($g m^{-2}$) and LMA ($g m^{-2}$) are also shown.

surements shows extensive leaf-trait variation within species and plant functional types (PFTs) (Kattge et al., 2011; Meng et al., 2015). Trait gradient analysis allows trait variation to be partitioned into a component due to variation within species and a component due to species replacement.

2 Materials and methods

Our analyses are based on 442 leaf measurements representing all species found in a 100 m × 100 m plot at each of 27 sites on a broad north–south transect across Australia (Fig. 1). We performed a regression analysis to test the relationships of N_{area} with mean annual temperature (MAT), irradiance, plant trait leaf mass per area (LMA), $c_i : c_a$ ratio, and N-fixation capacity. We also fitted a statistical model in which N_{area} was treated as the sum of a metabolic component proportional to predicted (optimal) photosynthetic capacity at standard temperature (based on temperature, irradiance, and $c_i : c_a$ ratio) and a structural component proportional to LMA. Finally, we carried out a trait gradient analysis in order to quantify the contributions of environment vs. species identity to variation in N_{area}, $c_i : c_a$ ratio, and LMA.

2.1 Climate data and analysis

Climatological data for the 27 sites were obtained from the eMAST/ANUClimate data set (www.emast.org.au), which extends from 1970 to 2012 with 1 km spatial resolution across the entire continent. Mean annual precipitation (MAP) over this period at the sampling sites ranged from 154 to 1726 mm and mean annual temperature (MAT) from 14.1 to 27.6°. The moisture index ($MI = P/E_q$, where P is mean annual precipitation and E_q is equilibrium evapotranspiration, calculated with the STASH program: Gallego-Sala et al., 2012) varied from 0.07 to 0.82. The mean incident flux of photosynthetically active radiation (PAR) during daylight hours, expressed as photosynthetic photon flux density (μmol m^{-2} s^{-1}), was also calculated using STASH. This incident flux (at the top of the canopy) was averaged through the canopy using Beer's law, as follows. First leaf area index (L) was estimated from a remotely sensed (MODIS NBAR-derived using MOD43A4: http://remote-sensing.nci.org.au/u39/public/html/modis/fractionalcover-clw) fractional cover of photosynthetic vegetation (f_v) at 1 km resolution at each site, from data assembled by the TERN AusCover facility (Guerschman et al., 2009):

$$L \approx -(1/k)ln(1 - f_v), \quad (1)$$

where $k = 0.5$. Then absorbed PAR per unit leaf area (I_L) was calculated as

$$I_L \approx I_0(1-e^{-kL})/L \approx I_0 k f_v / \ln\left[1/(1-f_v)\right], \quad (2)$$

where I_0 is the incident PAR above the canopy. This calculation yields $I_L \approx I_0$ for sparse vegetation ($L<1$), but I_L becomes progressively smaller than I_0 as foliage density increases, reflecting the fact that the irradiance experienced by the average species is much lower in, say, a closed woodland than in an open shrubland, even if the PAR incident at the top of canopy is the same. In dense vegetation I_L will underestimate the PAR exposure of canopy dominants and overestimate the PAR exposure of understorey species. However, the use of a canopy average in this way was a necessary approximation (because we did not have quantitative information about the canopy position of each species) and considered preferable to using I_0, which will systematically overestimate PAR exposure for most species in a dense community.

2.2 Foliage sampling and analysis

Mature outer-canopy leaves of each species were sampled during the growing season using the AusPlots methodology (White et al., 2012). (Note that in denser vegetation many species sampled are in the understorey, so their "outer-canopy" leaves are still shaded by the overstorey. Many species thus receive considerably reduced sunlight compared to the overstorey, implying that the canopy-average irradiance I_L is more suitable than the top-of-canopy value I_0 as a community measure of irradiance.) In total, the 27 selected sites included 442 unique species, of which 37 were C_4 plants (not analysed further here). LMA was measured on the archived leaf samples by scanning and weighing the leaves. Subsamples (a mixture of material from at least two replicates) were analysed for C and N contents and bulk $\delta^{13}C$ at the Stable Isotope Core Laboratory of Washington State University, USA. N_{area} was calculated from N content and LMA. Carbon isotope discrimination (Δ) values were derived from the reported $\delta^{13}C$ values using the standard formula

$$\Delta = (\delta_{air} - \delta_{plant})/(1+\delta_{plant}), \quad (3)$$

where δ_{air} is the carbon isotope composition of air and δ_{plant} is the carbon isotope composition of the plant material. Because of the different diffusion rates and biochemical rates of carboxylation between $^{13}CO_2$ and $^{12}CO_2$, Δ can be used to estimate the $c_i : c_a$ ratio as

$$c_i : c_a \approx (a+\Delta)/(b-a), \quad (4)$$

where the recommended standard values are $a = 4.4$ ‰ and $b = 27$ ‰ (e.g. Cernusak et al. 2013).

2.3 Analysis of V_{cmax}

Values of V_{cmax} were predicted based on the co-ordination hypothesis, by equating the carboxylation- and electron transport-limited rates of photosynthesis and, as a simplifying assumption, treating the electron transport-limited rate as proportional to absorbed PAR (i.e. ignoring the saturation of the electron transport rate at high irradiances). These assumptions lead to the following estimate:

$$V_{cmax} \approx \varphi_0 I_L (c_i + K)/(c_i + 2\Gamma^*), \quad (5)$$

where φ_0 is the intrinsic quantum efficiency of photosynthesis (0.093: Long et al., 1993), c_i is the leaf-internal concentration of CO_2, K is the effective Michaelis–Menten coefficient of Rubisco, and Γ^* is the photorespiratory compensation point. Values of both these quantities and their activation energies (governing their temperature responses) are based on the empirical in vivo determinations by Bernacchi et al. (2001) widely used in photosynthesis research. Both K and Γ^* were evaluated at standard atmospheric pressure and oxygen concentration, and site MAT. Predicted values of V_{cmax} were adjusted to 25°, because the amount of N allocated to Rubisco and other enzymes involved in carboxylation should be proportional to V_{cmax} at a standard temperature, not at the growth temperature.

2.4 Statistical methods

All statistics were performed in R3.1.3 (R Core Team, 2015). Linear regressions were fitted using the *lm* function, partial residual plots were generated using the *visreg* package, and the relative contributions of different predictors were quantified using the Lindeman et al. (1980) method as implemented in the *relaimpo* package. In a first, exploratory statistical analysis, a linear model was fitted for ln N_{area} with $c_i : c_a$, MAT, ln I_L, ln LMA, and the factor "N-fixer" as predictors. The regression slopes of ln N_{area} against $c_i : c_a$, MAT and ln I_L can all be independently predicted from the co-ordination hypothesis by differentiation of Eq. (5) (see Appendix A; note that these formulae explicitly predict the slopes for ln N_{area}). These predicted values were compared with the fitted values and their 95 % confidence limits in order to assess support for the co-ordination hypothesis.

In a second analysis, community-mean values were calculated as simple averages across the species in each plot, omitting the factor "N-fixer". A linear model was fitted to the community means of ln N_{area} as a function of $c_i : c_a$, MAT, ln I_L, and ln LMA to assess the predictability of leaf N at the community level.

In a third analysis, N_{area} was modelled as a linear combination of the predictors Rubisco N, $N_{rubisco}$ (derived from predicted V_{cmax} at 25°), and structural N, $N_{structure}$ (derived from LMA using the empirical relationship $N_{structure} = 10^{-2.67}$ $LMA^{0.99}$, in g m^{-2}: Yusuke Onoda,personal communication 2015), including "N-fixer" as a factor and allowing interactions of the predictors with this factor.

2.5 Trait gradient analysis

Trait gradients were generated for ln LMA, ln N_{area}, and $c_i : c_a$ following the analysis method of Ackerly and Cornwell (2007), again using simple averages across species to estimate community means. In this analysis species trait values were plotted against site-mean trait values. By definition, the regression of the species trait values against site-mean trait values has a slope of unity. For a perfectly plastic trait, regression of trait variation within species against the site-mean trait values would also yield a slope of unity. The common within-species slope that this approach provides is a measure of the fraction of trait variation due to phenotypic plasticity and/or genotypic variability. Its one-complement measures the fraction due to species turnover. Natural log transformation was applied to LMA and N_{area} because of their large variance and skewed distributions, but not to $c_i : c_a$, because of its small variance and approximately normal distribution.

3 Results

3.1 Leaf N variations with climate and leaf traits

Significant partial relationships were found for ln N_{area} vs. $c_i : c_a$, MAT, and ln I_L (Table 1, Fig. 2). The relationship was negative for $c_i : c_a$, as expected, because lower $c_i : c_a$ implies that a greater photosynthetic capacity is required to achieve a given assimilation rate (or equivalently: a stronger CO_2 drawdown is enabled by a higher V_{cmax}). The relationship was also negative for MAT, as expected, because there is an inverse relationship between temperature and the quantity of leaf proteins required to support a given value of V_{cmax}. The relationship was positive for ln I_L (PAR), as expected, because the higher the irradiance, the greater the carboxylation capacity required for co-limitation with the rate of electron transport.

Theoretical slopes for these relationships (derived in Appendix A) are compared with the fitted slopes in Table 1. For ln N_{area} vs. ln I_L, the theoretical slope is unity. The fitted slope of 0.874 (95 % confidence limits: 0.685, 1.063) was statistically indistinguishable from unity. (A slope significantly greater than unity was found for ln N_{area} vs. ln I_0, i.e. top-of-canopy PAR, as expected, as this measure underestimates the change in mean canopy PAR along the gradient from sparse, high-PAR to dense, lower-PAR communities.) For ln N_{area} vs. $c_i : c_a$, the fitted slope of -0.611 (-1.107, -0.115) was fortuitously close to the theoretical slope of -0.615, although the value was only weakly constrained for these data. For ln N_{area} vs. MAT, the theoretical slope was obtained by subtracting the "kinetic" slope of ln V_{cmax} vs. temperature (from the activation energy of carboxylation as given by Bernacchi et al., 2001) from the shallow positive slope implied by Eq. (5). The kinetic effect was dominant and results in an overall predicted negative slope of -0.048. The fitted slope of -0.047 (-0.060, -0.034) was indistinguishable from this theoretical slope, indicating acclimation to temperature by diminished allocation of N to metabolic functions at higher temperature, offsetting the increased reaction rate predicted by the Arrhenius equation. However, this slope was shallower than would be predicted by the Arrhenius equation alone, reflecting the reduced quantum efficiency of assimilation (a higher V_{cmax} is required to support a given assimilation rate) at higher temperatures.

The proportion of leaf N allocated to Rubisco has generally been found to decline, while the total N allocated to cell walls increases with increasing LMA (Hikosaka and Shigeno, 2009). Figure 2 shows a strong positive partial relationship between ln N_{area} and LMA. N-fixers had generally higher N_{area} than non-N-fixers (Fig. 2e: $p<0.001$). The predictors together explained 55 % of the variation in leaf N across species and sites.

Fully 82 % of the variation in the community-mean value of ln N_{area} could be explained by the combination of community-mean LMA and environmental variables. Significant partial relationships of community-mean ln N_{area} with MAT, ln I_L, and ln LMA (Table 2) were consistent with the results obtained at species level. The fitted slopes of ln N_{area} against ln I_L and MAT were again indistinguishable from the theoretical values, albeit with wide error bounds due to the much smaller sample size (27 as opposed to 405). The community-level partial relationship between ln N_{area} and $c_i : c_a$ showed a negative slope as predicted, although this relationship was barely significant ($p \approx 0.1$) due to the small sample size.

3.2 Leaf N as the sum of metabolic and structural components

Highly significant ($p< 0.001$) positive relationships were found between N_{area} and the predicted Rubisco-N content per unit leaf area ($N_{rubisco}$), and the predicted cell-wall N content per unit leaf area ($N_{structure}$) (Fig. 3). A priori we would expect the regression coefficient for $N_{structure}$ to be close to unity, and that for $N_{rubisco}$ to be about 6 to 20 (if Rubisco constitutes about 5 to 15 % of total leaf protein: Evans, 1989; Evans and Seemann, 1989; Onoda et al., 2004). The fitted slopes of 1.2 ($p< 0.001$; 95 % confidence limits: 1.0, 1.4) and 9.5 ($p< 0.001$; 7.6, 11.5) in Table 3 respectively were consistent with these expectations.

There was no significant main effect of the factor "N-fixer" and no significant interaction between $N_{rubisco}$ and the factor "N-fixer". The co-ordination hypothesis predicts that the metabolic component of N_{area} should be environmentally optimized and therefore independent of N supply. This could not be tested without direct measurements of V_{cmax} or $N_{rubisco}$, which were precluded by the design of this study. However, N-fixers showed a steeper relationship between N_{area} and $N_{structure}$. This was manifested as a significant inter-

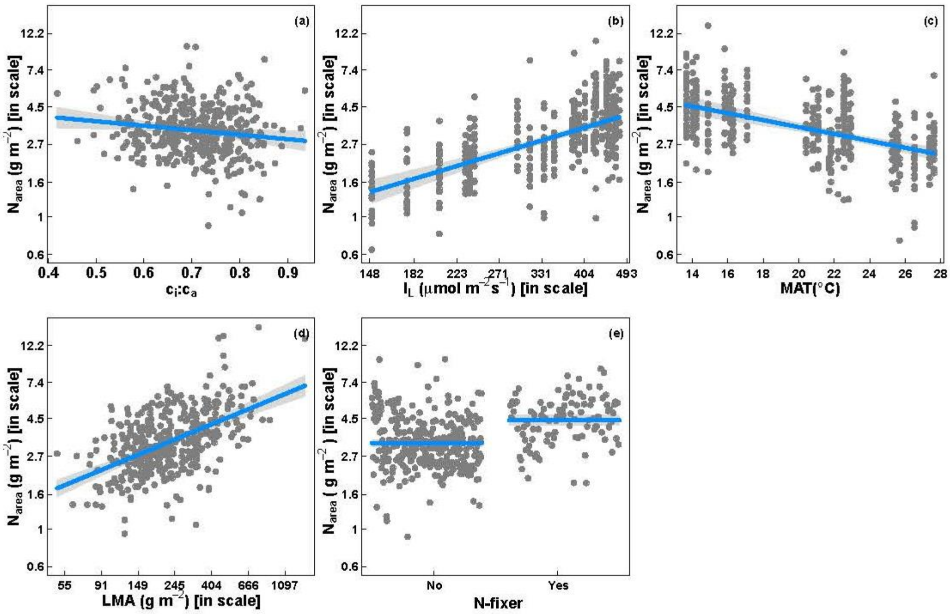

Figure 2. Partial residual plots for the regression of ln N_{area} ($g\,m^{-2}$) as a function of $c_i : c_a$ (from $\delta^{13}C$), ln (mean canopy PAR, I_L) ($\mu mol\,m^{-2}\,s^{-1}$), MAT (°), ln LMA ($g\,m^{-2}$), and the factor "N-fixer" at species level. Note the logarithmic scale of the y axis.

Table 1. Linear regression coefficients for ln N_{area} ($g\,m^{-2}$) as a function of $c_i : c_a$ (from $\delta^{13}C$), ln (mean canopy PAR, I_L) ($\mu mol\,m^{-2}\,s^{-1}$), MAT (°), ln LMA ($g\,m^{-2}$), and the factor "N-fixer" at species level.

	Estimated	Predicted	p	Relative importance	R^2
$c_i : c_a$	-0.611 ± 0.252	-0.615	< 0.01	14 %	
ln I_L	0.874 ± 0.096	1	< 0.001	19 %	
MAT	-0.047 ± 0.007	-0.048	< 0.001	9 %	55 %
ln LMA	0.415 ± 0.036	n/a	< 0.001	39 %	
"N-fixer"	0.306 ± 0.041	n/a	< 0.001	19 %	

n/a: not applicable.

action between the factor "N-fixer" and $N_{structure}$ ($p < 0.01$). This model, in which N_{area} was decomposed into a metabolic component predicted by the co-ordination hypothesis and a structural component proportional to LMA, explained 52 % of the variance in N_{area} across species and sites. The relative importance of variations in the metabolic and structural components was determined to be 39 and 61 % respectively, showing inter alia the importance of variation in LMA in determining leaf N content.

3.3 Quantifying trait plasticity vs. species turnover

In total, 243 C_3 species were sampled at two or more sites. These species allowed calculation of a common slope, being an estimate of trait plasticity *sensu lato* (that is, phenotypic plasticity or genetic adaptation or both) across species (Fig. 4), for the traits $c_i : c_a$, ln LMA, and ln N_{area}. Contrasting results were obtained for the three traits. It appeared that $c_i : c_a$ is perfectly plastic, with a common (within-species) slope indistinguishable from unity. The common slope of N_{area} was close to 0.5, indicating approximately equal contributions of plasticity and species turnover to the total variation. In the case of LMA, however, there was significant heterogeneity ($p < 0.05$) among the within-species slopes, with *Marsdenia viridiflora* showing a significantly steeper slope than the other species. After excluding this species, the common slope for LMA was also close to 0.5. A positive common slope indicates the ability of species to adapt their leaf morphology to environment. The positive common slope found for N_{area} is consistent with this trait's nature as a combination of metabolic and structural components; its similar-

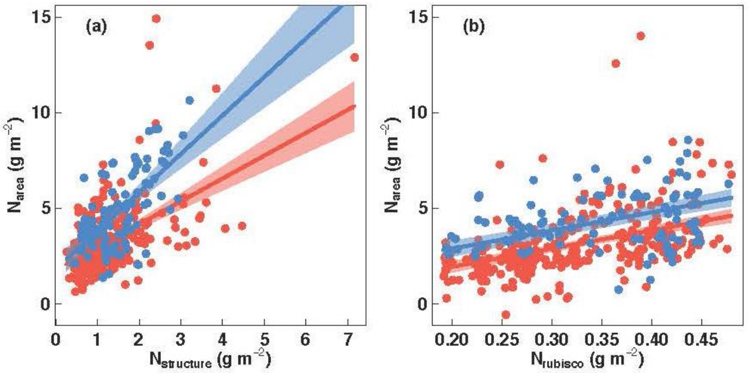

Figure 3. Partial residual plots for the linear regression of $\mathrm{N_{area}}$ as a function of independently predicted values of $\mathrm{N_{rubisco}}$ and $N_{\mathrm{structure}}$ (all in $\mathrm{g\,m^{-2}}$) at species level. Blue: N-fixers; red: non-N-fixers.

Table 2. Linear regression coefficients for community-mean (simple average) values of ln $\mathrm{N_{area}}$ ($\mathrm{g\,m^{-2}}$) as a function of $c_i : c_a$ (from δ^{13}C), ln (mean canopy PAR, I_L) ($\mathrm{\mu mol\,m^{-2}\,s^{-1}}$), MAT (°), and ln LMA ($\mathrm{g\,m^{-2}}$).

	Estimated	Predicted	p	Relative importance	R^2
$c_i : c_a$	-1.60 ± 0.94	-0.615	n.s.	42 %	82 %
ln I_L	0.70 ± 0.23	1	<0.001	20 %	
MAT	-0.035 ± 0.016	-0.048	<0.001	11 %	
ln LMA	0.57 ± 0.19	n/a	<0.001	27 %	

n.s.: no significance. n/a: not applicable.

ity to the slope for LMA is consistent with the importance of variations in structural N in determining total N.

