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Enhanced species distribution models: a case study using essential population data from *Actinotus helianthi* (flannel flower)

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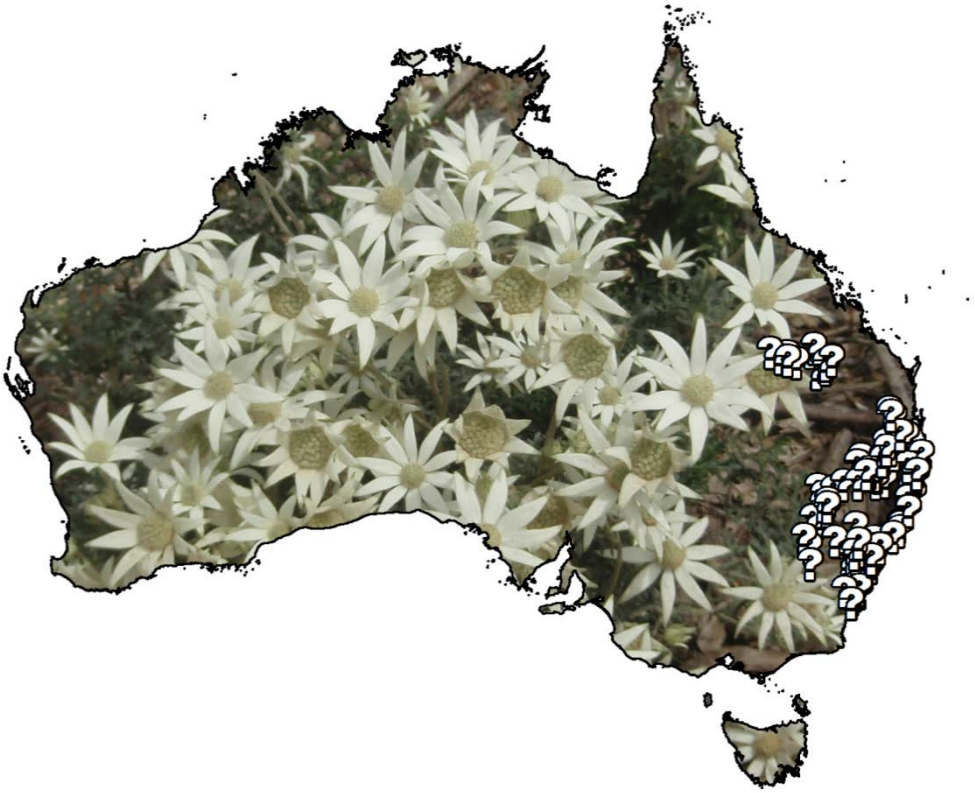

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To Mum and Dad
For giving me so many opportunities

Preface

This thesis is the work of Nathan Emery, and supervised by A/Prof. Glenda Wardle and A/Prof Murray Henwood at the University of Sydney, and Dr. Catherine Offord at the Australian Botanic Garden, Mount Annan. I am grateful to my supervisors for their assistance with the design of experiments and constructive comments on the manuscripts. All of the research reported in this thesis is my own work.

The thesis contains five data chapters that contribute towards enhancing the current capacity of Species Distribution Models (SDMs) to predict future changes to the spatial distribution of species. As is customary, the thesis has an introductory chapter and a general discussion. The five data chapters were each written as stand-alone manuscripts. Although the thesis was written entirely by me with guidance from my supervisors, the manuscripts when submitted for publication may include the supervisors as co-authors. For the thesis version of these chapters, the references have been pooled into one section to make it easier to find them and to reduce duplication.

Financial support for the thesis work is as follows. Glenda Wardle provided financial assistance through Australian Research Council funding for data collection in the field for Chapters 2, 4 and 5. Catherine Offord provided financial support for the experiment in Chapter 3, as well as for having soil samples externally analysed in Chapter 5. Lisa Popenhagen, an undergraduate student supervised by Murray Henwood and I, assisted in the data collection used in Chapter 6. The common garden experiment in Chapter 3 was partially funded by the Valette Williams Scholarship in Botany, awarded by the Australian Plant Society, North Shore Group.

On the following page is a short list of definitions for key words throughout this thesis. All literature and online references for each chapter have been amalgamated at the end of thesis to help with the overall flow and appearance of this thesis.