4 Discussion

4.1 Leaf N and environment

The variety of environments provided in this study by the long transcontinental transect, and the number of species sampled, allowed us to statistically separate the effects of $c_i : c_a$, irradiance, temperature, and LMA on $\mathrm{N_{area}}$. The relationships with $c_i : c_a$, irradiance, and temperature were in the directions and magnitudes predicted by the co-ordination hypothesis. The relationship with site mean irradiance had a slope as predicted by the co-ordination hypothesis (i.e. close to 1), but a strong relationship, with a steeper slope as expected, was found when top-of-canopy irradiance was used instead of the canopy mean – indicating that both spatial variations and within-canopy shading were contributing to the relationship with site mean irradiance. We performed an additional regression using leaf nitrogen content per unit mass ($\mathrm{N_{mass}}$) which showed, as expected, identical fitted coefficients for all predictors except LMA (Appendix B). However, because of the regression coefficient of ln $\mathrm{N_{area}}$ with respect to ln LMA < 1, the regression coefficient of ln $\mathrm{N_{mass}}$ with respect to ln LMA < 0, i.e. $\mathrm{N_{mass}}$, declines with increasing LMA – as has been widely reported. We also tried a regression of $\mathrm{N_{mass}}$ on the same set of predictors but without the inclusion of LMA; this yielded a much poorer fit and is not shown.

High $\mathrm{N_{area}}$ in plants from arid environments has been described often, and has traditionally been explained as a consequence of high N supply in environments with low rainfall (reducing leaching losses) and restricted plant cover (reducing total vegetation N demand) (e.g. Field and Mooney, 1986). This explanation would imply that plants in wetter environments have lower (and suboptimal) $\mathrm{N_{area}}$ due to low availability of N. However, the negative relationship commonly found between $c_i : c_a$ and $\mathrm{N_{area}}$ supports an alternative, adaptive (plant-centred) explanation. The least-cost hypothesis (Wright et al., 2003; Prentice et al., 2014) predicts lower $c_i : c_a$ in drier environments. This is because the drier the atmosphere, the greater the flux of water required to support a given rate of assimilation, which in turn shifts the balance of costs and benefits towards investment in photosynthetic capacity (V_{cmax}) and away from water transport capacity. When $c_i : c_a$ is lower, the co-ordination hypothesis predicts that a higher V_{cmax} (and therefore higher $\mathrm{N_{area}}$) will be optimal, in order for the leaves to fully utilize the available light. The co-ordination hypothesis also predicts a further in-

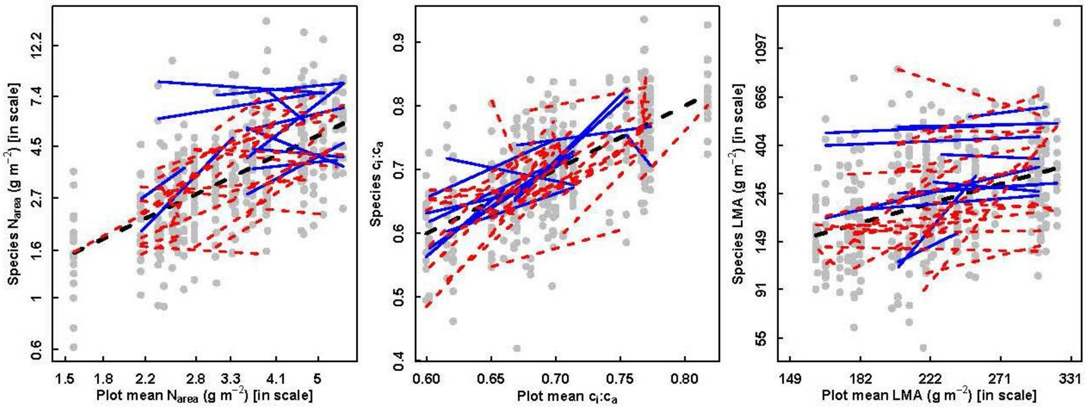

Figure 4. Trait means and regression lines for all 243 C_3 plant species in the 27 study sites. Note the logarithmic scales for N_{area} ($g\,m^{-2}$) and LMA ($g\,m^{-2}$). Thin red dashed lines represent individual within-species regression lines of non-N-fixer species. Thin blue lines represent individual within-species regression lines of N-fixer species. The black dashed line represents the overall regression line, which has a slope of unity by definition. Grey dots denote individual species–site combinations. Common within-species slopes are 0.53 ± 0.11 (ln N_{area}), 1.02 ± 0.12 ($c_i : c_a$), and 0.55 ± 0.11 (ln LMA).

Table 3. Linear regression coefficients for N_{area} as a function of independently predicted values of $N_{rubisco}$ and $N_{structure}$ (all in $g\,m^{-2}$) at species level.

	Estimated	Predicted	p	Relative importance	R^2
$N_{rubisco}$	9.5 ± 2.0	6–20	< 0.001	39 %	
$N_{structure}$	1.2 ± 0.2	1	< 0.001	61 %	52 %
$N_{structure}$: "N-fixer"	1.0 ± 0.3	n/a	< 0.01	n/a	

n/a: not applicable.

crease in N_{area} with increasing aridity due to reduced cloudiness and reduced shading by competitors, both factors tending to increase I_L (and both apparently contributing to the fitted relationship of N_{area} with I_L). Thus the co-ordination hypothesis could account for independent positive effects of site irradiance and aridity on N_{area}, as previously reported by Wright et al. (2005). The fitted relationship of N_{area} with temperature, PAR, and $c_i : c_a$ is consistent with our theoretical prediction, which implicitly includes all of these effects.

Despite the large within-site variation in LMA found at all points along the aridity gradient, there is a significant tendency for LMA to increase with aridity, perhaps because of the resistance to dehydration conferred by stiffer leaves (Niinemets, 2001; Wright and Westoby, 2002; Harrison et al., 2010) and/or the need for leaves to avoid overheating under transient conditions of high radiation load and low transpiration rates combined with low wind speed (Leigh et al., 2012). This increase in LMA is inevitably accompanied by an increasing structural N component.

Thus, several distinct aspects of plant allocation tend to increase N_{area} along gradients of increasing dryness. The predicted response of $N_{rubisco}$ to temperature is a result of opposing effects: the declining efficiency of photosynthesis with increasing temperature (due to the temperature dependencies of K and Γ^*) is offset by the increased catalytic capacity of Rubisco at higher temperatures. The latter effect is predicted to be stronger, implying reduced N_{area} with increasing temperature, as observed.

4.2 The predictability of leaf N

Predicted $N_{rubisco}$ and $N_{structure}$ together explained more than half of the variation in total N_{area} across species and sites. Our approach to predicting these two quantities invokes a simplified formula, Eq. (5), which is based on the co-ordination hypothesis for $N_{rubisco}$, assuming proportionality with Rubisco capacity, and assumes a simple proportionality with LMA for $N_{structure}$. Our finding of highly significant multiple regression coefficients for both variables indicates that the prediction obtained when taking both into account is more accurate than could be obtained from either variable alone. Osnas et al. (2013), analysing a large global leaf-trait data set and applying a novel method to determine the extent to which different traits are area- vs. mass-proportional, found leaf N to be an intermediate case. This is to be expected if leaf N is, as our results suggest, a composite of an area-proportional ($N_{rubisco}$) and a mass-proportional ($N_{structure}$) component. The two predictors (Rubisco capacity and LMA) are not fully independent, because leaves with higher photosynthetic capacity tend to have higher LMA for structural reasons. But such leaves must have increased structural N as well. By showing independently significant regression coefficients for modelled $N_{rubisco}$ and LMA, the multiple regression results establish that successful prediction of N_{area} requires consideration of both components, and that each has an independent effect, irrespective of their correlation ($r^2 = 0.28$ in this data set). Osnas et al. (2013) also fitted various statistical models for the relationships among leaf traits. Their "model LN" for ln N_{area} vs. ln LMA yielded a slope of 0.38 (95 % confidence interval 0.36 to 0.40). This value, based on a global data set, can be compared directly with – and is indistinguishable from – our fitted partial regression coefficient of ln Narea vs. ln LMA, which is 0.42 (0.34 to 0.49) (Table 1).

In reality, however, leaf N does not consist exclusively of Rubisco and cell-wall constituents. Leaf N includes multiple additional components, including other photosynthetic proteins, proteins of the light-harvesting complexes and electron transport chains, cytosolic proteins, ribosomes and mitochondria, nucleic acids (which account for about 10–15 % of leaf N: Chapin III and Kedrowski, 1983), and N-based defensive compounds. It is possible that the higher N found for N-fixers resides in N-based osmolytes (Erskine et al., 1996) or defence compounds (Gutschick, 1981). Nonetheless, our simplifications suggest that N_{area} – especially at the community level, which is key for large-scale modelling – is, to first order, inherently predictable from leaf morphology and the physical environment. A corollary is that limitation in N supply may act primarily by changing plant allocation patterns (reducing allocation to light capture by leaves while increasing allocation to N uptake by roots), rather than by altering leaf stoichiometry.

4.3 Trait variations within and between species

By testing for acclimation along spatial gradients, the design of our study did not allow phenotypic plasticity to be distinguished from genetic adaptation. Phenotypic plasticity is the ability of a genotype to alter its expressed trait values in response to environmental conditions (Bradshaw, 1965; Sultan, 2000). A part of the observed variation in trait values within species could be due to shifts in the occurrence and frequency of different genotypes, producing different preferred trait values. Thus, when we refer to traits as "plastic", this should be understood in a broad sense to allow the possibility of a genetic component of the observed adaptive differentiation within species. Seasonal acclimation within individual plants can provide more direct evidence for phenotypic plasticity (Togashi et al., 2017), whereas in this study we disregard possible seasonal variations and instead relate trait variations to the mean annual environment. However, by sampling all of the species present at each site and including measurements on species at multiple sites, we could distinguish between the contribution of plasticity sensu lato (phenotypic plasticity and/or genetic adaptation) vs. species turnover, i.e. the progressive replacement of species with different mean trait values, to spatial variation in the community-mean values of a given trait. We found that $\delta^{13}C$ was perfectly plastic, perhaps not surprisingly, as variations in $c_i : c_a$ are under stomatal control. In contrast, LMA and N_{area} showed approximately equal contributions from plasticity and species turnover.

4.4 Implications for modelling

There has been a surge of interest in schemes to predict continuous trait variation in DGVMs (e.g. Scheiter et al., 2013; Fyllas et al., 2014; van Bodegom et al., 2014; Ali et al., 2015; Fisher et al., 2015; Meng et al., 2015; Sakschewski et al., 2015). Some trait-based modelling approaches have relied on empirical information on trait–trait and trait–environment covariation, but others (e.g. Scheiter et al., 2013) have aimed to represent the adaptive nature of trait variation explicitly. Our focus has been on testing an explicit adaptive hypothesis for the controls of one key trait, N_{area}, which in addition to a structural component (necessarily linked to LMA) includes an important metabolic component, reflecting the leaf-level investment in photosynthetic proteins. All models that attempt to represent the coupling between C and N cycles in terrestrial ecosystems require a method to calculate leaf N content, given other environmental and plant characteristics. Some models prescribe fixed values of V_{cmax} (per plant functional type), but this approach does not take account of the observed variation in V_{cmax} with environmental conditions. Models that assume proportionality between V_{cmax} and N_{area} neglect the important variation in leaf structural N. We have shown that N_{area} is predictable, to a degree that is useful for modelling, when both metabolic and structural components are taken into account. Our prediction is based on LMA, $c_i : c_a$, and a theoretically predicted value of V_{cmax} based on the co-ordination hypothesis – for which there is strong independent evidence (e.g. Maire et al., 2012). The partial responses of N_{area} to $c_i : c_a$, irradiance, and temperature are consistent with predictions of the co-ordination hypothesis, and the inclusion of predicted V_{cmax} adds significantly and substantially to the predictive power of LMA and $c_i : c_a$ alone. As both LMA (Wright et al., 2005) and $c_i : c_a$ (Prentice et al., 2014) show relationships with environment, our results suggest a possible route towards a general adaptive scheme for the prediction of major leaf traits in DGVMs, which would be an improvement on models that assume a one-to-one relationship between photosynthetic capacity and N_{area} (see e.g. Adams et al., 2016, who showed that there is considerable variation in N_{area} among N-fixers that is unrelated to photosynthetic capacity). Our results also suggest some priorities for trait data collection and analysis: to test the predicted controls of N_{area} over a wider range of environments, and to test the predicted environmental controls of V_{cmax} directly in the field.

Our application of trait gradient analysis also points out a way towards process-based treatments of functional trait diversity in next-generation models. It is increasingly accepted that models could, and should, sample "species" from continuous gradients of traits rather than fix the traits associated with discrete PFTs. A hybrid approach to modelling N_{area} based on the present analysis would consider N_{area} explicitly as the sum of metabolic and structural components. The metabolic component would be treated as plastic and subject to environmental optimization (in space and time), consistent with the least-cost and co-ordination hypotheses. The structural component would be tied to LMA, which is a key variable of the "leaf economics spectrum" (Wright et al., 2004), strongly expressed both within and between environments and therefore requiring a broad range of values to be assigned to model "species".

Finally, we note that if our results can be corroborated more widely, this would point to the need for a shift in the way N "limitation" is treated – both in models and in analyses of field data. In studies of the relationship between V_{cmax} and leaf N, for example, it is conventional to plot N on the x axis and V_{cmax} on the y axis, and it is then often stated that the positive relationship found shows that variation in leaf N "causes" variation in V_{cmax}. But all that is shown on the graph is a correlation, and our "plant-centred" interpretation is the opposite of the conventional one: that is, V_{cmax} is adaptively matched (acclimated) to environmental conditions, and the metabolic component of leaf N is a consequence of this acclimation. Low N availability would then result in reduced allocation of C (and N) to leaves, and increased allocation below ground – which is also an adaptive response, but at the whole-plant rather than the leaf level.

Appendix A: Theoretical responses of N_{area} to environmental predictors

We estimate optimal V_{cmax} by $\varphi_0 I_{\mathrm{L}}(c_i + K)/(c_i + 2\Gamma^*)$ (Eq. 5). Holding other variables constant, the sensitivity of this estimate to absorbed PAR is given by the derivative of its natural logarithm with respect to ln I_{L}:

$$\partial \ln V_{\mathrm{cmax}}/\partial \ln I_{\mathrm{L}} = 1. \tag{A1}$$

Similarly, the sensitivity of this estimate to c_i is given by

$$\partial \ln V_{\mathrm{cmax}}/\partial c_i = (2\Gamma^* - K)/[(c_i + K)(c_i + 2\Gamma^*)] \tag{A2}$$

and its sensitivity to the $c_i : c_a$ ratio is smaller than this by a factor c_a.

Temperature-dependent reaction rates are described by the Arrhenius equation:

$$\ln x(T) - \ln x(T_{\mathrm{ref}}) = (\Delta H/R)(1/T_{\mathrm{ref}} - 1/T), \tag{A3}$$

where x is the rate parameter of interest, T is the measurement temperature (K), T_{ref} is the reference temperature (here 298 K), ΔH is the activation energy of the reaction ($\mathrm{J\,mol^{-1}\,K^{-1}}$), and R is the universal gas constant ($8.314\,\mathrm{J\,mol^{-1}\,K^{-1}}$). Linearizing Eq. (A3) around T_{ref} yields

$$\ln x(T) - \ln x(T_{\mathrm{ref}}) \approx (\Delta H/RT_{\mathrm{ref}}^2)\Delta T, \tag{A4}$$

where $\Delta T = T - T_{\mathrm{ref}}$. Thus, from Eq. (5),

$$\ln V_{\mathrm{cmax25}} \approx \ln V_{\mathrm{cmax}} - (\Delta H_{\mathrm{v}}/RT_{\mathrm{ref}}^2)\Delta T, \tag{A5}$$

where ΔH_{v} is the activation energy of V_{cmax}. The sensitivity of V_{cmax25} to T is then

$$\begin{aligned}\partial \ln V_{\mathrm{cmax25}}/\partial T &= \partial \ln V_{\mathrm{cmax}}/\partial T - (\Delta H_{\mathrm{v}}/RT_{\mathrm{ref}}^2)\\ &= (\partial K/\partial T)/(c_i + K) - 2(\partial \Gamma^*/\partial T)/\\ &\quad (c_i + 2\Gamma^*) - (\Delta H_{\mathrm{v}} R/T_{\mathrm{ref}}^2),\end{aligned} \tag{A6}$$

where $K = K_{\mathrm{c}}\,(1 + O/K_{\mathrm{o}})$, hence

$$\begin{aligned}\partial K/\partial T &= \partial K_{\mathrm{c}}/\partial T\\ &\quad + [(\partial K_{\mathrm{c}}/\partial T)\,K_{\mathrm{o}} - (\partial K_{\mathrm{o}}/\partial T)\,K_{\mathrm{c}}]O/K_{\mathrm{o}}^2,\end{aligned} \tag{A7}$$

where O is the atmospheric concentration of oxygen and $\Gamma*$ and the Michaelis–Menten coefficients for carboxylation (K_{c}) and oxygenation (K_{o}) respectively have values at T_{ref} (in $\mathrm{\mu mol\,mol^{-1}}$) and activation energies as given by Bernacchi et al. (2001).

Appendix B: Partial responses of N_{mass} to environmental predictors

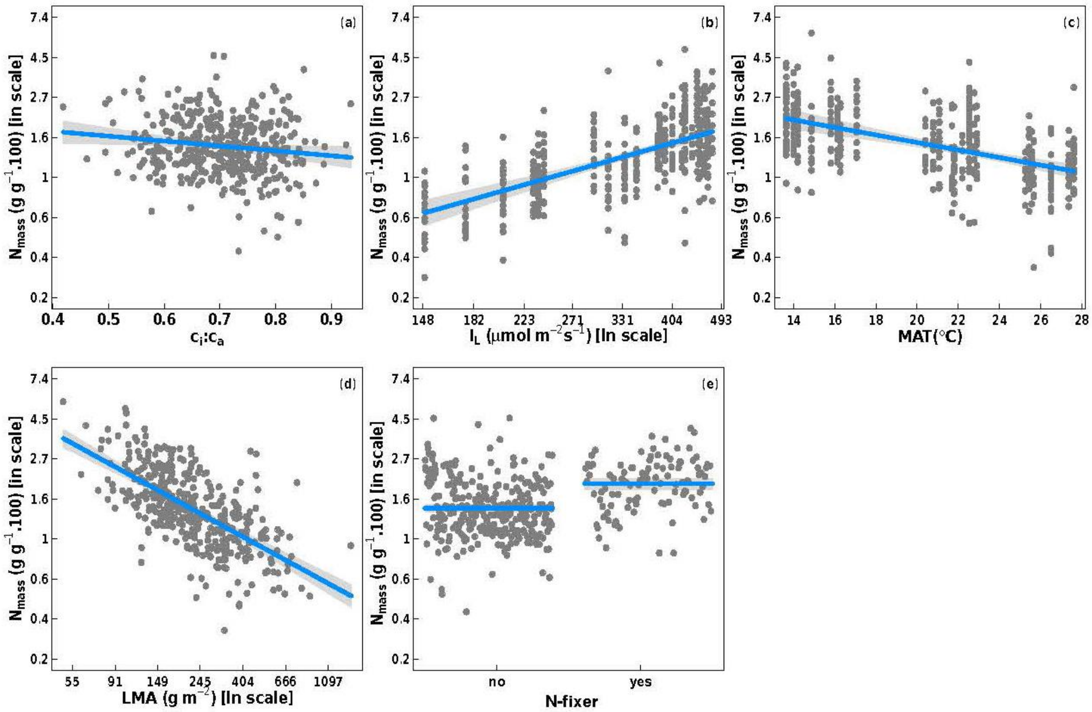

Figure B1. Partial residual plots for the regression of ln ($N_{mass} \times 100$) ($g\,g^{-1}$) as a function of $c_i : c_a$ (from $\delta^{13}C$), ln (mean canopy PAR, IL) ($\mu mol\,m^{-2}\,s^{-1}$), MAT (°C), ln LMA ($g\,m^{-2}$), and the factor "N-fixer" at species level.

Table B1. Linear regression coefficients for ln ($N_{mass} \times 100$) ($g\,g^{-1}$) as a function of $c_i : c_a$ (from $\delta^{13}C$), ln (mean canopy PAR, I_L) ($\mu mol\,m^{-2}\,s^{-1}$), MAT (°), ln LMA ($g\,m^{-2}$), and the factor "N-fixer" at species level. Note that N_{mass} was multiplied by 100 before logarithmic transformation.

	Estimated	Predicted	p	R^2
$c_i : c_a$	-0.611 ± 0.252	-0.615	< 0.01	
ln I_L	0.874 ± 0.096	1	< 0.001	
MAT	-0.047 ± 0.007	-0.048	< 0.001	
ln LMA	-0.585 ± 0.036	n/a	< 0.001	51 %
"N-fixer"	0.306 ± 0.041	n/a	< 0.001	

n/a: not applicable.

Information about the Supplement

Species analyzed in this study can be found in Supplement S1.

The Supplement related to this article is available online at doi:10.5194/bg-14-481-2017-supplement.

Author contributions. Iain Colin Prentice, Ning Dong, and Andrew J. Lowe planned and designed the study; Ning Dong carried out all the field measurements and performed the data analyses. Ning Dong and Iain Colin Prentice wrote the first draft; Bradley J. Evans supported the study through provision of climate data; Ian J. Wright assisted with data interpretation, contributed with ideas throughout, and suggested important improvements to the text. Stefan Caddy-Retalic contributed important ideas to improve the text. All authors contributed to subsequent versions.

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Establishment of an ecosystem transect to address climate change policy questions for natural resource management

DEWNR Technical report 2016/04

Government of South Australia
Department of Environment, Water and Natural Resources

Establishment of an ecosystem transect to address climate change policy questions for natural resource management

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Foreword

The Department of Environment, Water and Natural Resources (DEWNR) is responsible for the management of the State's natural resources, ranging from policy leadership to on-ground delivery in consultation with government, industry and communities.

High-quality science and effective monitoring provides the foundation for the successful management of our environment and natural resources. This is achieved through undertaking appropriate research, investigations, assessments, monitoring and evaluation.

DEWNR's strong partnerships with educational and research institutions, industries, government agencies, Natural Resources Management Boards and the community ensures that there is continual capacity building across the sector, and that the best skills and expertise are used to inform decision making.

Sandy Pitcher
CHIEF EXECUTIVE
DEPARTMENT OF ENVIRONMENT, WATER AND NATURAL RESOURCES

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Summary

- In this report we present a real world example from a research institution–government partnership in South Australia for climate change biodiversity conservation planning.
- Climate change is expected to be a significant driver of ecosystem change and, given a range of additional anthropogenic impacts (e.g. habitat clearance), evidence-based management is crucial to minimise biodiversity loss during this change. However, whether science can effectively guide biodiversity management through climate change has been a long-standing question, as individual ecological studies often lack immediate policy relevance or direct policy recipients.
- The program, Transects for Environmental Monitoring and Decision Making (TREND), which was completed in 2014, used a range of iterative processes starting with policy drivers and questions that informed the scientific program, two-way dialogue on the research and its relevance to policy, translation of peer-reviewed findings into policy relevant products, and the identification of gaps for future activities.
- Specifically the science–policy integration model involved a seven stage process:
    - Determine policy drivers
    - Develop scientific framework
    - Generate initial data
    - Review approach
    - Major research phase
    - Primary policy translation phase
    - Program review
- At the inception of TREND, environmental agencies in South Australia had high-level climate change policies but a perceived lack of specific data on ecosystem climate sensitivity. TREND provided these data via policy fora that ensured the project research was directed towards relevant policy imperatives and established research–policy connections at the time of the work. The project research, which was based on existing data and field measurements, suggested climate change may result in significant changes to the species composition of terrestrial ecosystems, and identified species and habitats that are climatically adaptable or that have limited tolerances.
- To help derive practical and useful evidence-based guidelines, specific policy relevant questions developed by the project team were answered using project results and associated knowledge. These questions included:
    - What drives species composition and how will this be affected by climate change?
        - What species or ecosystems could provide early indicators of stress?
        - What species and ecological communities are most and least at risk from climate change and what are the expected impacts?
        - How will climate change interact with other disturbance to influence ecosystem attributes?

    - What adaptation strategies could improve the resilience of key species and communities?
        - What shifts in distribution, species composition and ecological characteristics can we expect?
        - What are the implications for conservation planning and landscape design?

- TREND successfully established partnerships, generated policy-relevant data on climate sensitivity, effectively leveraged other research and scientific infrastructure funding (more than 10x the original project costs for TREND), formed the blueprint for a national climate change ecosystem monitoring network (the Australian Transect Network - part of the Terrestrial Ecosystem Research Network; http://www.tern.org.au), and produced excellent quality scientific knowledge and research results (published over 25 peer reviewed papers). In addition, an independent review of all science outputs relevant to climate change ecosystem resilience planning for the Adelaide & Mt Lofty Ranges NRM region found that the TREND project outputs were able to be directly applied to the region's on-ground management.
- A full breakdown of the project outputs and outcomes can be found http://www.trendsa.org.au
- This report has outlined some of the co-creation processes, iterative design feedback frameworks and science/policy translation communications that were used in an attempt to bridge the gap between science, policy, and implementation with respect to climate change adaptation. This report outlines the practical steps taken at each of these phases to achieve the outputs and outcomes of the TREND project. It is also important to acknowledge, however, that the challenges relating to science-policy translation are complex and multi-layered, and include a range of strategies, including improved general acknowledgement of the business drivers of academia and government; broad collaboration across all elements of knowledge development; and dedicated resourcing of knowledge brokers in government and research institutions. DEWNR have begun to develop some of these approaches in collaboration with the South Australia research sector, using mechanisms such as those developed with the NRM Research and Innovation Network (NRM RaIN). Continuing to acknowledge and develop these solutions will further improve the application of science into NRM policy and delivery, with benefits to both natural resource managers and researchers.

1 Introduction

There has been lively debate about how ecological science could better link to biodiversity policy to inform the management of natural systems and ecosystem services in the face of anthropogenic impacts (Jones *et al.* 1999; Watson 2005; Moser and Luers 2008; Perrings *et al.* 2011). Climate change is recognised as a significant concern for the management of biodiversity, and is already influencing the function of ecosystems (Moser and Luers 2008; Grimm *et al.* 2013; Stein *et al.* 2013; Svenning and Sandel 2013). Despite numerous research papers on climate change ecology, questions remain over the relevance of the science for managers, and whether existing policy processes can use new data (Jones *et al.* 1999; Moser and Luers 2008).

For scientific research to be useful to policy, it must be relevant (e.g. in terms of time scales), credible (e.g. peer reviewed) and assist decision-making in the presence of uncertainty, while not being policy prescriptive (Jones *et al.* 1999; Cash *et al.* 2003; Watson 2005). For policy processes to make use of science, policy makers must be aware of, and receptive to, the science, and have the capacity to translate it into policy and action (Moser and Luers 2008; Sutherland *et al.* 2013). For the science–policy interface to be realised, both sides must reach an adequate level of maturity (Jones *et al.* 1999) and integrate policy development with research, monitoring and assessment (Perrings *et al.* 2011).

At a strategic level, a lack of either relevant data, or political will to address climate change ecology concerns, could disrupt the process. A practical constraint may be that differences in cultural norms, drivers and reward systems limits the effectiveness of research-policy partnerships (Kinzig *et al.* 2003; Kueffer *et al.* 2012). Impediments to effective partnerships include the two sectors operating within different timeframes. For example, there is often an emphasis on quick solutions in government, and a variety of demands for policy development that vary over time, and may be inconsistent with the long-term focus of science (Briggs 2006). The sectors may also differ in which natural resource management questions are considered important or answerable (Cash *et al.* 2003), which suggests that the collaborative development of appropriate questions may be a good starting point. Targeted questions designed to inform evidence-based policy have been identified previously through researcher–government and non-government organisation partnerships. For example, representatives of 28 UK-based organisations identified 100 policy questions directly relating to climate warming to influence the ecological research agenda for informing policy development in the UK (Sutherland *et al.* 2006).

A synthesis of ecological research relevant to climate change adaptation for South Australia (AECOM 2013) only makesgeneric, high-level conclusions about promoting ecosystem resilience. Similarly, in an overview of a landscape assessment framework used by South Australia's Department of Environment, Water and Natural Resources (DEWNR), Rogers *et al.* (2012) stated that "... among those stressors that are impacting a landscape's biodiversity, climate change may be one that we can do the least about." In the absence of detailed data on the climate sensitivity of South Australian ecosystems, they concluded that climate change impacts on biodiversity were best addressed by increasing general resilience. While this is an important and low risk strategy for dealing with critical threats (Heller and Zavaleta 2009; Dawson *et al.* 2011), an important question for researchers and policy makers in South Australia became: Can we do better than a generic 'improve resilience' approach to climate change?

This paper presents a model for science–policy integration, with particular reference to climate change conservation planning, and to assess the implementation of this model. This partnership focused on the development of research objectives to inform existing government strategies, and implemented a research program designed to address key policy questions. Our case study in South Australia lies within the Mediterranean Biome, which is one of the most globally vulnerable systems to climate change due to limited geographic extent and high land-use impacts (Mouillot *et al.* 2002; Bardsley and Sweeney 2010). The need to integrate scientific research into climate change policy with practical management actions in such regions has been recognised previously (Moser and Luers 2008; Bardsley and Sweeney 2010).

2 The TREND transect as a framework for climate change science–policy partnership

The South Australian Transects for Environmental Monitoring and Decision Making (TREND) is a collaboration between university and government (The University of Adelaide, Primary Industries and Regions SA, South Australian Research and Development Institute, Department of Environment, Water and Natural Resources [DEWNR]). TREND was established with funding from the Government of South Australia and later expanded by the Australia-wide Terrestrial Ecosystem Research Network, which has integrated TREND into a national network of ecosystem transects (the Australian Transect Network). The broad aim and scope of TREND was determined at its inception: to establish baseline monitoring transects in South Australia to assess the impact of climate change on the composition of the state's natural systems, primarily through the concept that space can be used as a proxy for time. Data were collected to assist natural resource managers to better incorporate climate change into their planning.

The benefits partners hoped to achieve by participating in TREND included gaining a greater understanding of the influence of climate on ecosystems, but also to better integrate science and policy on a long-term basis. Baseline data from the project were expected to aid in the identification of systems and species most susceptible to climate change, and those already undergoing change. Information collected was therefore intended to improve climate change science, while supporting government policy and decision-making (Fig. 1).

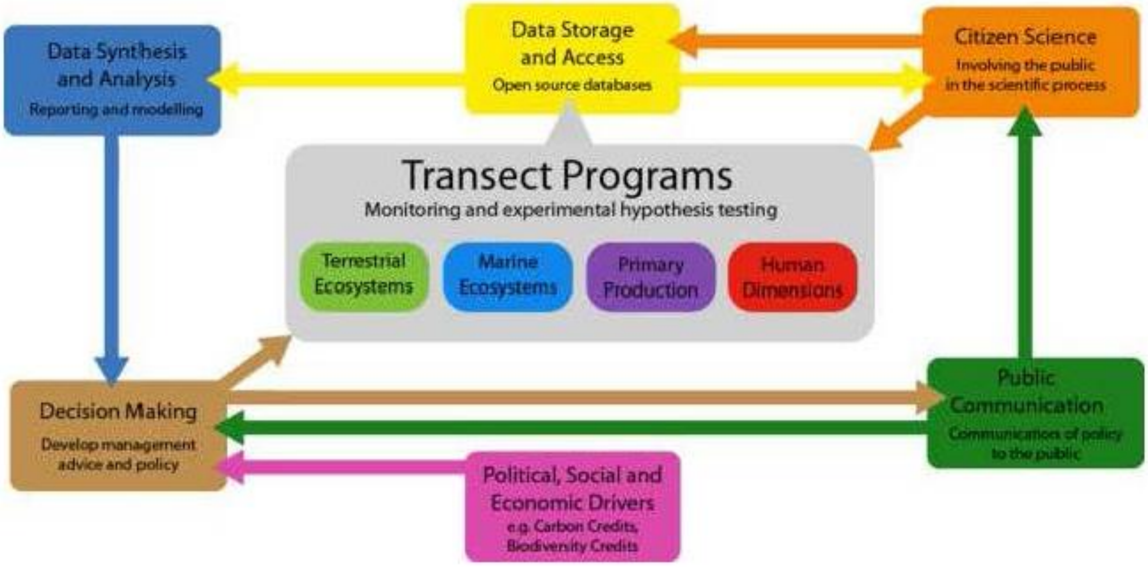

Fig. 1. Envisaged framework for climate change policy–science information flow for the TREND project, as devised at the inception of the project.

The framework involves an iterative cycle of scientific data generation via Transect Programs and implementation of monitoring and experimental stations (central box). These data are stored in open access databases for longevity and to maximise their use (yellow). Citizen Science programs (orange) allow members of the public to submit data (e.g. on selected species occurrences) to supplement those collected centrally. A range of collected and open access data undergo Synthesis and Analysis (blue) and results relevant to climate change adaptation inform Decision Making (brown), which includes the development of management recommendations, updates to policy and the opportunity to direct future research priorities. Policy is of course directed not only by science from the transect, but also by a range of Political, Social and Economic Drivers (pink). The final element of the framework is two-way communication with the wider public (green).

We focus here on the science–policy integration process undertaken for the terrestrial ecosystems transect of TREND (marine ecosystems, productive terrestrials systems and human dimensions were also considered in parallel but are not reported here), located in the Mount Lofty Ranges (south) to Flinders and Gammon Ranges (north) regions of South Australia (Fig. 2). A generalised model was developed to capture the main elements of the science–policy workflow (Fig. 3), which describes an iterative process whereby high level policy drivers and policy gaps lead to a set of specific, collaboratively developed, policy relevant questions. Researchers gather data to answer these questions during several stages, including initial gathering of available information and pilot data, a review of the approach and its relevance to the policy questions, followed by a major research phase. Results were then translated back onto policy needs and further gaps identified. The implementation of each of these phases for TREND is described in the following sections.

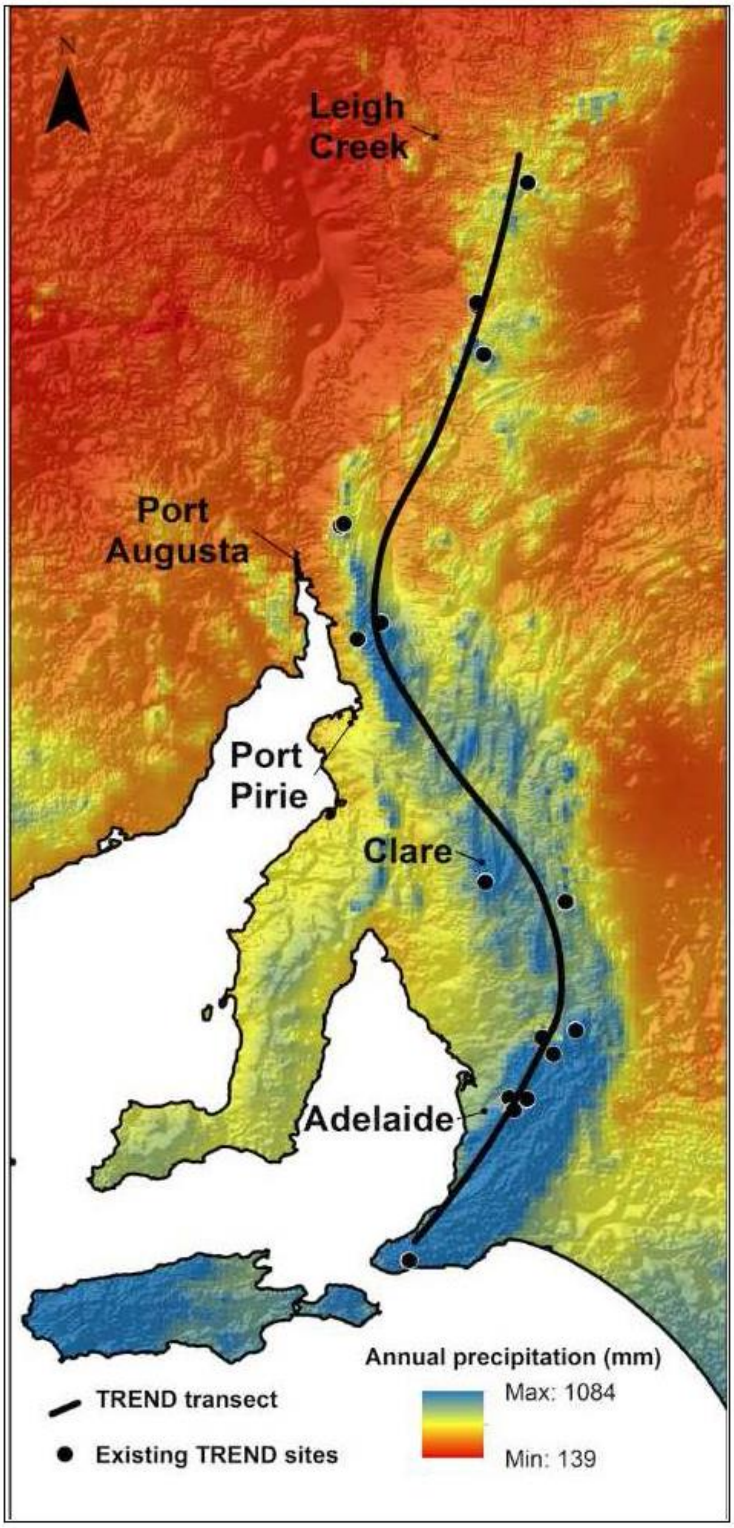

Fig. 2. Map of the case study region (in southern South Australia) with the **TREND** transect highlighted by the bold line and some key monitoring locations marked.

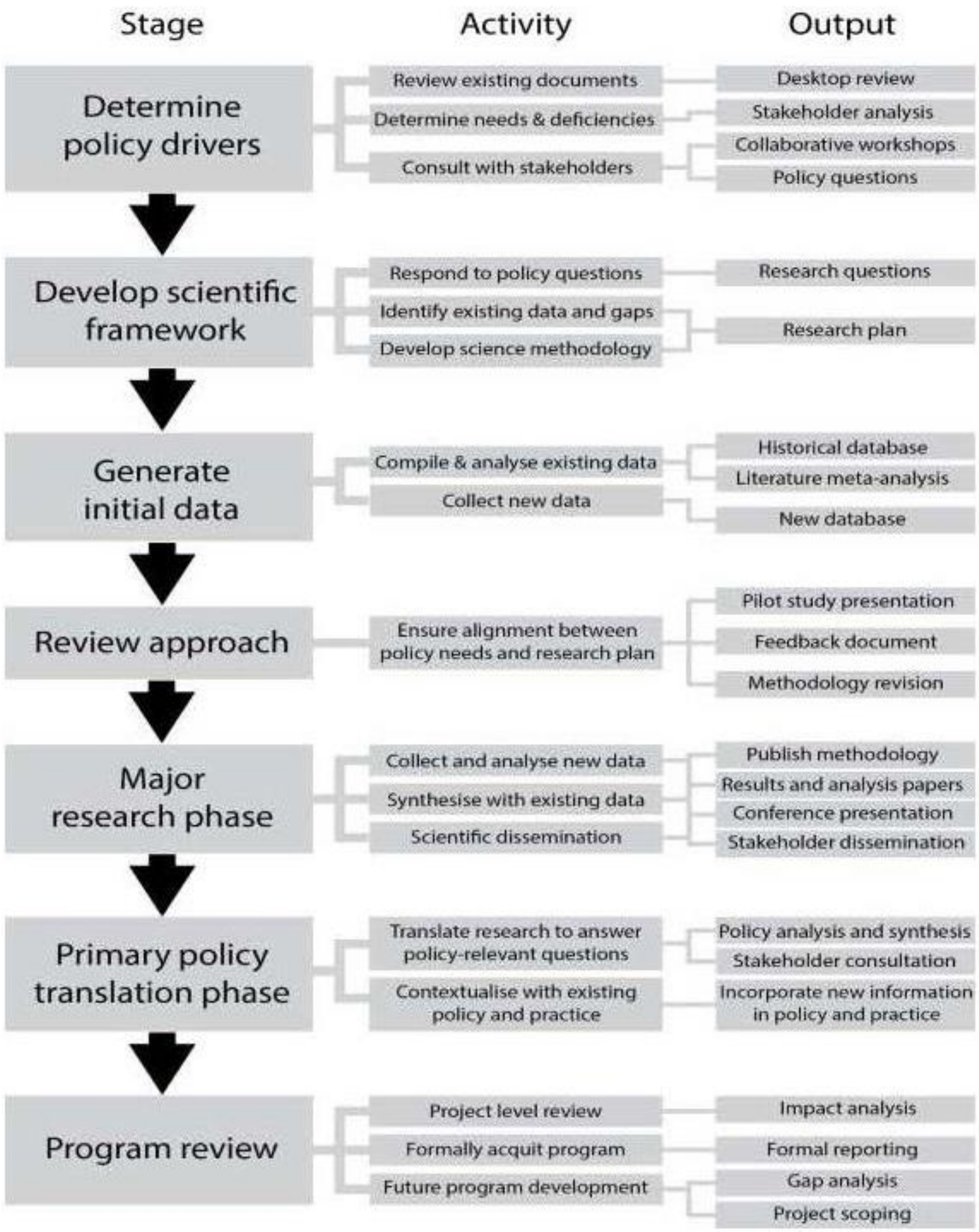

Fig. 3 **A generalised model framework for maintaining the science–policy interface, which was applied to the TREND project.**

The model is iterative and involves two-way communication between researchers and policy makers. The model is not intended to be linear or determinate, in that further gaps identified at the project review stage lead to new cycles. Summaries of actions taken at each step for TREND are given in the text.