Definitions

The following are key terms for this thesis. These terms have been found in the literature to have slightly different definitions. To minimize confusion and to help with the overall flow of the thesis, these terms are defined below.

Abundance:	the number of individual plants (counted or estimated) within a population.
Bioclimatic model:	a model which correlates species occurrence records with climate variables to predict the extent of climatic conditions within which a species can survive.
Climate envelope:	the spatial polygon within which the climate is suitable for a species to survive.
Fruit:	mature ovary containing the seed. This is what I refer to in thesis for *Actinotus helianthi*, as the seed was not required to be isolated for germination or burial experiments.
Macroclimate:	a set of broad-scale climate variables.
Population:	a number of individuals plants growing in an area with identifiable margins/boundaries. There are many methods and quantitative analyses available for defining margins (Krebs 1999). However I used a simple approach while surveying populations in the field and determined the boundary to occur when no other plants were found within a 50 m distance away from the population. This was undertaken as the plant were easily identified when in flower.
Seed:	the fertilized ovule containing the embryo, stored food material (endosperm) and surrounded by an outer seed coat.
Species distribution model:	a model which correlates species occurrence records with environmental variables (climate and nonclimate) to predict the extent of suitable environmental conditions within which a species can survive.
Traits:	Phenological or physical characteristics of individual plants that can be measured or recorded under field or laboratory conditions. Traits recorded or measured in this thesis include plant height, number of umbels, leaf length, stem width, germination, and seedling height.
Umbel:	A cluster of flowers forming part of an inflorescence. For *Actinotus helianthi*, this does not include the surrounding petaloid involucral bracts.

Thesis abstract

Species distribution models (SDMs) are predictive, numerical models that relate climate and other environmental data to species distributions. These models are useful for quantifying the spatial configuration or change of suitable habitat for a given species. This makes SDMs indispensable for conservation planning and climate adaptation management. It is timely, therefore, to examine the underlying model assumptions more carefully. The models typically use data on the known localities of individuals of the species as an indication of what environmental conditions the species will persist under. Each confirmed record is treated equally as an indication of suitability, thereby assuming that all populations are equal. However, populations vary considerably in the number of individuals, ranging from a few individuals to many thousands with implications for how they might persist. The number of individuals may also indicate another dimension to how suitable the environment is to the growth, survival and reproductive success of that species. A second assumption is that within each population all individuals are equivalent in their requirements from the environment. My thesis focuses on testing these assumptions by performing field and laboratory experiments, which incorporated population level data to determine whether populations are equivalent in their response to current or future environments. I then incorporate some of the limiting environmental factors identified in my experiments to produce a new SDM. My approach demonstrates great promise for further enhancing the use of predictive models to assess the impact of future climate on species distributions.

I begin my thesis by introducing and summarizing Species Distribution Models (SDMs) and Bioclimatic Models (BMs). Here, I discuss that, given the appropriate ecological question, climate-only models can be an integral part of research and management. I then discuss the benefits and issues associated with most distribution models. Specifically, I argue

that important limiting factors, such as species traits, species interactions and environmental barriers, should be examined to determine the capacity for these to be included as factors in distribution models.

Given the assumption of SDMs to treat each population the same, I use an exploratory analysis to examine whether plant traits within current field populations can be predicted, in part, by climate. This approach also tests the assumption inherent in BMs that climate is the main factor influencing species distributions. In Chapter 2, therefore, I examine whether climate envelopes, generated by overlaying temperature and rainfall variables onto geographic species occurrence records, reflect the ecological processes that determine whether an individual plant will survive. To achieve this, I sampled plants from 40 field populations of *Actinotus helianthi* across the known geographic distribution of the species. Climate envelopes were generated using a Hierarchical Cluster Analysis to group populations which had ≥ 84% similarity in the local climate. To determine the extent to which plant traits are influenced by current local climate, I used multivariate analyses to compare plants with similar phenological traits with their bioclimatic data. Plant traits within climatically similar populations were highly dissimilar. Therefore, SDMs which rely solely on climate factors may be over-generalizing potential distribution shifts and may not encompass more local effects. This raises the question of what factors are contributing to this variation in species traits.