3 Determine policy drivers

3.1 Climate change policy context in South Australia

The high-level policy background to TREND is multi-layered. Significant climate change policy drivers for natural resources management (NRM) planning in South Australia include the state's Strategic Plan, DEWNR Corporate Plan, the State Natural Resources Management Plan, as well as relevant Australian Government initiatives. While setting the broad objectives of climate change NRM policy, existing policies alone do not lead directly to specific management actions (Paton *et al.* 2010). As an example, the Climate Change Adaptation Framework for South Australia focuses on promoting generic strategies for increasing resilience of biodiversity, and on developing new policy for biodiversity conservation and sustainable use of land and water resources under climate change. A key emphasis in the climate change policies is the need to understand vulnerabilities within and across sectors and to identify or create knowledge to underpin management decisions. The TREND project therefore did not set out to supersede existing policy, but to fill data gaps at a practical level. In addition, information need to be in a form that it can be practically applied and is appropriate for on-ground delivery.

3.2 Development of policy questions

The policy translation work directed research within the scope of the established climate change transect towards policy questions relevant for evidence-based decision making by Government (Fig. 3; Box 1). The initial phase consisted of identifying the broad policy questions that government needed answered, to ensure the ensuing data collection was relevant. Policy makers, including a diverse group of government policy specialists, applied scientists and land managers, were also encouraged from the start to respond directly to new scientific information as it became available.

Box 1. Policy-relevant questions for 'TREND', terrestrial ecosystems, South Australia

1. What drives species composition and how will this be affected by climate change?
    - What species or ecosystems could provide early indicators of stress?
    - What species and ecological communities are most and least at risk from climate change and what are the expected impacts?
    - How will climate change interact with other disturbance to influence ecosystem attributes?
2. What adaptation strategies could improve the resilience of key species and communities?
    - What shifts in distribution, species composition and ecological characteristics can we expect?
    - What are the implications for conservation planning and landscape design?

Prior to an initial workshop, invitees were provided with information about TREND and the main policy drivers directing climate change adaptation research in South Australia. Invitees were asked to provide draft policy questions, which were compiled and synthesised. At the workshop, researchers provided a description of the aims and methodology of the proposed research and a policy officer delivered an overview of the policy translation expectations. Workshop participants discussed and prioritised the previously compiled draft policy questions, with guidance from researchers as to what was realistic. Therefore, policy issues were identified prior to the workshops and the workshops focussed on the synergies between the policy issues and the planned research.

4 Develop scientific framework and generate initial data

4.1 Scientific framework

In response to the policy questions, researchers developed a conceptual model highlighting where science can provide data, and management can influence outcomes, relating to climate change influences on ecosystem composition, and this became a context for specific data gathering and analysis (Fig. 4). Predicting the species composition of an ecosystem under climate change based on the responses of individual species is fraught with complexity (Shipley *et al.* 2006). One way to reduce complexity is to start with shifts in higher-level community constraints, such as potential biomass or functional properties, which are to some degree determined by the environment, and from this, determine the likely species composition (Shipley *et al.* 2006; Guerin *et al.* 2014a). The contributions of intrinsic (e.g. changes in species relative abundances in situ) versus extrinsic responses (e.g. changes in species composition) to a shifting community constraint determine the resilience of the community, and hence the magnitude of expected changes. Therefore, data on potential ecosystem responses inform decision-making to enhance resilience and adaptation. For example, intrinsic resilience is dependent upon maintenance of genetic variation within populations – a function of population size, historical factors (e.g. refugia during historical periods of climate flux) and the potential for gene flow between populations (Guerin *et al.* 2014a). Adaptive potential can be enhanced via landscape planning and appropriate seed selection for restoration (Breed *et al.* 2013). Adaptive potential also relates to concepts of ecological resilience that describe a system's capacity to be placed under stress, but still essentially retain its fundamental structure and function (Walker and Salt 2006, 2012)

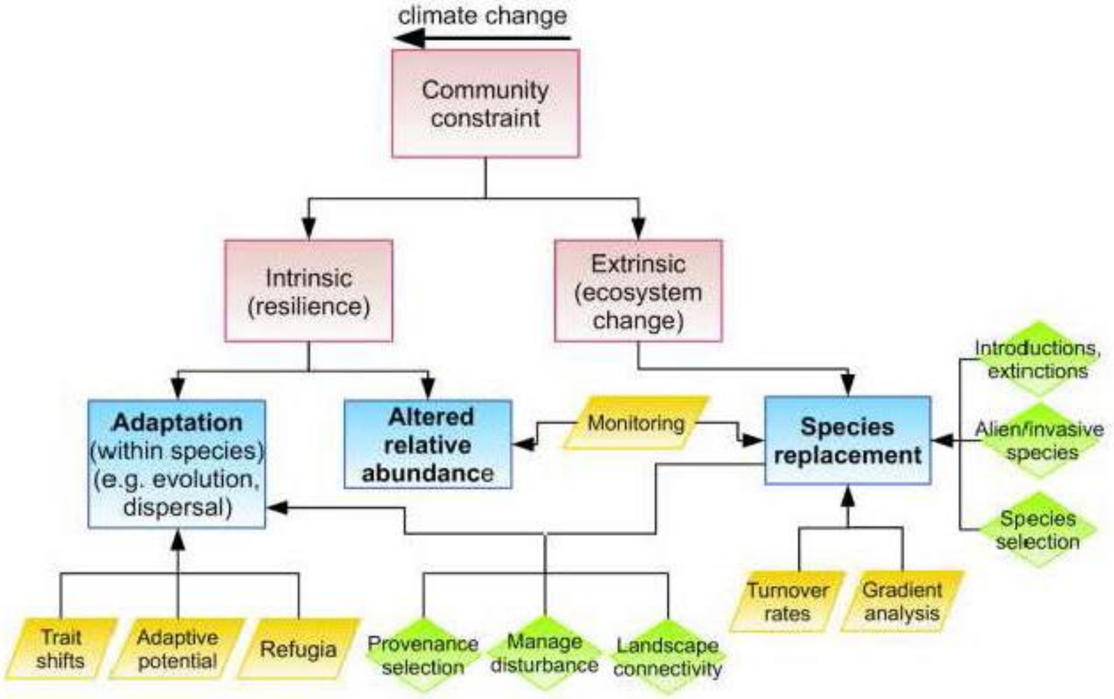

Fig. 4 The conceptual scientific framework for ecosystem adaptation to climate change that was developed as context for specific TREND research projects:
Where yellow boxes represent scientific information and green boxes management actions. An ecological community level constraint (such as functional properties within a patch of vegetation) shifts with climate change (top red box), driving changes at lower levels of organisation. The community level response can be intrinsic or extrinsic (blue boxes). Intrinsic resilience can include changes within species (e.g. evolution-adaptation, phenotypic plasticity) and changes in relative abundance, to match the new constraint. Resilience levels can be informed by studies identifying refugia and species adaptive potential (e.g. landscape genetics). Resilience can be manipulated in restoration via provenance selection and management of landscapes. Extrinsic responses involve changes in species composition. Gradient analysis can inform rates of species replacement across heterogeneous landscapes. Restoration can pre-empt species replacement via species selection and management of alien species. Managing at community level avoids the complexity of predicting responses among diverse species. However, information on individual species sensitivity and adaptive capacity is useful for understanding vulnerability

4.2 Generate initial data

In conjunction with the development of conceptual approaches, researchers accessed existing relevant ecological and environmental datasets. These included opportunistic records of target species, data from vegetation survey plots established for the Biological Survey of South Australia in the vicinity of the transect, herbarium collections from the transect and environmental data such as climate surfaces. Researchers subsequently conducted pilot field studies such as methodological trials and baseline survey at monitoring locations and population-based sampling of species along the transect, for various functional and genetic analyses. With these data, researchers developed approaches for desktop analysis that were informative of ecosystem resilience, including modelling the climatic distribution of individual species and changes between plant communities sampled at different points along climate gradients.

5 Review approach

Once researchers had implemented the first phase of fieldwork and desktop analysis, they presented early results and conclusions, plus an outline of planned approaches, to a follow-up workshop with a large gathering of scientific and policy officers, mainly from DEWNR. The session included discussion of results and their relevance to the policy questions and was an opportunity for a face-to-face question and answer session on the technical detail, but also for policy officers to give feedback and direct future work, advice which was subsequently incorporated into work programs.

6 Major research phase

With initial results and practical feedback from the formative review in hand (Section 5), researchers reviewed scientific approaches. A range of individual analyses were then completed and published in the scientific literature, ensuring that the evidence base intended to inform practical management outcomes had gone through peer review and was therefore more likely to be perceived as credible. Individual studies focused on areas such as vegetation monitoring methods, spatial modelling of existing plot-based data, exploratory analysis of empirical data from new field plots, analysis of historical herbarium collections and population genetics. Relevant literature on the region generated externally to TREND was also reviewed (e.g. Crossman *et al.* 2012).

At the core of the major research phase was the establishment of a field-based ecosystem transect over a distance of ~750 km, including 120 plots (Guerin and Lowe 2013c; Guerin *et al.* 2014b; Keith *et al.* 2014), covering strong latitudinal and altitudinal gradients in temperature and rainfall and a range of vegetation types. The field transect allowed for spatial analysis of abiotic drivers of community composition, while establishing a monitoring baseline (Guerin *et al.* 2014b).

Ecological climate sensitivity was determined through analyses of new and existing data, such as correlative species distribution modelling (supplemented in some cases with population genetic data) and modelling of community composition with respect to environmental and geographic differences (e.g. Guerin and Lowe 2013a; Guerin *et al.* 2013; Guerin *et al.* 2014b; McCallum *et al.* 2014). The recurrent conclusion from these studies was that climate is a significant driver of species occurrences and ecological community composition. However, these studies found ecological changes with climate are not uniform across the landscape: while suitable habitat for many species was predicted to persist with modest climate change in the Mount Lofty Ranges, south, and upwards shifts of suitable habitat may generate sharper species turnover in the Flinders Ranges.

In parallel to studies on variation in the species composition of ecosystems, researchers conducted studies on individual species along the transect, and detected significant associations between ecologically relevant traits and spatial and temporal changes in climate. For example, leaf width has ecophysiological significance because narrower leaves better tolerate heat in arid climates (Yates *et al.* 2010), and leaves were found to be narrower in populations of Dodonaea viscosa subsp. angustissima (DC.) J.G.West at warmer locations (more northern latitudes) but also to have become narrower over time, based on herbarium samples spanning a century, consistent with a physiologically relevant response to climate change (Guerin *et al.* 2012, but see also Duncan 2013 and Guerin and Lowe 2013b). Orchids of the genus Diuris Sm. were found to have flowered significantly earlier in spring since around 1972, in association with El Niño events and a strong warming trend, based on herbarium records (MacGillivray *et al.* 2010).

These and other individual peer-reviewed research projects became the scientific basis for answering the policy questions (Box 1; Section 7).

7 Primary policy translation phase

7.1 Overview and synthesis

Individual research projects were completed and published in the primary scientific literature. Specific results were disseminated to attendees of the previous workshops and a wider range of policy officers and conservation practitioners in the form of discussions, presentations, reports and journal articles. While specific studies initially focused on the transect, some have been extended statewide or have wider relevance, at least for the southern agricultural regions of South Australia. This is important, as relatively little has been reported on ecosystem sensitivity to climate across the state. Data from outside TREND were also considered during the translation phase, such as a study of the exposure of plant species in South Australia's Murray–Darling Basin (immediately east of the TREND transect) to climate change (Summers *et al.* 2012). Broad assessments of spatial conservation priorities are useful because data on the adaptive capacity of individual species are sparse and translation seeks to inform conservation planning across regions and ecosystems.

An example of the relevance of the science to conservation planning is that ecosystems in the transitional zone between mesic and arid biomes were found to be climate-sensitive (rapid changes with respect to prevailing temperatures, for example), whereas landscapes that are less fragmented, and contain refugia (or heterogeneous habitats), such as mountain ranges, are likely to be relatively resilient. The translation of this knowledge for policy is that landscape planning must balance attempts to increase adaptive capacity and resilience with predictive provenancing and species selection in climate-sensitive ecosystems (Breed *et al.* 2013). For example, in the absence of specific data on climate sensitivity and genetic diversity, it could be assumed that species with small, isolated populations, or with restricted climatic ranges, will be at greater risk than phenotypically variable and widespread species (McCallum *et al.* 2014; Christmas *et al.* 2015). In general, research provides guidance for the practical interpretation of climate responses of biodiversity, by highlighting the importance of combining current knowledge about resilience (or adaptive capacity) with data on climate exposure (Prober *et al.* 2012; Gillson *et al.* 2013).

7.2 Relevance to policy questions

Following dissemination of research results, studies were synthesised to provide answers to the policy questions. For illustrative purposes, we provide brief summaries of these answers below, based on research data and general principles developed within TREND and wider supporting literature.

1. What drives species composition and how will this be affected by climate change?

Composition is determined by a complex set of factors, including history, niche conservatism, abiotic environments, species interactions, disturbance regimes and ecological drift (Guerin *et al.* 2014a). Climate is a fundamental abiotic driver, determining biome boundaries and how species are sorted across landscapes, although history, chance, landscape change and increasing concentrations of atmospheric CO2, among others, remain important (Guerin *et al.* 2014a). Climate change is expected to drive changes in composition by directly influencing species potential distributions, altering fire regimes and compounding landscape change. Management responses need to focus on different levels of biological organisation and on ecological processes that drive change.

– What species or ecosystems could provide early indicators of stress?

Early ecosystem indicators include phenotypic responses, such as in flowering phenology. Early signs of stress, such as decreased population size, biomass or reproductive output, would be expected in populations of vulnerable species (defined based on climate sensitivity or resilience in terms of population demography) in ecotones. The earliest changes detectable at community level will be in species relative abundance, as species replacement involves longer time lags (Davis 1986; Svenning and Sandel 2013).

– What species and ecological communities are most and least at risk from climate change, and what are the expected impacts?

Species least at risk have wide climatic tolerances or high adaptive capacity or phenotypic plasticity (Guerin *et al.* 2012). Communities least at risk are those within their limit of intrinsic resilience, given their climate sensitivity. Species most at risk have small, isolated populations, narrow climatic preferences or low adaptive capacity (McCallum *et al.* 2014). Communities most at risk are those within ecotones or with poor resilience relative to their sensitivity, due to landscape modification (Guerin *et al.* 2013). Within the study region, ecosystems in the central Mount Lofty Ranges are the most stable with spatial changes in climate, but have undergone significant habitat fragmentation, reducing their resilience. Policy makers need to decide how to respond to early warning signs of stress, and to evidence of risk. For example, decisions need to be made about continued investment of management effort into the most vulnerable species and communities.

– How will climate change interact with other disturbance to influence ecosystem attributes?

Historical disturbance in the study region includes habitat clearance, which has resulted in just 13% of pre-European (1836) vegetation remaining in the Mount Lofty Ranges (Armstrong *et al.* 2003). Habitat fragmentation promotes inbreeding (Breed *et al.* 2012) and restricts dispersal (McConkey *et al.* 2012), which together inhibit adaptation to climate change (Fig. 4; Christmas *et al.* 2015) and may push populations under stress due to historical change further towards collapse. On-going disturbance (i.e. periodic destruction of biomass via fire, grazing) has complex synergies with climate (de Bello 2005), while multiple threats from habitat fragmentation, altered disturbance regimes and climate change decrease the likelihood of persistence of range-restricted species (Lawson *et al.* 2010). Climate change is increasing the frequency and severity of fires (Mouillot *et al.* 2002), which opens up resources such as space for native and alien colonisers and, in conjunction with other aspects of global change, modifies vegetation composition, which itself affects fuel dynamics (Thomson and Leishman 2005; Cary *et al.* 2012; Guerin *et al.* 2014a). Fire management can be controversial due to conflicting management objectives and the need to minimise the impacts of unplanned fires on human lives and built assets (Gill *et al.* 2013). The challenges for fire management are particularly acute in peri-urban settings such as the Adelaide–Mt Lofty Ranges, but also at the rural–wildland interface (Gill and Stephens 2009). Key challenges remain around how to manage fire to achieve conservation objectives under changing climate in historically altered landscapes.

2. What adaptation strategies could improve the resilience of key species and communities?

The sensitivity and resilience of ecosystems to climate change varies. For individual species, concerns for promoting resilience include maintaining population sizes and genetic diversity (Sgrò *et al.* 2011; Christmas *et al.* 2015) and the use of quality seed of appropriate provenance in restoration (Breed *et al.* 2013). Community level resilience can be supported through landscape restoration to improve habitat area and connectivity between isolated remnants (Christmas *et al.* 2015). Restored ecosystems in ecotones may be more resilient if species adapted to warming conditions are used, rather than strictly historical composition (Guerin *et al.* 2013). This suggests that NRM managers need to experiment with alternative designs for habitat restoration using an adaptive management approach (Sabine *et al.* 2004).

– What shifts in distribution, species composition and ecological characteristics can we expect?

Widespread species are expected to contract south and/or to higher altitude. Distribution shifts are predicted to be more pronounced in the Flinders Ranges and other parts of South Australia's Mediterranean–desert biome ecotone due to higher climate sensitivity (Guerin and Lowe 2013a; Guerin *et al.* 2013). In the Mediterranean–desert ecotone, there is expected to be pressure towards a major ecological shift from e.g. sclerophyllous woodland vegetation to more open vegetation dominated by arid-zone taxa. An unknown factor is the degree to which these shifts can occur without management interventions such as corridor creation and assisted translocations.

- **What are the implications for conservation planning and landscape design?**

Landscape connectivity can promote gene flow and maintenance of metapopulations (Sgrò *et al.* 2011; Christmas *et al.* 2015). Species and seed sources for restoration can be selected to enhance adaptive capacity or pre-empt which genotypes and species will prosper (Breed *et al.* 2013; Guerin *et al.* 2013). In areas likely to undergo species turnover, selection of species for habitat restoration could include a higher proportion of provenances or even species from warmer habitats, whereas refugia could be restored using historical composition (Guerin *et al.* 2013). Strict adherence to historical composition in conservation objectives is likely to be counter-productive. However, we recognise that the issue of whether to implement restoration of novel ecosystems in the face of climate change remains a subject of debate in the literature and that there are unknowns about practical application (Hobbs and Suding 2009;).