To examine the existing variation in plant traits associated with the reproductive niche, the reproductive ecology of *A. helianthi* is described to better understand how it might impact on the species reproductive success. Specifically, in Chapter 3, I examine the floral phenology and breeding system of the species to determine whether the type of pollination affects seed set and germination. Four pollination types – intra-umbel geitonogamy, inter-geitonogamy, xenogamy and open control – were tested in two populations. Intra-umbel

geitonogamy produced very low seed set. By contrast, seed set and germination were not significantly different between the three other pollination treatments. The results indicate that geitonogamy does not adversely affect overall seed set and germination.

It is then necessary to quantify the extent of variation that exists among current populations of *A. helianthi*. Specifically, I test whether early plant performance traits are genetically-fixed by location and if reproductive success is impacted by site-specific interactions with insect visitors. The first experiment in Chapter 4 involved growing plants from seed in a common garden environment. Plants from 17 populations of *A. helianthi* were tested for germination, early growth and survival. The germination, seedling growth and early survival were found to vary by population. Variation in these early performance traits existed at multiple levels from the maternal plant to biogeographic regions. The second experiment examined localized species interactions by recording insect visitors to umbels on plants from nine populations of *A. helianthi* across New South Wales (NSW). The abundance and diversity of insect visitors also varied among populations, and seed set was subsequently site specific. These results indicate the likelihood that populations are adapted to the local environment.

Soil is one of the most commonly known local environmental factors that influence plant growth and survival. However, the edaphic environment is rarely included in predictive models. In Chapter 5, I build on my previous results, by growing plants from seed in local and nonlocal soils to examine the interactive effect of climate and soil on predicted future distributions. Soil samples were collected at intervals of 1 km, 10 km, and 40 km from local populations, following the direction that favourable climate was predicted to shift in the future using a bioclimatic model in MaxEnt. Seed emergence was significantly influenced by population and origin of soil. The model that best explained the variation in seedling growth contained pH, sodicity, salinity and phosphate. Soil pH and sodicity were added as spatial

environmental predictors to the original bioclimatic MaxEnt model. Following the initial experimental evidence, the climate-only model was determined to be over-predicting areas with high suitability (60-100% suitability) for *A. helianthi*.

Trait data has been demonstrated to be an important predictor for the variation between *A. helianthi* populations. However, collecting enough trait data in the field can be costly and time consuming. Therefore, in Chapter 6, I present a novel experiment in which plant trait data collected from herbarium specimens of *Actinotus forsythii*, *A. minor* and *A. suffocatus* are analyzed using the methodology described in Chapter 2. I employ this method to illustrate the capacity to detect patterns between plant traits and climate factors that are likely to depend on the geographic extent or scale. The results presented in this chapter provide insights into whether bioclimatic models should, or could, be performed using herbarium data.

I summarize the main findings of my thesis in Chapter 7. I discuss some of important questions which arise from my results. I outline several directions that future work should take to further enhance, and compliment, the findings of my thesis. My thesis creates a means to enhance SDMs by testing some of the assumptions inherent in these models. I have successfully illustrated that ecologically limiting factors, such as soil, can be incorporated into a SDM to further enhance predictions. Since plant traits differed among populations from climatically similar regions, it is important to include these in SDMs as they will have a different response to climate across a geographic distribution. The choice of predictors in SDMs is crucial to their success, as the model assumes that all relevant factors are included. My thesis is an important foundation for experimentally testing the assumptions inherent in most SDMs, while at the same time, illustrating how these factors can be added to, or combined with, the initial model.