8 Program review

The first cycle of TREND was completed in terms of finalising studies and acquitting funding. Participants considered how the research had addressed policy and research gaps and a range of associated research projects were initiated that would be further informative of ecological resilience and the functional consequences of climate change. For example, it was identified that basic information on the spatial location of biodiversity (e.g. in terms of levels of species diversity and endemism) within South Australia could be overlayed with climate sensitivity and habitat fragmentation data to provide an insightful resource relevant to landscape planning. Consequently, a research project has been initiated that seeks to answer basic questions such as: What kind of biodiversity does South Australia have, where is it, and how does it interact with areas of high climate sensitivity and landscape modification?

There is an emerging opportunity for uptake of research, with recent Australian Government investment in improving the climate change content of NRM plans, which places emphasis on identifying spatially explicit targets for investment in adaptation actions. This highlights the multi-layered nature of policy: a range of climate change NRM adaptation actions are not delivered by high-level strategic policy, but by operational policies embedded in planning documents, such as regional NRM plans, which take the extra step of developing practical approaches following the synthesis and interpretation of data.

9 Assessment of model implementation

The aim of this report was to assess the usefulness of our model (Fig. 3) in bridging the gap between ecological research and policy in the context of climate change. The envisaged framework for the TREND project (Fig. 1) was successfully implemented in terms of the flow of information, and NRM practitioners are starting to take up this information within practical programs. The project successfully established these partnerships and generated policy-relevant data on climate sensitivity. In addition, these partnerships facilitated the effective leveraging of additional research and scientific infrastructure funding (more than 10x the original project costs for TREND), and produced excellent quality scientific knowledge and research results (over 25 peer reviewed papers). The project has also formed the blueprint for a national climate change ecosystem monitoring network, the Australian Transect Network, part of the Terrestrial Ecosystem Research Network (http://www.tern.org.au).

The process highlighted research gaps as a foundation for developing evidence-based policy, which could otherwise remain generic. One of the biggest challenges for land managers is to determine where to take action, and TREND provided spatial analysis to highlight vulnerable systems and pointed land managers towards options for building landscape resilience. In fact an independent review of all science outputs relevant to climate change ecosystem resilience planning for South Australia, undertaken by the Adelaide and Mount Lofty Ranges region Natural Resources Management Board in 2015, found that the TREND project outputs used the best data, appropriate scientific methods and presented information at an understandable and relevant scale to make policy and management relevant decisions.

Importantly, two-way dialogue between researchers and decision-makers – a key aspect of the model – is on-going, allowing new research to feed policy development and changing policy priorities to inform the research agenda. Individual research projects can form part of the evidence base for sustainability, but one-way communication from researchers to government on perceived important questions may not lead to the best practical outcomes (Cash *et al.* 2003). For this reason, while the research component of TREND produced peer-review publications on the climate sensitivity of local ecosystems, it is useful to consider which factors influence the integration of specific research findings with policy objectives. A summary of such an analysis is given in Table 1.

Table 1. Examples of factors that influence the success of the science–policy integration model (see Fig. 3), with example outcomes from the TREND project

Project phase (Fig. 3)	Factor	Possible response	Actual TREND outcome
Determine policy drivers	Scale of policy needs compared to individual research projects	Researchers up front about limitations of what can realistically be achieved, and set research priorities	Set of precise and answerable policy questions developed
Determine policy drivers	Limitations of funded research scope and researcher expertise	Relevant match between research options (e.g. scope of funding and area of expertise) and policy recipients	Initial research scope limited to plant community composition along a pre-defined transect
Determine policy drivers	Breadth of developed policy relevant questions	Develop questions collaboratively with practical objectives to ensure breadth is neither too general, nor trivial	Developed questions were broad enough to be useful for policy but detailed enough to seed research projects
Develop scientific framework	Short-term research funding cycles	Focus on spatial analysis, historical data and establishing ecological baselines	Took advantage of retrospective data for temporal analysis and focused on modelling spatial climate change proxies
Major research phase	Scientific credibility of research findings	Research published in peer-reviewed journals and explained to decision-makers	A number of journal articles resulted from TREND, providing a sound basis for supporting policy change
Primary policy translation phase	Informing landscape-scale planning via research projects on specific species or sites	Develop general principles from specific research projects and wider literature. Implement research at a range of scales, e.g. population to region	TREND research framed within a model of climate change ecology based on literature and filled-in with local empirical data. Results synthesised and placed in context of wider literature
Primary policy translation phase	Integrating specific science into practical management regimes	Treat as iterative process. More realistic if earlier phases provided realistic policy questions and directed research towards applicable outcomes	On-going process via NRM planning. Other avenues being explored include trials of predictive species composition and provenancing for restoration
Program review	Availability of funding to address further policy needs identified	Use track record of practical science–policy links plus established ecological monitoring infrastructure as a platform	TREND was extended through a range of additional funding sources to build on initial gains

Limitations to the success of science–policy translation were evident. For example, the goals of the project had pre-defined boundaries, and the time and resources available were modest, leaving unanswered questions. Some relevant components of research will develop over a longer timeframe than the initial three-year funding cycle, requiring a long-term commitment to the partnership for these data to be integrated into policy. While information flow between TREND partners was useful, implementing a specific research project within a practical planning framework remains complex. Challenges remain in making primary scientific research truly policy relevant, i.e. exchanging and interpreting results in a useful format. For example, while scientific papers are useful for a technically knowledgeable audience, we also found that less formal reports with information presented spatially was accessible to a wider policy audience. The individual research studies – and ecology generally – tend to focus on specific components of ecosystem function, whereas managers need to make decisions across entire landscapes (McConkey *et al.* 2012) and to consider regional and local processes (Paton *et al.* 2010), which requires synthesis. In addition managers and scientists increasingly need to understand the limitations of the scientific data in the context of the social and economic systems within which they work, particularly the highly complex, and sometime conflicting priority, environment of natural resource management.

While no model is perfect, or simple to implement in the real world, the TREND model provided real potential for on-going research to be directed towards specific policy needs and opened up direct communication between researchers and policy makers. There are, of course, inherent limitations to the policy questions that science can credibly answer (Cash *et al.* 2003; especially on a short-term basis) and to the potential for science to become directly useful for conservation planning and on-ground implementation.

10 Conclusions

We have proposed a practical model for climate change science–policy integration, populated with real world examples from a transect through terrestrial ecosystems from the TREND project in South Australia. TREND was successful in its stated goals of: 1) establishing baseline monitoring transects to assess the influence of climate on ecosystem composition and; 2) collecting policy-relevant data on climate change ecology specific to the region. The process was centred on research but brought researchers, policy makers and natural resource managers into a collaborative environment. We conclude that the model contributed to bridging the gap between research and policy in that two-way dialogue guided research and provided NRM practitioners with guiding principles, based on local examples, and spatial information on climate sensitivity. Limitations of the process included practical constraints on what could be achieved and the on-going challenge of translating specific science into on-ground action. NRM planners now have some basic local information on the some likely impacts of climate change and their spatial and taxonomic idiosyncrasies.

The inherent difficulties in implementing evidence-based biodiversity management under climate change have been discussed at length (Jones *et al.* 1999; Moser and Luers 2008; Bardsley and Sweeney 2010; Stein *et al.* 2013). Climate change in coming decades is considered inevitable, regardless of action taken to limit greenhouse emissions (Stein *et al.* 2013) and therefore adaptation is required, because climate change, combined with impacts such as habitat fragmentation and invasive species, may exceed ecosystem resilience (Grimm *et al.* 2013; Stein *et al.* 2013). These changes may cross the threshold between ecosystems persisting in their present-day form, or entering transitional states (Grimm *et al.* 2013; Guerin *et al.* 2013; Stein *et al.* 2013), in fact, ecosystem shifts linked to climate change have already been documented (Peñuelas and Boada 2003; Grimm *et al.* 2013).

Adapting biodiversity management to climate change could involve promoting resilience to protect important biodiversity, or actively promoting change to enhance adaptation (Stein *et al.* 2013). The broadest policy-relevant conclusion of climate change ecology is that ecosystems are dynamic so that changes in climate will likely be reflected in changes to ecosystem composition and function. This suggests attempting to maintain ecosystem fidelity to historical states as a default may be unrealistic and counter-productive (Harris *et al.* 2006; Guerin *et al.* 2013; Stein *et al.* 2013). Policy makers can take advantage of insights from research if there is a long-term commitment to fostering and maintaining the type of partnership demonstrated by the TREND project.

Bridging the gap between scientific research and NRM decision-making continues to pose a challenge for the application of evidence in natural resource management, and remains a barrier to the effective application of scientific evidence in decision making. Here we have presented a case study of how scientific questions regarding climate change were designed by bringing together scientists and policymakers. Since this work was undertaken, DEWNR has been working with research organisations to actively develop mechanisms to improve the relationship between science generation and NRM decision making. The NRM Research and Innovation Network (www.nrmrain.org.au), a partnership between the three South Australian universities, SA Water, the regional NRM Boards, PIRSA and DEWNR, has been specifically designed to address the challenge of bridging the science-policy interface. The Network facilitates genuine collaborative partnerships between researchers and research institutions, and NRM policymakers and practitioners, such that scientific questions are designed and implemented in a way that the information can be most effectively applied to natural resource management issues. More broadly, DEWNR is increasingly placing emphasis on the importance of science translation into policy, and the need to actively engage with the research sector to achieve this.

11 Glossary

Abiotic driver — Non-biological/ecological factor that influences ecosystem function, such as landscape properties and climate

Adaptive management — A management approach often used in natural resource management where there is little information and/or a lot of complexity, and there is a need to implement some management changes sooner rather than later. The approach is to use the best available information for the first actions, implement the changes, monitor the outcomes, investigate the assumptions, and regularly evaluate and review the actions required. Consideration must be given to the temporal and spatial scale of monitoring and the evaluation processes appropriate to the ecosystem being managed.

Biodiversity — (1) The number and variety of organisms found within a specified geographic region. (2) The variability among living organisms on the earth, including the variability within and between species and within and between ecosystems

Biome — Major ecological regions defined by their climatic and ecological properties

Composition — The make-up of ecological communities, particularly the species that are present

Demography — The nature/make-up of populations of species

DEWNR — Department of Environment, Water and Natural Resources (Government of South Australia)

Ecological community — The set of species, generally within a particular taxonomic or trophic group (such as plants) that occur together within a habitat location

Ecological processes — All biological, physical or chemical processes that maintain an ecosystem

Ecology — The study of the relationships between living organisms and their environment

Ecosystem — Any system in which there is an interdependence upon, and interaction between, living organisms and their immediate physical, chemical and biological environment

Ecosystem services — All biological, physical or chemical processes that maintain ecosystems and biodiversity and provide inputs and waste treatment services that support human activities

Ecotone — A boundary between different ecological regions or habitats

Endemism — The restriction of species to a certain locality or region

Habitat fragmentation — Loss of habitat resulting in smaller, more isolated remnants

Metapopulation — A set of populations that interact with each other

Phenology — The timing of biological events such as flowering in plants

Phenotypic — Relating to species morphology/traits or observable characteristics

Phenotypic plasticity — Short-term phenotypic changes, for example in response to environmental conditions

Provenance — The region of origin, for example of seed

Relative abundance — The number of individuals or amount of biomass of species in a habitat in relation to other species

Species replacement — Ecological change involving the loss of some species from an ecological community and the appearance of additional species

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REPORT

SWATT Foliar Carbon Isotope Pilot Study

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Executive Summary

The leaf stable carbon isotope ratio ($\delta^{13}C$) of C_3 plants has potential to provide an integrated measure of plant responses to water stress. Recent work comparing the carbon isotope responses of a wide range of species on different aridity gradients provides evidence that individual species and landscapes have different carbon isotope responses to available moisture (Caddy-Retalic et al. In preparation). These findings refute the claim that a "universal scaling" relationship exists between leaf $\delta^{13}C$ ratios and measures of moisture (Prentice et al. 2011a). The variation observed suggests instead that the slope of this regression reflects the sensitivities of individual species and entire landscapes to aridity. This information can be used to inform projections of biotic responses to climate change, and may provide early indications of the species most and least at risk of future changes in available moisture.

To further investigate the potential utility of these relationships, we were engaged by the Australian Transect Network and Western Australian Department of Biodiversity, Conservation and Attractions to investigate a suite of plant species on the South-West Australian Transitional Transect (SWATT). In total, 663 plant tissue samples were analysed from across the SWATT (Figure 1) for leaf carbon isotope ratio, nitrogen isotope ratio, carbon and nitrogen content.

The leaf carbon isotope ratios of plants analysed here on SWATT show a weak but statistically significant correlation with mean annual precipitation (MAP). The weakness of the correlation likely results from the relatively narrow range of precipitation. The slope of the regression is similar to that on the South Australian Transect for Environmental monitoring and Decision making (TREND), but shallower than that on the North East China Transect (NECT) (Caddy-Retalic et al. In preparation).

Of the 49 species analysed from six or more sites, two exhibited statistically significant correlations (Bonferroni adjusted $p<0.05$) with mean annual precipitation. These slopes were steeper than the community slope, providing evidence against universal scaling.

Two sympatric species, *Eucalyptus salmonophloia* and *Eucalyptus salubris*, were analysed with higher sampling intensity ($n>50$). In spite of high sample numbers, they did not exhibit a statistically significant correlation between $\delta^{13}C$ and mean annual precipitation. They did, however, exhibit statistically significant differences in $\delta^{13}C$ and carbon content which provides evidence that these species display functional physiological differences despite their apparently similar habit and leaf morphology.

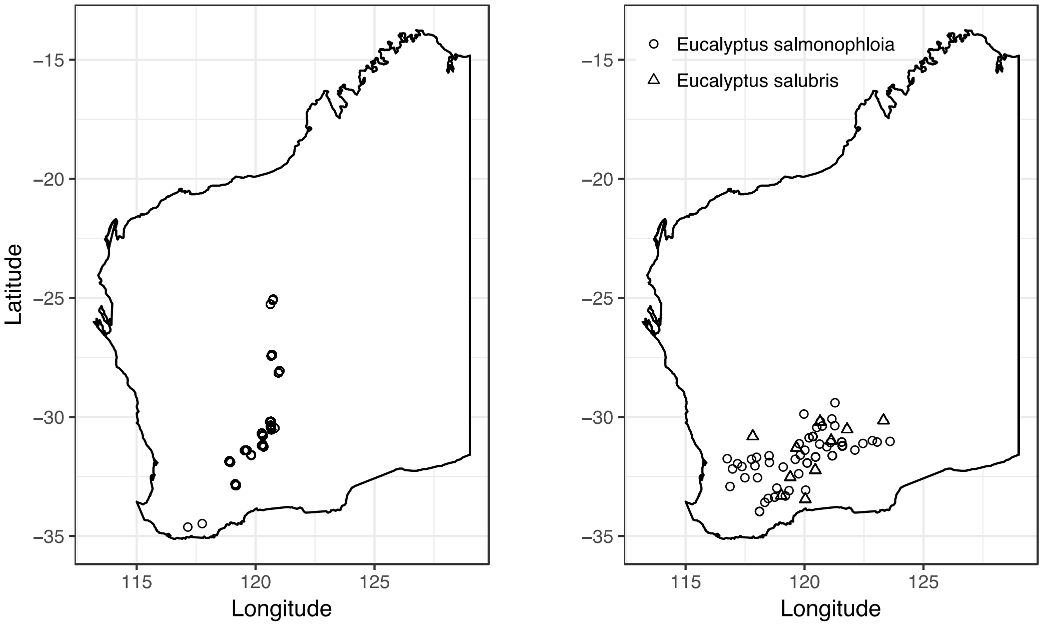

Figure 1: SWATT sampling locations for species-community comparison (left) and *Eucalyptus* study (right).

Background and Motivation

Most Australian plants, including all trees and most shrubs and forbs, utilise the C_3 photosynthetic pathway. Some Australian plant species use the alternative C_4 (primarily warm season grasses and some chenopods) or Crassulacean Acid Metabolism (CAM; primarily xeric succulents) photosynthetic pathways, which are subject to different biochemical processes and are not addressed here.

For C_3 plants, the photosynthetic uptake of carbon and the associated fractionation of carbon isotopes been widely applied to understanding water management. RuBisCO, the enzyme responsible for converting carbon dioxide to organic molecules, preferentially utilises the lighter and more abundant ^{12}C and discriminates against the heavier ^{13}C. This discrimination results from a combination of differences in diffusion rates and fixation rates for $^{12}CO_2$ and $^{13}CO_2$ and results in photosynthetic products that are even further ^{13}C-depleted than the atmosphere (Cernusak et al. 2013; Farquhar et al. 1989; Werner et al. 2012).

Carbon isotope values are expressed in delta notation, where the ratio of carbon isotopes in a sample are compared to that of a standard:

$$\delta^{13}\mathrm{C} = \left(\frac{\frac{^{13}\mathrm{C}}{^{12}\mathrm{C}}_{sample}}{\frac{^{13}\mathrm{C}}{^{12}\mathrm{C}}_{standard}} - 1\right) * 1000 \qquad \text{(Equation 1)}$$

The reference standard used is the Vienna Pee Dee Belemnite (VPDB) and $\delta^{13}C$ values are expressed in per mille, or parts per thousand (‰).

Carbon isotope fractionation during photosynthesis is presented as the discrimination ($\Delta^{13}C$) between the isotopic signature of the plant ($\delta^{13}C_p$) and that of the atmosphere ($\delta^{13}C_a$):

$$\Delta^{13}\mathrm{C} = \frac{(\delta^{13}\mathrm{C_a}\text{-}\delta^{13}\mathrm{C_p})}{(1+\delta^{13}\mathrm{C_p})} \qquad \text{(Equation 2)}$$

Plants discriminate against ^{13}C during photosynthesis, and the degree of discrimination in C_3 plants depends largely on the ratio of the concentration of CO_2 inside the intercellular air spaces (c_i) to that outside the leaf (c_a) (Farquhar et al. 1982). The simplified model of what controls photosynthetic carbon isotope discrimination is

$$\Delta^{13}\mathrm{C} = a + (b - a)\frac{c_i}{c_a} \qquad \text{(Equation 3)}$$

where *a* is the fractionation during diffusion of CO_2 in air (4.4‰), *b* is the fractionation due to carboxylation in C_3 plants (approx. 27‰) and c_i and c_a are the partial pressure of CO_2 inside the leaf (sub-stomatal) and in the atmosphere, respectively (Farquhar et al. 1982). There are more complex models that include numerous additional corrections, but the simplified model sufficient for many applications including the approach taken here (Cernusak et al. 2013).

This model implies that the primary control on the carbon isotope fractionation in a plant relative to the atmosphere is the ratio of the concentration of CO_2 within the intercellular air spaces of the leaf to that of the atmosphere (c_i/c_a). This ratio is a function of the supply of gases through stomata (stomatal conductance, g_s) and the demand for photosynthetic assimilation of carbon (A). Because water loss through transpiration is also controlled by the flow of water out through stomata, $\Delta^{13}C$ varies with mean annual precipitation at a global scale (Diefendorf et al. 2010; Kohn 2010).

The $\delta^{13}C$ of atmospheric CO_2 ($\delta^{13}C_a$) displays small seasonal variations, especially in the northern hemisphere, a general long-term decline due to the burning of fossil fuels, and localised decreases proximal to fossil fuel combustion sources. In addition, closed canopy forests can trap soil respired ^{13}C-depleted CO_2, causing a significant departure from the open atmosphere. However, due to the lack of concentrated industry and lack of dense, closed canopies, we have regarded spatial and temporal variation in $\delta^{13}C_a$ as negligible. Therefore, rather than considering carbon isotope discrimination between the plant and the atmosphere ($\Delta^{13}C$), we report plant tissue results in terms of leaf carbon isotope ratios ($\delta^{13}C_p$).