Chapter I

GENERAL INTRODUCTION: NEW APPROACHES TO MODELLING SPECIES DISTRIBUTION

1. Introduction

Climate change presents a significant threat to the function of ecosystems (Bakkenes *et al.* 2004; Hughes 2003; Stocker *et al.* 2013), especially when combined with other disturbances, such as habitat fragmentation and invasive species (Mokany and Ferrier 2011; Williams *et al.* 2008). Ecosystems typically experience some level of environmental variability, but global climate change presents pervasive, rapid, challenges that may transform all ecosystems. To survive and reproduce, organisms must adapt or face local extinction. Therefore, the ability of a species to withstand future environmental conditions will be dependent on the plasticity or fitness of the individuals in its populations, as well as having the necessary traits/mechanisms to find and colonize alternative suitable environments. Environmental factors, in particular temperature and moisture availability, are known to influence plant traits, including flowering time (Dahlgren *et al.* 2007; Rumpff *et al.* 2010), seed maturation (Chambers and Keatley 2010), seed dispersal (Venable and Brown 1988), and seed dormancy (Baskin *et al.* 1998; Cochrane and Probert 2006; Scholten *et al.* 2009; Schütz and Milberg 1997; Vandelook *et al.* 2007). These traits represent population-level differences. However, how the data is included in a model will be dependent on the biota studied. For example, predictive studies which model animal species have used models to assess species vulnerability under climate change, such as thermal tolerance (Helmuth *et al.* 2002; Pörtner 2001). Furthermore, there is recent evidence that suggests mobile species should be modelled at the landscape scale (Harris *et al.* 2014).

Given the influence of environmental factors on different species, current research has focused on the development of quantitative methods to predict the impact of climate change on species distribution and species persistence (Elith *et al.* 2006; Kearney *et al.* 2010; Mokany and Ferrier 2011). The most common approach to predicting the impact of environmental variation is to model distribution changes to individual species in response to climate, and this is referred to as either a Species Distribution Model (SDM) or Bioclimatic Model (BM). These models have great potential to inform management and policy as the application of SDMs has demonstrated the potential magnitude of climate effects on the future distributions of species. However, a number of studies have recently criticized the usefulness of BMs and have discussed several assumptions which violate environmental processes, including species traits and interactions, as well as other landscape factors (Araújo and Peterson 2012; Heikkinen *et al.* 2006; Luoto *et al.* 2005). Consequently, these models could create an inaccurate evaluation of a species ability to adapt under changing climate as they do not incorporate population trait differences (Albert *et al.* 2010a). For example, despite showing a correlation between a species and an environmental variable, it remains unknown whether this is due to a direct relationship, a biotic or abiotic interaction giving an indirect effect, or a response to another factor not included in the model (Kearney and Porter 2009; Keith *et al.* 2008; Mac Nally 2000). Therefore, whether broad-scale BMs can accurately represent the major contributing forces that shape species traits within populations and their distributions has not been fully tested. This is due to a paucity of experimental data concerning the effects of other non-climatic factors on a species. These factors should be most relevant at fine scale projections since climate is classified as a broad, macro-influential factor (Austin and Van Niel 2011b; Petrů and Tielbörger 2008).

From the emerging literature, it is propitious to evaluate the ability of BMs to accurately depict a species distribution. In this Chapter, I review and summarize SDMs and BMs by initially outlining the data required to build a model, and then define the concept of these models in ecological niche theory. In doing so, the limitations of BMs are made apparent within the framework of complex ecological systems. However, it is important to discuss that under the appropriate ecological questions, BMs form an integral part of ecological research and management. In this thesis, several novel approaches are combined to address some of the issues associated with SDMs, including quantifying several plant traits from the 'reproductive niche'. This work is among the first to combine several population reproductive niche traits and constraints with distribution modelling, and this aspect of the project is timely. However, several recent publications have demonstrated that including population traits, such as phenological and other species attributes, generates significantly different model outputs (Chuine 2010; Hanspach *et al.* 2010). Albert *et al.* (2010b) show that significant variation can exist for a plant trait within a population, and this may account for up to 30% of the total trait-based variation for a species (Albert *et al.* 2010a). Adding to this evidence of within-population variation, Wright *et al.* (2001) report several leaf traits to display greater variation within a single habitat than across habitats. That traits may be more influenced by their local climate than other factors will be examined in this thesis. By examining how traits vary across populations in one species permits a greater understanding of how life-history factors can influence distribution.

2. Building SDMs

2.1. Defining the model

BMs can be defined as testing predictions by correlating species occurrence records with climate variables to spatially depict the extent of the climatic conditions within which a species can survive (i.e. the climate 'envelope') (Booth *et al.* 2014; Walker and Cocks 1991).

The basic modelling approach is achieved by superimposing spatial grids (of different sizes depending on resolution) over the study area. The initial layer contains biological data, often in the form of presence/absence or presence-only data. Each environmental predictor is added as a separate layer to the grid. The most common and widely available environmental data are temperature and precipitation (and their derivatives) (Hijmans *et al.* 2005), and these are usually stored through a geographic information system (GIS) (Goodchild 2003; Skidmore 2002). These models can then be applied to future climate scenarios to enable a predictive assessment of changes to species distributions across regions or countries, either as range expansions, range contractions or range splits (Sommer *et al.* 2010).