The $\delta^{13}C_p$ of C_3 leaf tissue generally ranges between -34 to -24‰. Plants under water stress close their stomata to limit stomatal transpiration, isolating the intercellular CO_2 pool from the atmosphere (Cernusak et al. 2013; Farquhar et al. 1982). When stomata are closed, RuBisCo is forced to fix more $^{13}CO_2$, decreasing the observed discrimination as compared to plants that are not under water stress.

This trade off between water loss and carbon uptake leads to a negative relationship between water availability and $\delta^{13}C_p$. It is important to note that there are other environmental factors that have the potential to influence carbon isotope discrimination in

C_3 plants, including light, temperature, altitude and soil nutrient availability. However, water availability is a major determinant in carbon isotope discrimination, which allows plant $\delta^{13}C$ values to be used as an integrated proxy for water stress in C_3 plants (Cernusak et al. 2013).

The general relationship between water availability and leaf $\delta^{13}C$ values in C_3 plants has led to the hypothesis of a universal scaling relationship between $\delta^{13}C$ and moisture across all species (Prentice et al. 2011a). This hypothesis predicts that the $\delta^{13}C$ response to available moisture (precipitation or moisture index) is the same for individual species as the community as a whole, and that this is a universal response. This hypothesis can be tested by examining the slope of the regression for individual species (m_s) and comparing it to the community slope (m_c) along a moisture availability gradient and by comparing different gradients. These tests have begun with the work on the South Australian Transect for Environmental monitoring and Decision making (TREND) and North East China Transect (NECT) (Caddy-Retalic et al. In preparation) and could be expanded through comparison with other bioclimatic gradients.

The competing hypothesis is that, through different expression of individual traits, species have the potential to display a range of relationships between $\delta^{13}C_p$ and moisture availability ($m_{s1}{\neq}m_{s2}$), which may not match the response of the community as a whole ($m_s{\neq}m_c$). Additionally, landscape-level responses of plants on different gradients could produce different relationships ($m_{c1}{\neq}m_{c2}$). This hypothesis has been supported by the development and comparison of m_s values for 186 species on the TREND (150) and NECT (36), many of which are different to the community mean (m_s) (Caddy-Retalic et al. In preparation). In addition, the community mean slopes for the two transects differ (Figure 2a and b). These data show that different species and gradients appear to have different $\delta^{13}C$ responses to precipitation and supports the hypothesis that m_s and m_c values could be used to compare the relative sensitivity of species and landscapes to aridity.

The findings that m_s and m_c values could be used to infer sensitivity to aridity has motivated interest in testing to what degree these values vary within semi-arid Australian ecosystems. Much of the work to date has occurred on the TREND in South Australia, which spans ~150-1000mm mean annual precipitation (MAP) and transitions from arid grasslands and chenopod shrublands to increasingly dense shrublands and woodlands at the mesic end. The development of a second semi-arid Australian gradient, the South West Australian Transitional Transect (SWATT) with floristic data and plant leaf tissue samples has motivated this study. The SWATT spans a similar rainfall gradient to the TREND, from ~250mm MAP at Weebo to 740mm MAP at Mt Roe but is entirely based on sand-plain communities. The transect spans the South West Australian global biodiversity hotspot and Central and Eastern Avon Wheatbelt Australian biodiversity hotspot, both of which are at risk under climate change.

Study Design and Aims

This purpose of this study was to examine the relationship between $\delta^{13}C_p$ and precipitation in the most common C_3 species present on the SWATT. The study was comprised of two components.

The first component sampled the most commonly sampled species on the SWATT in order to establish both a suite of m_s values for common species, as well as an m_c value for the entire gradient. Well-collected species were selected based on our experience conducting a similar study on the TREND in order to ensure the SWATT m_c was representative of common species and to maximise the robustness of the m_s values for those species. m_s values were calculated for all species sampled from at least six sites (49 species from 127 sites, Figure 1a).

The evidence from earlier work on the TREND and NECT suggests that species have variable m_s values, potentially relating to different leaf traits and/or water use strategies. The second component of the study compared m_s values from two similar eucalypts found on the SWATT, *Eucalyptus salmonophloia* and *Eucalyptus salubris,* with a much higher sampling intensity (*E. salubris* n= 50; *E. salmonophloia* n=51, Figure 1b). The intention of this strategy was to quantify the $\delta^{13}C_p$ range expressed by these species across their natural ranges and maximise the robustness of calculated m_s values, in order to determine whether it is possible to distinguish these species based on their isotopic signatures.

In summary, the aims of this study are to determine whether:

1) the community δ^{13}C-MAP slope (m_c) of SWATT is similar to or different from TREND, NECT and a global compilation;
2) the species δ^{13}C-MAP regression slopes (m_s) are similar to or different from the community slope (m_c) on SWATT; and
3) the geographically overlapping *Eucalyptus salubris* and *E. salmonophloia* demonstrate different isotopic ranges and m_s values in order to determine whether these species can be distinguished isotopically.

Methods

160 individual sites were surveyed for the SWATT using the methodology described by Gibson et al. (2017), augmented by a number of AusPlots surveys using the methodology described by White et al. (2012). Surveys occurred at ten locations spanning the SWATT gradient (Figure 1), with full floristics data available for download from the AEKOS data repository.

Plant materials were collected and dried in the field either as pressed specimens (SWATT plots and AusPlots) or as leaf samples stored in gauze bags and dried on silica (AusPlots only). Pressed voucher specimens were identified at the Western Australian Herbarium. Previous work on the TREND indicated that at least seven plant sampling locations were required to reasonably examine the slope of the δ^{13}C-MAP regression. 46 C_3 species collected at seven or more sites were selected, with one species (*Grevillea hookeriana*) having only six collections (Appendix A). The limited number of sites at the mesic end of the SWATT gradient, coupled with the high species turnover in this region meant that most selected species were from the semi-arid zone, particularly the Coolgardie bioregion, with a relatively narrow precipitation range (250-350mm MAP). Single specimens of *Eremophila ionantha*, *Grevillea haplantha* and *Hibbertia rostellata* were also inadvertently processed – these singletons were not able to be used in calculating m_s values, but were incorporated in to the calculation of the m_c value for the entire gradient.

Additional leaf samples of *Eucalyptus salmonophloia* (n=51) and *E. salubris* (n=50) were provided by the Western Australian Department of Biodiversity, Conservation and Attractions. These samples included replicate or closely-located samples to better quantify variability (5 replicates for E. salubris; 1-2 replicates from more closely located sites for *E. salmonophloia*). Individual m_s values were calculated for these species, and their data integrated in to the whole SWATT m_c value.

Leaf samples were taken from WA Herbarium vouchers and AusPlots leaf tissue collections stored in gauze bags on silica gel. Approximately 20mg of dried leaf tissue was placed in a 2mL screw top eppendorf tube with two 5mm steel ball bearings. Eppendorf tubes were loaded into a Retsch ball mill fitted with a Qiagen Tissuelyser adaptor plate (2 x 24 tubes/plate) and ground for 2 to 5 minutes. If samples were not ground after two minutes, the partially ground leaf material was transferred to a 5 mL stainless steel grinding jar and ground for 30 to 90 seconds in 30 second intervals.

Ground material (2.5 mg ± 10%) was weighed into tin capsules. Samples were analysed for $\delta^{13}C$, $\delta^{15}N$, %C and %N at the University of Adelaide on a EuroVector Euro elemental analyser inline with a Nu Instruments Continuous Flow Isotope Ratio Mass Spectrometer (CF-IRMS). Internal isotope standards run alongside were glycine ($\delta^{13}C$, $\delta^{15}N$), and glutamic acid ($\delta^{13}C$, $\delta^{15}N$). Certified reference material for elemental concentration was Triphenyl Amine (TPA; C:N). The uncertainty for carbon isotope measurements was ±0.09 ‰ and for nitrogen isotope measurements was ±0.12 ‰.

Climate data for all sites was extracted from long-term (1960-2012) 0.01 degree (~1km) gridded BioClim layers based on ANUCLIM v6, provided by CSIRO Ecosystem Services and published by the Atlas of Living Australia (www.ala.org.au).

Data analysis was undertaken in R using the methodology developed for analysis of TREND and NECT isotopic datasets (Caddy-Retalic et al. in prep). Species level responses (m_s) were calculated as the linear regression of $\delta^{13}C_p$~MAP for all observations of that species. Because multiple linear regressions were calculated, significance (p) values were adjusted using a Bonferroni correction based on the number of analyses undertaken per transect.

Results and discussion

Isotope measurements and slopes for species are presented in Appendix A.
Species, sites, and climate information for sampling sites are presented in Appendix B. Notably, the MAP for the entire dataset ranges from 220-746 mm/year, with most data being between 250-350mm MAP, which is a more restricted range than previous studies that produced community slopes (Caddy-Retalic et al. in prep; Diefendorf et al. 2010; Prentice et al. 2010).

Aim 1: Determine whether the community δ^{13}C~MAP slope (m_c) of SWATT was similar to or different from TREND, NECT and global compilations.

The SWATT δ^{13}C~MAP slope (m_c) was 0.004 which was similar to that recorded for the TREND and site-averaged global compilation data (Figure 2, Table 1). While statistically significant ($p<0.05$), the R^2 value for this correlation is very low, indicating that the data is a poor fit for the overall regression and predictive power is low. This limitation is due to the concentration of samples analysed within 250-350mm MAP. While this range has been sufficient to display an aridity effect, complementary sampling at the more mesic end of the gradient would make this relationship far more statistically robust. Given the high species turnover in this region, additional mesic sampling would require either additional surveys to accumulate >6 occurrences of species, or a lowering of the threshold below six occurrences with the current available sample set.

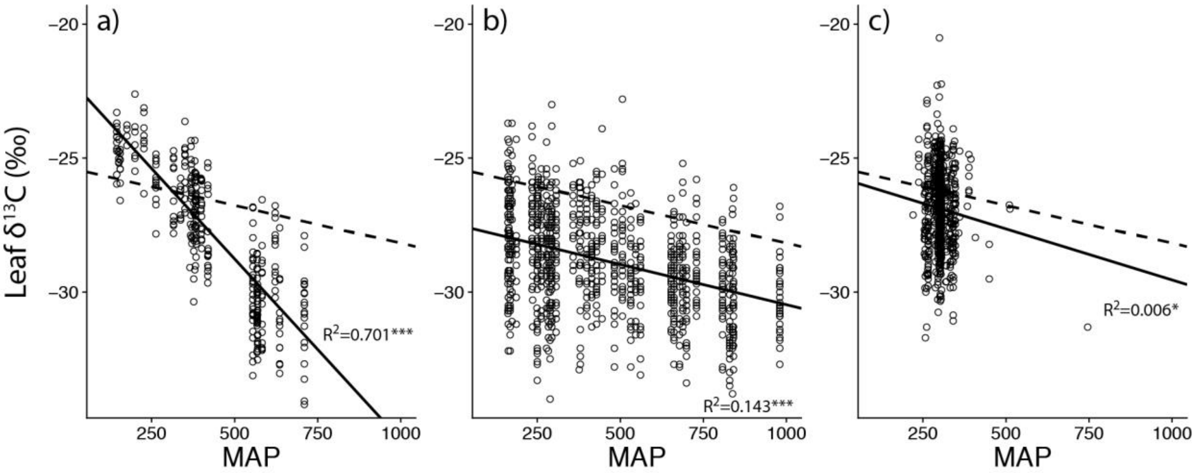

Figure 2: Gradient-level relationships between leaf δ^{13}C and mean annual precipitation (MAP) for a) the NECT; b) the TREND and c) the SWATT. Dashed lines represent a global MAP~leaf δ^{13}C linear regression derived from a global dataset of site-averaged leaf δ^{13}C values restricted to the MAP ranges of the gradients presented here. Solid lines show MAP~leaf δ^{13}C linear regressions for each gradient. Linear model statistics are shown in Table 1. *p<0.05, ***p<0.001.

Table 1: Gradient level statistics for the NECT, TREND, SWATT and global compilation

Gradient	Observations	Min MAP	Max MAP	MAP range	Slope	Intercept	R
Global compilation	392	140	980	830	-0.0028	-25.3571	0.171***
NECT	333	145	710	565	-0.0135	-22.0081	0.701***
TREND	996	162	980	818	-0.0030	-27.4683	0.143***
SWATT	652	220	746	526	-0.0040	-25.6973	0.006*

$^{*}p<0.05$, $^{***}p<0.001$. All MAP figures in mm.

Aim 2: Determine whether species δ^{13}C-MAP regression slopes (m_s) are similar to or different from the community slope (m_c) on SWATT.

Slopes for individual species were highly variable along the SWATT, mirroring the results found on the TREND (Figure 3). Six species recorded statistically significant m_s values: *Allocasuarina spinosissima*, *Dianella revoluta*, *Eucalyptus leptopoda*, *Eucalyptus salubris*, *Platysace trachymenioides* and *Ptilotus obovatus* (Appendix A). Following Bonferroni adjustment, where p values are multiplied by the number of tests in order to account for the increased likelihood of $p<0.05$ results occurring via chance, only *D. revoluta* and *P. trachymenioides* retained statistically significant m_s values (Appendix A).

Dianella revoluta is a perennial forb that is also present on the TREND in South Australia, and also produced a statistically significant m_s on TREND, where no other species exhibited a statistically significant m_s. This may point to *D. revoluta* exhibiting traits that make the species more sensitive to changes in c_i/c_a and/or less sensitive to other potential effects (e.g. mesophyll conductance). The *D. revoluta* m_s on the SWATT (-0.0109) was similar to that the species' TREND m_s of -0.0074, suggesting the species probably displays a similar response to aridity throughout its range.

Both *D. revoluta* and *P. trachymenioides* exhibited species slopes that were steeper than the community slope providing further evidence against universal scaling. Furthermore, it provides some evidence that these species are more isotopically responsive to changes in aridity than the entire tested flora at a landscape level.

P. trachymenioides exhibited a steeper m_s (-0.0146) than *D. revoluta* (-0.0109), indicating it is more isotopically responsive to changed MAP and suggests that it is unlikely to be able to maintain its current photosynthetic profile in environments more arid than those tested. This lower value may be an artefact of the limited sampling range, as when checked against the distribution of this species in the Australian Virtual Herbarium, its occurrence in MAP ranges of 208-436mm indicates it is able to persist in drier conditions than in which we sampled. Nevertheless, if the m_s we have calculated for this species based on its occurrence on the SWATT is correct, it is likely to be very sensitive to aridification in the future.

The sampling of >6 individuals across the SWATT did not produce numerous significant δ^{13}C-MAP relationships, likely due to the narrow range of MAP values. Nonetheless, the sample set does provide a measure of the average carbon isotope ratios of these species across a large geographic and climatic range (Figure 4), which could be easily expanded upon in the future. The differences among species could reflect differences in water use efficiency, with more positive values indicating greater water use efficiency (assimilation relative to water loss). However, we caution that other factors may contribute such as sampling of shaded versus fully sunlit leaves, and differences in mesophyll conductance.

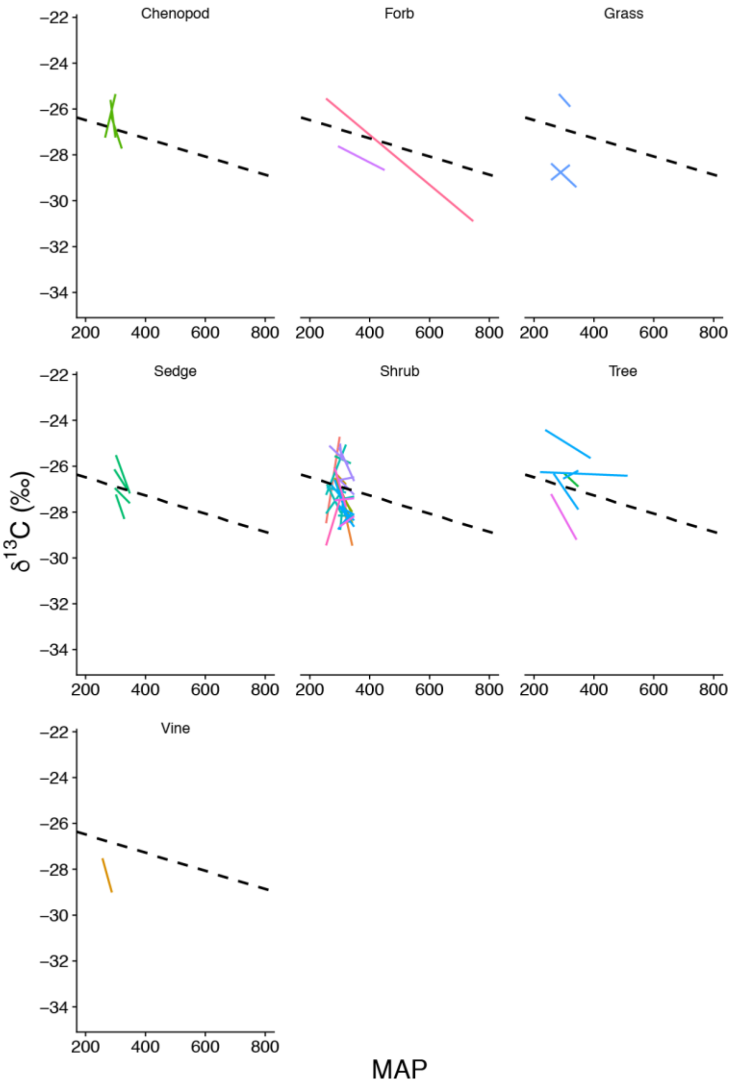

Figure 3: Species regressions for $\delta^{13}C$ vs. MAP for 49 species sampled on the SWATT, grouped by growth form. All species returned non-significant regressions except *Platysace trachymenioides* (shrub) and *Dianella revoluta* (forb).

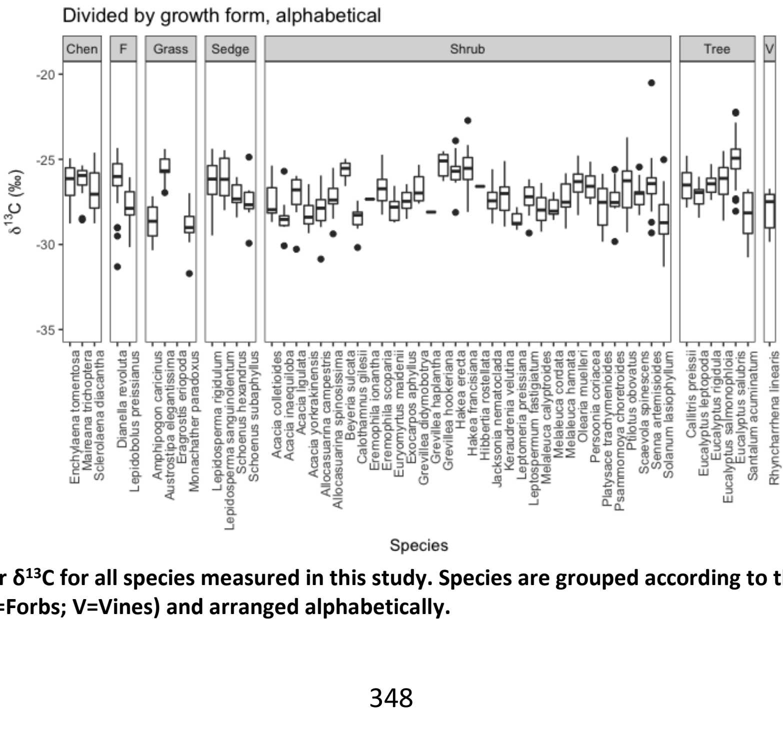

Figure 4: Box plots for $\delta^{13}C$ for all species measured in this study. Species are grouped according to their growth form (Chen=Chenopods; F=Forbs; V=Vines) and arranged alphabetically.