Predicting how distributions might change can be problematic as climate-environmental-species interactions can be spatially and temporally dynamic. To avoid complications, one of the most commonly used SDMs has been to predict distributions of individual species (Ferrier and Guisan 2006). Models rely heavily on the validity and integrity of input data. Most models operate under the classic niche concept (realized vs fundamental niche) described by Hutchinson (1957), which states that species distributions are restricted by their interactions with other organisms (Sinclair *et al.* 2010). For example, Davis et al. (1998b) used captive *Drosophila melanogaster* to demonstrate that changes in temperature had a significant interaction with competition. However, the adopted premise of these models is that the current climatic geographic distribution is the most accurate representation of a species' tolerance to climate. In other words, the species is at equilibrium with climate as it only occurs in areas that are climatically suitable while being absent from non-suitable environments (Hutchinson 1957). This is unlikely to be true, as complex relationships exist at multiple levels for most species (Araújo and Pearson 2005). This principle will be tested in the following Chapters of this thesis.

2.2. Data inputs/outputs

2.2.1. Biological data

Biological data can be gathered from ecological surveys, from specimen collections and/or from historical observations. Due to practical, ethical, financial or logistical restrictions, surveys are often sparse, incomplete, and biased. This can mean that the collected data may cover a smaller area than originally planned. Data paucity is a major concern for validating model accuracy and has been previously reported to directly affect model predictions for plants and animals (Hernandez *et al.* 2006; Kadmon *et al.* 2003; Wisz *et al.* 2008). There are several reasons why sample size is important, as outlined by Wisz *et al.* (2008), including: (1) uncertainties surrounding statistical parameters (e.g. means) that decrease with increasing sample size; (2) outliers are highly influential in small data sets, and more importantly; (3) small sample sizes often will not capture niche variation. Therefore, to survey biological data efficiently, an environmentally stratified design is required where abundance data is then recorded (Austin 1998; Elith *et al.* 2006).

Despite sometimes involving large numbers of individuals, models which use biological data from historical (e.g. herbarium) records also suffer from several problems (Austin 1998; Ferrier and Guisan 2006). Firstly, since the intent of collections/collectors may be unclear (Elith *et al.* 2006), the records are presence-only and often lack any information from sites visited where the species was absent, or other environmental data. Secondly, the locations of older records may be too inaccurate to be of value (Austin 1998). Finally, the localities where species have been recorded from are often clumped in areas with easy accessibility (e.g. roads and fire-trails), representing a haphazard or biased survey at best. However, historical specimen records provide physical confirmation that a species was present in an area, compared to anecdotal observational data. It is also suggested that including ‘pseudo-absences’ derived from the presence of other species in the community or

ecosystem provide the best supplementary information for building models using presence-only data (Ferrier and Guisan 2006).

2.2.2. Distribution predictors

Each environmental predictor is stored as a GIS grid layer. In contrast to species occurrence or trait data, environmental data are stored in every grid cell, which can be set to different resolutions or sizes. Environmental factors can have a direct effect on species through physiological restrictions, such as temperature and pH, or as resource-limiting, such as water availability, light, competition and food. Indirect factors, including slope, elevation and latitude, may have no immediate influence on a species' distribution, but are still important and should also be considered in SDMs when the data is available (Austin 2007; Austin 1998).

With the increasing availability of species presence data, access to high-resolution environmental data has also become widely available from online databases. This information can be derived from satellite images and constructed from detailed historical and contemporary climate records (Elith *et al.* 2006; Hijmans *et al.* 2005; Turner *et al.* 2003). However, which environmental data to include, how to obtain it, and at what resolution is decided by the modeler. Whatever the combination of environmental factors selected, the data for that model are then assumed to be the main spatial limiting factors.