Aim 3: Determine whether the geographically overlapping *Eucalyptus salubris* and *E. salmonophloia* demonstrate different isotopic signature ranges and m_s values in order to determine whether these species can be distinguished isotopically.

Two *Eucalyptus* species (*Eucalyptus salmonophloia* and *E. salubris*) were selected for a high-resolution study to assess the value of more intensive sampling (n=50) within species, to quantify the natural $\delta^{13}C$ ranges of these species, and to determine whether these two similar and sympatric species exhibit different isotopic profiles.

While *E. salubris* did return a statistically significant m_s value (p=0.0442), this was not significant following Bonferroni correction (adj. p=2.1369; Appendix A). *E. salmonophloia* did not return a statistically significant m_s (p=0.8700). Thus, we were unable to statistically validate the isotopic response to changed MAP for either species, although this may be possible with either further sampling or additional control over other potential determinants of $\delta^{13}C_p$. In particular, as the leaves used for this analysis were initially collected for genetic analysis, it is unclear whether collections were standardised to ensure only sunlit leaves were collected to minimise the effect of shade altering photosynthetic demand.

When comparing the range of $\delta^{13}C_p$ values for *E. salmonophloia* and *E. salubris*, there is a clear offset between the two species (Figure 5), with *E. salmonophloia* displaying a lower $\delta^{13}C$ signature (mean $\delta^{13}C$ = -26.31 ‰, standard deviation = 1.10 ‰) than *E. salubris* (mean $\delta^{13}C$ = -24.98 ‰, standard deviation = 1.17 ‰). An analysis of variance (ANOVA) confirms that these two species are statistically distinguishable (Table 2). A similar result is evident for leaf carbon content (Figure 5, Table 3).

Isotope ratios of nitrogen are routinely measured along with carbon isotopes. As with carbon, stable nitrogen isotope measurements are a ratio of the heavier isotope (^{15}N) to the lighter isotope (^{14}N) compared to a standard (atmospheric nitrogen) and expressed in delta notation ($\delta^{15}N$). Nitrogen isotopes in plants are controlled by a potentially complex mix of drivers including soil chemistry and mycorhizzal associations (Craine et al. 2015). The lack of separation in $\delta^{15}N$ values between *E. salmonophloia* and *E. salubris* provides some evidence that soil-based processes are not influencing $\delta^{13}C$ in these species and one or more other environmental variables, or traits of the plants themselves, are driving the carbon isotope offset.

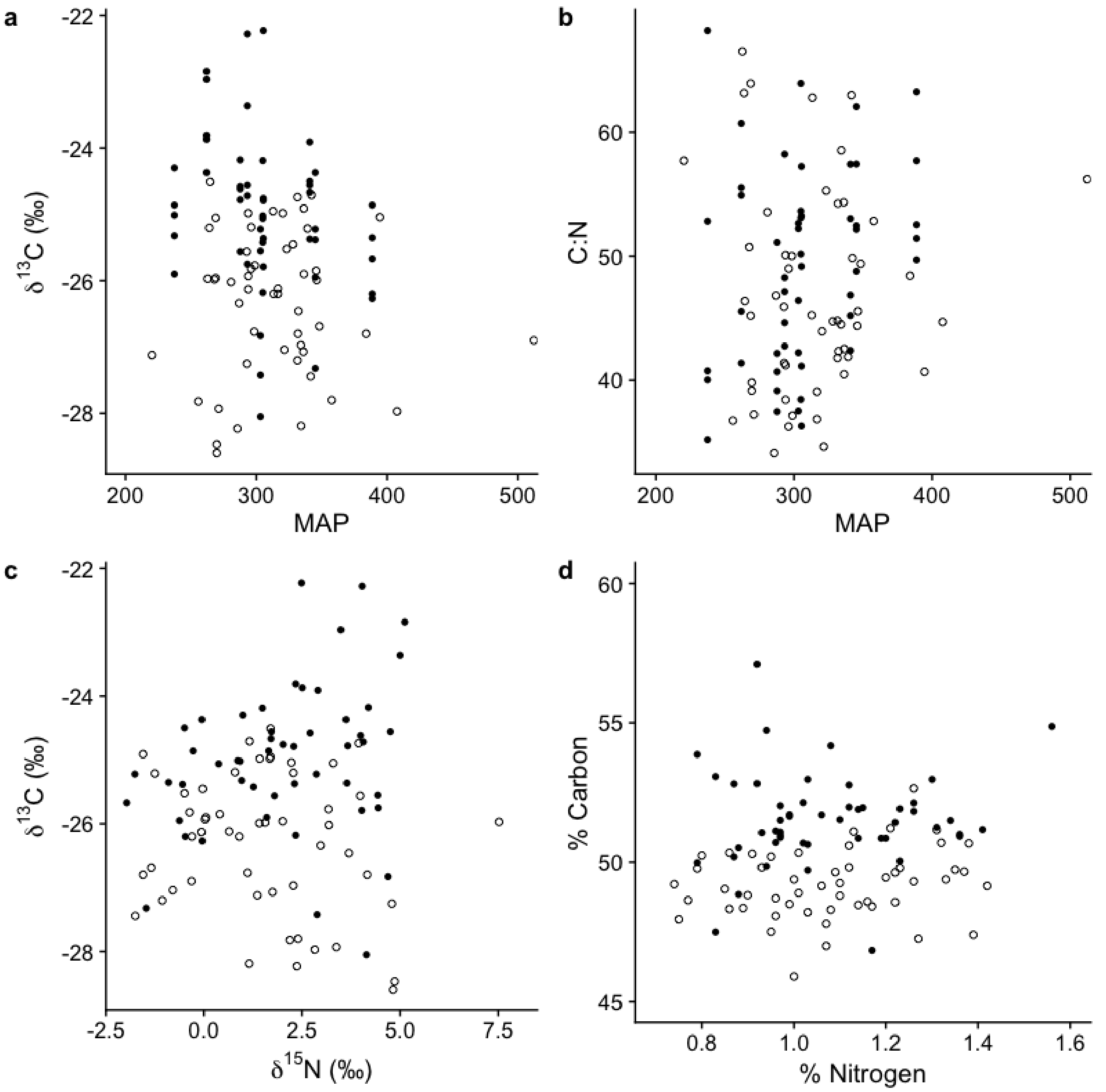

Figure 5: Despite having similar leaf morphology and overlapping distributions, *Eucalyptus salmonophloia* (open circles) and E. *salubris* (filled circles) were readily distinguishable based on leaf carbon isotope ($\delta^{13}C$) signatures (a,b) and carbon content (d), but not based on leaf nitrogen isotope ($\delta^{15}N$) signature (b) or C:N (b).

Table 2: Species level ANOVA results of $\delta^{13}C$ difference

	Degrees of freedom	Sum of Squares	Mean Squares	F value	p value
Species	1	44.38	44.38	34.57	<0.0001***
Residuals	99	127.10	1.28		

Table 3: Species level ANOVA results of % C differences

Degrees of freedom	Sum of Squares	Mean Squares	F value	p value
1	137.5	62.38	3.97	<0.0001***
99	218.3	2.2		

Conclusion

- Significant δ^{13}C-MAP relationships have been identified in a global compilation and on the TREND and NECT bioclimatic transects which span large ranges of MAP.
- This is also true of the SWATT, despite our samples being concentrated primarily in a much smaller MAP range (200-400 mm/year).
- With only two exceptions, individual species failed to produce significant relationships between leaf δ^{13}C and MAP.
- The small range of MAP likely hindered detection of significant species slopes in spite of efforts to sample more intensively than previously on either TREND or NECT.
- The two significant species slopes are steeper than the community slope, providing evidence against universal scaling occurring at different scales on the SWATT.
- The high intensity *Eucalyptus* study shows that finding significant species slopes is not simply a function of sampling intensity.
- The offset in δ^{13}C and carbon content between two sympatric eucalypts suggest that species-specific traits play a measurable role in leaf δ^{13}C, even when those species are very similar.
- Likewise, the similar response of *Dianella revoluta* on two different transects suggests species are able to respond similarly even in quite different environment (sandplains vs the TREND soil mosaic).
- The diversity of m_s (albeit without statistical significance) could be due to narrow range or precipitation, but could also reflect diversity or approaches to managing water limitation that are not evident on other transects.
- The calculation of a gradient-wide δ^{13}C-MAP regression (m_c) for the SWATT that does not closely resemble those calculated for other subcontinental gradients (NECT) provides additional support for different landscape-level responses and further refutes the concept of universal scaling operating at different locations.

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Appendix A: Species level carbon isotope statistics. Significance indicated by shading of p value (p), and Bonferroni adjusted p (adj.p)

Species	n	Family	Form	MAP		$\delta^{13}C_p$		$\delta^{13}C_p$~MAP regression				
				Min	Max	Min	Max	Slope (m_s)	Intercept	R^2	p	Adj.p
Acacia colletioides	8	Fabaceae	Shrub	254.20	320.63	-28.68	-25.37	0.0183	-32.7053	-0.0165	0.3828	18.7584
Acacia inaequiloba	9	Fabaceae	Shrub	301.49	311.94	-30.08	-25.7	0.1351	-69.5183	-0.0028	0.3557	17.4270
Acacia ligulata	10	Fabaceae	Shrub	257.04	304.44	-30.28	-25.97	-0.0262	-20.0565	0.0192	0.3097	15.1765
Acacia yorkrakinensis	8	Fabaceae	Shrub	294.35	347.62	-29.34	-26.46	-0.0004	-28.0317	-0.1666	0.9869	48.3574
Allocasuarina campestris	9	Casuarinaceae	Shrub	299.07	341.72	-30.86	-25.95	-0.0284	-18.7617	0.0036	0.3442	16.8655
Allocasuarina spinosissima	17	Casuarinaceae	Shrub	283.07	341.72	-29.38	-25.5	-0.0269	-18.9108	0.2077	0.0377	1.8486
Amphipogon caricinus	16	Poaceae	Grass	257.04	340.90	-31.08	-27.17	-0.0123	-25.2196	0.0182	0.2774	13.5910
Austrostipa elegantissima	14	Poaceae	Grass	283.07	320.63	-26.96	-24.39	-0.0146	-21.2089	-0.0129	0.3789	18.5651
Beyeria sulcata	9	Euphorbiaceae	Shrub	283.07	336.69	-26.57	-24.97	-0.0059	-23.8895	-0.1175	0.7022	34.4058
Callitris preissii	15	Cupressaceae	Tree	299.07	347.70	-27.83	-24.79	-0.0122	-22.6291	-0.0330	0.4705	23.0569
Calothamnus gilesii	7	Myrtaceae	Shrub	294.35	340.08	-30.18	-27.43	0.0162	-33.5269	-0.1105	0.5535	27.1191
Dianella revoluta	23	Xanthorrhoeaceae	Forb	254.20	745.70	-31.31	-24.33	-0.0109	-22.7800	0.4023	0.0007	0.0337
Enchylaena tomentosa	8	Chenopodiaceae	Chenopod	265.07	299.65	-28.77	-24.94	0.0548	-41.7841	0.3527	0.0707	3.4636
Eremophila scoparia	9	Scrophulariaceae	Shrub	283.07	320.63	-28.25	-24.73	-0.0454	-13.2296	0.2525	0.0957	4.6908
Eucalyptus leptopoda	13	Myrtaceae	Tree	265.65	347.62	-28.43	-26.02	-0.0190	-21.2712	0.2811	0.0361	1.7709
Eucalyptus rigidula	10	Myrtaceae	Tree	297.33	347.70	-27.39	-25.28	0.0070	-28.6192	-0.0836	0.5954	29.1736
Eucalyptus salmonophloia	61	Myrtaceae	Tree	220.32	512.27	-28.6	-24.51	0.0005	-26.4950	-0.0165	0.8701	42.6358
Eucalyptus salubris	50	Myrtaceae	Tree	237.51	388.64	-28.05	-22.23	-0.0081	-22.4877	0.0626	0.0442	2.1639
Euryomyrtus maidenii	9	Myrtaceae	Shrub	297.33	347.70	-28.71	-26.56	-0.0184	-22.2341	0.0395	0.2869	14.0570
Exocarpos aphyllus	10	Santalaceae	Shrub	283.07	347.70	-28.5	-26.46	0.0014	-27.8882	-0.1230	0.9084	44.5131
Grevillea didymobotrya	20	Proteaceae	Shrub	294.35	347.70	-27.6	-25.32	-0.0134	-22.5672	0.0363	0.2068	10.1329
Grevillea hookeriana	6	Proteaceae	Shrub	301.19	306.26	-26.28	-24.52	-0.1409	17.4180	-0.0934	0.4912	24.0685
Hakea erecta	11	Proteaceae	Shrub	297.33	347.70	-28.12	-23.91	-0.0291	-16.5372	0.1585	0.1237	6.0630
Hakea francisiana	10	Proteaceae	Shrub	265.07	329.20	-28.09	-22.72	-0.0125	-21.8045	-0.0877	0.6147	30.1221
Jacksonia nematoclada	10	Fabaceae	Shrub	297.33	347.70	-28.78	-25.58	0.0027	-28.2529	-0.1208	0.8663	42.4480
Keraudrenia velutina	14	Malvaceae	Shrub	265.07	306.26	-28.95	-25.1	-0.0156	-22.5981	-0.0227	0.4154	20.3536
Lepidobolus preissianus	11	Restionaceae	Forb	294.35	449.67	-30.16	-26.05	-0.0067	-25.6544	-0.0489	0.4838	23.7048
Lepidosperma rigidulum	11	Cyperaceae	Sedge	296.00	340.90	-29.47	-24.38	-0.0204	-20.1109	-0.0689	0.5658	27.7260
Lepidosperma sanguinolentum	14	Cyperaceae	Sedge	301.19	347.70	-27.99	-24.46	-0.0359	-14.7200	0.2021	0.0605	2.9635
Leptomeria preissiana	9	Santalaceae	Shrub	299.07	347.70	-29.14	-27.81	0.0091	-31.3651	-0.0066	0.3627	17.7747
Leptospermum fastigiatum	12	Myrtaceae	Shrub	296.41	306.26	-29.33	-26.17	-0.0388	-15.5897	-0.0836	0.7057	34.5790
Maireana trichoptera	12	Chenopodiaceae	Chenopod	283.07	299.65	-28.56	-25.36	-0.0990	2.4163	0.1184	0.1466	7.1828
Melaleuca calyptroides	11	Myrtaceae	Shrub	299.65	347.70	-29.25	-26.39	-0.0118	-24.2489	-0.0493	0.4849	23.7603
Melaleuca cordata	16	Myrtaceae	Shrub	294.35	347.70	-28.56	-26.93	-0.0076	-25.4836	0.0029	0.3242	15.8850
Melaleuca hamata	9	Myrtaceae	Shrub	283.07	332.29	-29.07	-25.82	-0.0380	-15.8426	0.1148	0.1965	9.6281
Monachather paradoxus	11	Poaceae	Grass	257.04	319.11	-31.71	-26.99	0.0106	-31.8180	-0.0819	0.6340	31.0651
Olearia muelleri	12	Asteraceae	Shrub	283.07	320.63	-28.28	-24.78	-0.0138	-22.3660	-0.0677	0.5944	29.1278

Persoonia coriacea	12	Proteaceae	Shrub	297.33	347.70	-27.65	-25.14	0.0026	-27.3757	-0.0964	0.8605	42.1653
Platysace trachymenioides	12	Apiaceae	Shrub	283.07	341.72	-29.84	-25.85	-0.0582	-9.5830	0.7196	0.0003	0.0146
Psammomoya choretroides	8	Celastraceae	Shrub	297.33	340.90	-29.83	-25.6	-0.0203	-21.0521	-0.0906	0.5416	26.5365
Ptilotus obovatus	13	Amaranthaceae	Shrub	254.20	299.65	-29.29	-23.71	0.0828	-49.5416	0.4735	0.0056	0.2737
Rhyncharrhena linearis	7	Apocynaceae	Vine	256.21	287.66	-29.85	-26.73	-0.0473	-15.3926	-0.0514	0.4389	21.5080
Santalum acuminatum	14	Santalaceae	Tree	257.04	341.72	-30.76	-26.75	-0.0235	-21.1775	0.0409	0.2362	11.5754
Scaevola spinescens	10	Goodeniaceae	Shrub	256.21	320.63	-28.28	-25.45	-0.0035	-26.0233	-0.1161	0.8071	39.5489
Schoenus hexandrus	10	Cyperaceae	Sedge	297.33	347.70	-28.41	-26.04	-0.0130	-23.0814	-0.0276	0.4092	20.0499
Schoenus subaphyllus	7	Cyperaceae	Sedge	301.19	329.20	-29.93	-24.87	-0.0379	-15.8156	-0.1251	0.5889	28.8559
Sclerolaena diacantha	11	Chenopodiaceae	Chenopod	283.07	320.63	-28.76	-24.61	-0.0400	-14.9055	0.0956	0.1854	9.0835
Senna artemisioides	24	Fabaceae	Shrub	254.20	320.63	-29.32	-20.51	0.0329	-35.6260	0.1183	0.0556	2.7249
Solanum lasiophyllum	18	Solanaceae	Shrub	254.20	299.65	-31.31	-25.02	0.0432	-40.4366	0.1613	0.0554	2.7134

Appendix B: Site location and climate information

Site	Site type	Latitude	Longitude	Elevation	MAT	MaxT	MinT	MAP	MaxP	MinP	MAMI	MaxMI	MinMI
SWA0101	SWATT	-34.6192	117.1554	231.9	15.11	26.98	6.53	745.70	28.06	4.85	0.6380	1.0000	0.1262
SWA0301	SWATT	-32.8746	119.1580	329.8	16.46	31.84	4.46	332.29	10.87	2.89	0.2696	0.5985	0.0704
SWA0302	SWATT	-32.8752	119.1844	349.3	16.37	31.73	4.41	335.40	10.90	2.91	0.2728	0.6038	0.0716
SWA0303	SWATT	-32.8672	119.1927	354.3	16.36	31.74	4.40	336.69	10.93	2.92	0.2737	0.6053	0.0718
SWA0304	SWATT	-32.8147	119.1428	363.9	16.37	31.93	4.32	341.72	11.28	2.93	0.2789	0.6201	0.0711
SWA0401	SWATT	-31.8458	118.9052	440.1	17.10	33.78	4.09	347.70	11.64	3.33	0.2671	0.6211	0.0612
SWA0402	SWATT	-31.8532	118.8963	438.7	17.10	33.78	4.10	347.62	11.65	3.33	0.2674	0.6219	0.0612
SWA0403	SWATT	-31.8811	118.8934	402.3	17.27	33.93	4.22	340.08	11.35	3.26	0.2599	0.6052	0.0597
SWA0404	SWATT	-31.9023	118.9437	408.4	17.21	33.87	4.16	340.90	11.30	3.29	0.2608	0.6061	0.0602
SWA0502	SWATT	-31.4012	119.5396	432.6	17.49	34.15	3.81	329.20	9.78	3.34	0.2279	0.5101	0.0606
SWA0503	SWATT	-31.4014	119.5738	399.3	17.66	34.29	3.93	320.32	9.43	3.20	0.2191	0.4895	0.0589
SWA0504	SWATT	-31.4014	119.6405	378.0	17.79	34.37	4.04	311.94	9.06	3.08	0.2110	0.4701	0.0576
SWA0601	SWATT	-31.2032	120.3082	434.6	17.90	34.16	4.36	302.61	8.07	3.27	0.1910	0.4080	0.0650
SWA0701	SWATT	-30.7885	120.3150	421.7	18.46	34.78	4.53	294.35	7.75	2.94	0.1764	0.3775	0.0606
SWA0702	SWATT	-30.7783	120.3058	433.9	18.40	34.74	4.48	296.41	7.83	2.99	0.1783	0.3820	0.0610
SWA0703	SWATT	-30.7572	120.2699	461.1	18.28	34.66	4.37	301.19	8.06	3.10	0.1831	0.3937	0.0616
SWA0704	SWATT	-30.6854	120.2574	482.8	18.24	34.65	4.31	304.38	8.19	3.15	0.1852	0.3990	0.0619
SWA0802	SWATT	-30.5015	120.6600	522.7	18.34	34.58	4.52	304.44	8.24	3.00	0.1782	0.3767	0.0648
SWA0901	SWATT	-28.1431	120.9661	522.7	20.81	37.24	5.62	257.04	11.23	1.49	0.1220	0.2447	0.0418
SWA0902	SWATT	-28.1318	120.9625	512.4	20.87	37.30	5.66	256.21	11.26	1.47	0.1211	0.2425	0.0412
SWA0904	SWATT	-28.0651	121.0137	473.5	21.12	37.54	5.78	254.20	11.54	1.36	0.1177	0.2337	0.0385
SWA1001	SWATT	-27.3828	120.6911	530.4	21.19	37.97	4.90	266.08	11.55	1.17	0.1188	0.2275	0.0379
SWA1002	SWATT	-27.3892	120.6986	531.7	21.18	37.95	4.91	266.14	11.57	1.17	0.1189	0.2277	0.0380
SWA1003	SWATT	-27.3994	120.6822	531.6	21.17	37.95	4.90	265.65	11.44	1.18	0.1190	0.2284	0.0382
WAAAVW0001	AusPlot	-31.9022	118.9436	408.4	17.21	33.87	4.16	340.90	11.30	3.29	0.2608	0.6061	0.0602
WAAAVW0002	AusPlot	-31.8458	118.9051	440.1	17.10	33.78	4.09	347.70	11.64	3.33	0.2671	0.6211	0.0612
WAAAVW0003	AusPlot	-32.4804	116.9389	343.9	16.31	32.36	4.61	449.99	19.61	2.45	0.4278	0.9275	0.0615
WAAAVW0004	AusPlot	-32.4797	116.9357	341.4	16.32	32.38	4.61	449.67	19.60	2.44	0.4273	0.9269	0.0614
WAACOO0001	AusPlot	-30.4352	120.6441	497.7	18.54	34.79	4.60	299.65	8.32	2.91	0.1732	0.3658	0.0630
WAACOO0003	AusPlot	-30.3917	120.6483	487.3	18.64	34.89	4.64	297.33	8.42	2.87	0.1706	0.3600	0.0622
WAACOO0004	AusPlot	-30.4653	120.8064	413.6	18.97	35.09	4.97	283.07	8.24	2.61	0.1578	0.3286	0.0596
WAACOO0005	AusPlot	-31.6029	119.8096	393.4	17.56	33.97	4.11	319.11	9.39	3.42	0.2185	0.4816	0.0614
WAACOO0006	AusPlot	-31.5964	119.8241	407.4	17.50	33.91	4.07	320.63	9.41	3.46	0.2200	0.4847	0.0621
WAACOO0007	AusPlot	-31.6067	119.8075	394.2	17.55	33.96	4.11	319.36	9.41	3.43	0.2188	0.4822	0.0615
WAACOO0008	AusPlot	-31.5964	119.8241	407.4	17.50	33.91	4.07	320.63	9.41	3.46	0.2200	0.4847	0.0621
WAACOO0009	AusPlot	-31.2391	120.3261	391.5	18.09	34.30	4.50	295.53	7.91	3.16	0.1844	0.3926	0.0635
WAACOO0010	AusPlot	-31.2318	120.3298	395.2	18.08	34.29	4.49	296.00	7.92	3.16	0.1847	0.3933	0.0636
WAACOO0011	AusPlot	-31.2528	120.3428	383.5	18.12	34.31	4.54	294.22	7.88	3.14	0.1831	0.3892	0.0635
WAACOO0012	AusPlot	-31.2374	120.3323	390.4	18.10	34.30	4.51	295.24	7.91	3.15	0.1840	0.3916	0.0635

WAACOO0016	AusPlot	-31.2032	120.3082	434.6	17.90	34.16	4.36	302.61	8.07	3.27	0.1910	0.4080	0.0650
WAACOO0017	AusPlot	-31.1692	120.3055	429.8	17.97	34.23	4.38	301.19	8.04	3.22	0.1890	0.4040	0.0644
WAACOO0018	AusPlot	-31.1952	120.3184	435.1	17.91	34.16	4.37	302.46	8.07	3.26	0.1906	0.4068	0.0651
WAACOO0019	AusPlot	-31.2071	120.2651	452.1	17.80	34.09	4.27	306.26	8.17	3.36	0.1953	0.4188	0.0652
WAACOO0020	AusPlot	-30.1919	120.6551	436.3	19.13	35.39	4.85	287.35	8.86	2.65	0.1592	0.3343	0.0587
WAACOO0021	AusPlot	-30.1923	120.6506	437.6	19.12	35.39	4.84	287.66	8.86	2.66	0.1595	0.3350	0.0587
WAACOO0022	AusPlot	-30.1953	120.6328	448.8	19.05	35.33	4.80	289.85	8.85	2.70	0.1616	0.3399	0.0593
WAACOO0023	AusPlot	-30.1924	120.6587	435.6	19.13	35.40	4.85	287.19	8.87	2.65	0.1591	0.3339	0.0586
WAACOO0024	AusPlot	-30.1850	120.6447	443.8	19.09	35.37	4.82	288.79	8.89	2.67	0.1605	0.3372	0.0590
WAACOO0025	AusPlot	-30.1951	120.5988	475.4	18.91	35.22	4.69	294.90	8.87	2.79	0.1662	0.3509	0.0604
WAACOO0026	AusPlot	-30.5304	120.6657	502.3	18.42	34.63	4.59	301.27	8.14	2.93	0.1755	0.3702	0.0642
WAACOO0027	AusPlot	-30.5010	120.6606	522.7	18.34	34.58	4.52	304.44	8.24	3.00	0.1782	0.3767	0.0648
WAACOO0028	AusPlot	-30.4871	120.6587	520.0	18.37	34.61	4.53	303.82	8.27	2.99	0.1775	0.3752	0.0645
WAACOO0029	AusPlot	-30.4324	120.6272	507.1	18.49	34.75	4.56	301.49	8.34	2.95	0.1750	0.3701	0.0633
WAACOO0030	AusPlot	-30.4324	120.6272	507.1	18.49	34.75	4.56	301.49	8.34	2.95	0.1750	0.3701	0.0633
WAACOO0031	AusPlot	-30.6853	120.2574	482.8	18.24	34.65	4.31	304.38	8.19	3.15	0.1852	0.3990	0.0619
WAACOO0032	AusPlot	-30.7885	120.3149	421.7	18.46	34.78	4.53	294.35	7.75	2.94	0.1764	0.3775	0.0606
WAAESP0001	AusPlot	-34.4769	117.7543	171.6	15.73	27.13	6.78	509.98	17.83	4.23	0.4983	0.9200	0.1038
WAALSD0001	AusPlot	-25.0962	120.7245	609.3	22.56	39.08	5.02	261.01	18.77	0.73	0.0891	0.1344	0.0218
WAALSD0002	AusPlot	-25.0567	120.7397	575.3	22.76	39.27	5.15	257.62	18.96	0.67	0.0866	0.1330	0.0206
WAALSD0003	AusPlot	-25.2684	120.6280	639.6	22.29	38.87	4.89	258.96	17.57	0.81	0.0912	0.1408	0.0225
WAAMAL0002	AusPlot	-32.8147	119.1428	363.9	16.37	31.93	4.32	341.72	11.28	2.93	0.2789	0.6201	0.0711
WAAMAL0003	AusPlot	-32.8746	119.1580	329.8	16.46	31.84	4.46	332.29	10.87	2.89	0.2696	0.5985	0.0704
WAAMUR0001	AusPlot	-28.0651	121.0137	473.5	21.12	37.54	5.78	254.20	11.54	1.36	0.1177	0.2337	0.0385
WAAMUR0002	AusPlot	-28.1431	120.9661	522.7	20.81	37.24	5.62	257.04	11.23	1.49	0.1220	0.2447	0.0418
WAAMUR0028	AusPlot	-27.3887	120.6991	531.7	21.18	37.95	4.91	266.14	11.57	1.17	0.1189	0.2277	0.0380
WAAMUR0029	AusPlot	-27.3994	120.6822	531.6	21.17	37.95	4.90	265.65	11.44	1.18	0.1190	0.2284	0.0382
WAAMUR0030	AusPlot	-27.3823	120.6917	530.0	21.19	37.97	4.90	266.11	11.56	1.16	0.1188	0.2273	0.0379
WAAMUR0031	AusPlot	-27.4386	120.6555	539.6	21.10	37.87	4.88	265.07	11.18	1.22	0.1201	0.2318	0.0390
WAGCOO0001	AusPlot	-30.4361	120.6429	497.7	18.54	34.79	4.60	299.65	8.32	2.91	0.1732	0.3658	0.0630
WAGCOO0002	AusPlot	-30.3510	120.6427	499.2	18.62	34.89	4.61	299.07	8.53	2.90	0.1717	0.3629	0.0623
WAGCOO0004	AusPlot	-30.4653	120.8064	413.6	18.97	35.09	4.97	283.07	8.24	2.61	0.1578	0.3286	0.0596
BAN	Eucalypt	-30.3675	121.2720	421.1	19.07	35.17	4.95	271.15	8.59	2.57	0.1483	0.3081	0.0562
BEN	Eucalypt	-30.8101	117.8203	357.5	18.65	35.12	5.86	304.99	10.84	2.74	0.2245	0.5458	0.0463
BEV	Eucalypt	-32.1788	116.9765	211.3	17.44	33.99	4.86	394.45	17.01	2.05	0.3598	0.8366	0.0521
BOO	Eucalypt	-31.5953	119.8219	405.2	17.51	33.92	4.08	320.35	9.40	3.45	0.2197	0.4841	0.0620
BRR	Eucalypt	-32.0479	117.9129	289.0	17.42	33.62	4.86	321.57	12.38	2.36	0.2690	0.6465	0.0524
BUL	Eucalypt	-30.5200	121.7900	395.9	18.96	35.22	4.65	262.00	8.51	2.60	0.1450	0.3016	0.0516
BUN	Eucalypt	-32.9833	118.8333	301.7	16.39	31.71	4.51	331.91	11.84	2.87	0.2813	0.6350	0.0671
BUR	Eucalypt	-31.6276	118.5115	411.5	17.51	34.03	4.64	345.92	12.13	2.95	0.2642	0.6265	0.0565
CHF	Eucalypt	-30.9955	122.8492	282.5	18.75	34.74	4.58	255.89	9.23	2.87	0.1439	0.2754	0.0557

CHR	Eucalypt	-31.6304	121.1679	440.5	17.44	33.15	4.49	302.79	8.07	3.80	0.1942	0.3910	0.0777
CHR	Eucalypt	-33.9679	118.1103	249.9	15.84	28.89	5.92	360.38	12.32	3.05	0.3497	0.7253	0.0805
COO	Eucalypt	-31.0515	123.0540	357.3	18.25	34.25	4.25	264.00	9.24	3.06	0.1549	0.2993	0.0586
COW	Eucalypt	-31.1112	122.4534	292.1	18.65	34.57	4.65	267.80	9.34	3.13	0.1517	0.2924	0.0608
CRE	Eucalypt	-30.1900	120.6500	437.6	19.12	35.39	4.84	287.66	8.86	2.66	0.1595	0.3350	0.0587
CRN	Eucalypt	-30.3757	120.7467	428.7	18.98	35.16	4.89	285.57	8.43	2.66	0.1594	0.3336	0.0594
CRS	Eucalypt	-30.1908	120.6641	434.3	19.14	35.40	4.86	286.87	8.87	2.64	0.1588	0.3332	0.0586
CRW	Eucalypt	-30.4406	120.5165	444.7	18.78	35.07	4.69	292.65	8.09	2.79	0.1678	0.3550	0.0604
DAY	Eucalypt	-32.2300	120.4506	356.9	17.15	32.70	4.61	305.41	8.26	3.91	0.2107	0.4339	0.0731
DOO	Eucalypt	-31.6893	117.9820	321.5	17.78	34.04	5.09	323.31	11.28	2.55	0.2542	0.6050	0.0518
FHN	Eucalypt	-33.0684	120.0466	373.4	16.21	30.43	5.12	341.74	10.15	3.50	0.2597	0.5323	0.0861
FLF	Eucalypt	-32.1000	119.1000	400.0	17.05	33.61	4.10	346.40	11.40	3.43	0.2670	0.6132	0.0635
GOO	Eucalypt	-30.0800	121.1500	374.7	19.61	35.77	5.18	264.59	9.35	2.32	0.1397	0.2896	0.0534
HOL	Eucalypt	-31.6779	120.4634	442.5	17.34	33.34	4.31	310.23	8.12	3.86	0.2059	0.4315	0.0716
HOL	Eucalypt	-31.9299	120.1085	429.6	17.09	33.14	4.19	323.08	9.22	3.90	0.2256	0.4835	0.0700
HRK	Eucalypt	-31.3976	120.0112	439.3	17.58	33.97	4.08	312.89	8.63	3.46	0.2085	0.4547	0.0633
JAE	Eucalypt	-30.8198	120.3527	418.5	18.45	34.74	4.56	293.82	7.74	2.92	0.1757	0.3749	0.0612
JAE	Eucalypt	-30.8198	120.3527	418.5	18.45	34.74	4.56	293.82	7.74	2.92	0.1757	0.3749	0.0612
JDS	Eucalypt	-30.8728	120.1907	431.8	18.27	34.67	4.37	298.39	8.09	3.11	0.1834	0.3959	0.0603
KAH	Eucalypt	-30.9900	121.1200	420.0	18.44	34.15	5.18	293.15	8.29	2.51	0.1685	0.3401	0.0681
KAM	Eucalypt	-31.2000	121.6000	320.1	18.57	34.22	5.07	269.64	8.04	3.06	0.1558	0.3136	0.0660
KAM	Eucalypt	-31.2000	121.6000	320.1	18.57	34.22	5.07	269.64	8.04	3.06	0.1558	0.3136	0.0660
KAN	Eucalypt	-31.0536	121.5686	332.3	18.70	34.35	5.16	266.32	7.99	2.86	0.1509	0.3055	0.0631
KAN	Eucalypt	-31.2219	121.5887	325.2	18.53	34.17	5.05	271.37	8.06	3.10	0.1575	0.3168	0.0666
KHT	Eucalypt	-31.0683	121.0850	419.9	18.33	34.07	5.09	293.77	8.26	2.66	0.1711	0.3458	0.0693
KNT	Eucalypt	-33.3700	118.7400	317.8	15.96	30.31	4.92	339.33	11.13	3.14	0.2991	0.6479	0.0768
KOO	Eucalypt	-29.3996	121.2818	408.7	20.14	36.40	5.41	262.81	11.56	2.04	0.1334	0.2766	0.0509
KUL	Eucalypt	-32.5500	118.0200	326.7	16.67	32.73	4.57	334.36	12.54	2.63	0.2981	0.7011	0.0589
KWO	Eucalypt	-31.7743	117.7712	270.6	17.83	34.07	5.10	313.35	11.31	2.42	0.2542	0.6105	0.0492
LCN	Eucalypt	-32.3820	119.7584	382.7	16.84	32.62	4.36	334.20	10.22	3.60	0.2463	0.5335	0.0720
LGE	Eucalypt	-29.8771	119.9710	444.5	19.29	35.91	4.53	292.83	8.99	2.73	0.1656	0.3551	0.0556
LKG	Eucalypt	-33.0932	119.3495	347.9	16.14	30.80	4.68	336.54	10.57	3.27	0.2718	0.5861	0.0789
LOC	Eucalypt	-33.3000	119.0200	318.3	15.97	30.34	4.74	345.29	11.10	3.21	0.2926	0.6307	0.0792
MAD	Eucalypt	-31.3854	122.1136	336.3	18.15	33.90	4.56	280.68	8.35	3.46	0.1685	0.3317	0.0689
MOD	Eucalypt	-32.5260	119.3984	329.5	16.94	32.78	4.44	341.01	10.77	3.24	0.2590	0.5716	0.0702
MTH	Eucalypt	-32.9167	116.8667	369.9	15.59	31.30	4.41	512.27	22.29	2.59	0.4949	0.9804	0.0689
NEW	Eucalypt	-33.3090	119.1946	310.5	16.05	30.20	4.88	348.42	10.85	3.35	0.2875	0.6109	0.0826
NOR	Eucalypt	-31.7500	116.7500	211.7	17.71	34.36	5.02	407.67	17.56	1.81	0.3667	0.8570	0.0462
NYA	Eucalypt	-33.5833	118.3333	338.0	15.78	29.86	5.31	357.86	12.31	3.02	0.3389	0.7269	0.0799
PRS	Eucalypt	-31.7755	119.6125	399.0	17.34	33.78	4.05	336.41	10.66	3.54	0.2392	0.5331	0.0619
QUH	Eucalypt	-32.5463	117.4940	276.0	16.83	32.58	5.00	342.41	13.60	2.31	0.3185	0.7459	0.0540

QUS	Eucalypt	-32.0836	117.3683	223.1	17.59	33.87	4.96	328.19	12.79	2.25	0.2885	0.6904	0.0497	
QUW	Eucalypt	-31.9629	117.1795	330.1	17.13	33.64	4.59	383.94	15.67	2.35	0.3492	0.8172	0.0505	
QVS	Eucalypt	-30.1500	123.3200	355.4	19.17	35.42	4.43	237.51	9.52	2.32	0.1202	0.2246	0.0505	
RAV	Eucalypt	-33.4500	120.0300	283.7	16.31	29.27	5.97	388.64	11.07	4.17	0.2948	0.5800	0.1023	
SKP	Eucalypt	-33.4167	118.4667	280.9	16.22	30.55	5.34	332.32	11.19	3.06	0.3012	0.6592	0.0735	
VRN	Eucalypt	-31.2485	120.9390	421.0	18.06	33.88	4.84	296.09	8.12	3.03	0.1782	0.3632	0.0712	
WEL	Eucalypt	-31.1234	119.7792	382.1	18.11	34.69	4.14	298.85	8.76	2.96	0.1940	0.4299	0.0556	
WOG	Eucalypt	-31.9097	118.5214	331.8	17.62	34.17	4.74	336.16	11.34	2.93	0.2581	0.6055	0.0575	
WOO	Eucalypt	-31.1381	120.6342	418.5	18.15	34.18	4.70	296.01	8.02	2.95	0.1782	0.3710	0.0683	
YEL	Eucalypt	-31.2959	119.6544	380.1	17.89	34.50	4.03	303.18	8.90	2.86	0.2030	0.4530	0.0561	
ZAN	Eucalypt	-31.0276	123.5962	267.2	18.61	34.53	4.43	220.32	7.93	2.59	0.1226	0.2257	0.0512	

MAT= Mean Annual Temperature; MaxT=Mean maximum temperature of the warmest month; MinT=Mean minimum temperature of the coolest month; MAP=Mean Annual Precipitation; MaxP=Mean maximum precipitation of the wettest month; MinP=Mean minimum precipitation of the wettest month; MAMI=Mean Annual Moisture Index; MaxMI=Mean Moisure Index of the wettest quarter; MinMI=Mean Moisture Index of the driest quarter. All climate variables derived from 0.01° (~1km) gridded cells derived from ANUCLIM v6.0 extracted from the Atlas of Living Australia. Site type=SWATT (surveyed by Rachel Meissner during the establishment of the SWATT; AusPlot; Eucalypt (sampling location of *Eucalyptus salmonophloia* or *E. salubris* only).