Climate

Climatic conditions are widely acknowledged to be a major determinant for current species distribution patterns (Aitken *et al.* 2008; Helmuth *et al.* 2002; Sommer *et al.* 2010). Indeed, distribution shifts and adaptation for many woody plant species were a response to the climate as far back as the late Quaternary (Davis and Shaw 2001). This is because climate

directly influences species fitness. Clausen *et al.* (1941) noted that due to many plant species having latitudinal or longitudinal restrictions meant that there can then be physiological limitations to growth and survival due to the effects of climate. Therefore, it could be assumed that using climatic data extracted from a set of known occurrence points, should reasonably capture the climatic limitations of the species. This might then be useful for predicting where a species could be present across a large landscape in the future should the distribution follow any changes in the climate (Beaumont *et al.* 2005).

As explained above, predicting distribution shifts using climate alone can lead to significant overestimations of species loss (Araújo and Peterson 2012; Heikkinen *et al.* 2006; Luoto *et al.* 2005; Pearson and Dawson 2003). In a large-scale study, Thuiller *et al*. (2005) predicted that more than half of the 1,350 European plant species tested would become vulnerable or threatened by 2080. The authors point out, however, the impact of climate change lessens when species are more able to move across the landscape – such as runners (i.e. horizontal stems) vs. seed dispersers (Thuiller *et al.* 2005). There are hundreds of climatic factors that could be derived from data, including temperature, precipitation, solar radiation, so, together with their inherent variation between and within seasons, the interpretation of the data is a complex process (Franklin 2009). Furthermore, widespread species tend to exhibit regional or local differences in their ecological traits (Osborne and Suárez-Seoane 2002). Luoto *et al*. (2005) noted that model performance for any species is dependent on species prevalence, latitudinal range and spatial autocorrelation (i.e. if species presences are clumped in an area). Therefore, efforts should be made to improve the current methods used to model data, as well as the how the raw data itself is collected. The latter may be more important, as missing limiting data will be critical for model accuracy (Huntley *et al.* 2010b).

The absence of a species from climatically-suitable areas poses the question of how far from the climatic equilibrium is the current species distribution? In a broad sense, there are three main limitations that can inhibit model predictions as described by Mokany and Ferrier (2011). The first, the 'Linnaean shortfall' refers to lack of knowledge of how many species which have yet to be identified. The second, the 'Wallacean shortfall' refers to poor knowledge of a species distribution, often due to incomplete presence/absence data. The final, 'Hutchinsonian shortfall', states that for most species, we have a poor understanding of what species attributes or traits influence their niches. These terms are a newer way of referring to taxonomic distribution, and species traits or attribute limitations. When species locations are known, the 'Hutchinsonian shortfall' is arguably the most important limitation as this prevents mechanistic predictions of temporal changes using key population traits and interactions with other species in an ecosystem. Therefore, despite acknowledging the importance of the niche concept, many BMs are unable to compensate or account for this complexity (Sinclair *et al.* 2010). I will attempt to account for the complexity by performing several experiments to quantify traits associated with the 'reproductive niche' in subsequent chapters.

2.3. *MaxEnt* – a modeling program

With an increasing availability of environmental and climate data, there has been a rapid development of statistical programs capable of predicting and graphically illustrating geographic distributions of species. In this thesis, I use the commonly known SDM program, *MaxEnt* (maximum entropy) (Phillips *et al.* 2004). *MaxEnt* is a general-purpose SDM program which makes predictions of species distributions using incomplete or presence-only datasets (Phillips *et al.* 2006; Phillips and Dudík 2008). The premise of *MaxEnt* is to estimate the probability of a species' distribution using maximum entropy (i.e. the most spread out or closest clumped data), subject to a number of constraints (i.e. climate and/or environmental

data), thereby outputting the best estimate of an incomplete distribution since it agrees with all the known data and does not assume there is any unknown data (Phillips *et al.* 2006). Several advantages of *MaxEnt* over other modelling programs include, (1) requiring presence-only data, thus overcoming small unstratified sampling; (2) using both continuous and categorical environmental (restrictive) data as well as including any interactions between these variables (multivariate analysis); (3) optimized algorithms to give an optimal maximum entropy probability distribution, and: (4) being adaptive to allow for presence/absence data by using a conditional model (Phillips *et al.* 2006).

MaxEnt is now one of the most popular SDMs, and has been reported to produce one of the most accurate predictive outputs when compared with other models that use presence-only data (Elith *et al.* 2006). *MaxEnt* can also be 'tuned' to cope with model issues such as sampling bias (i.e. sampling along roads and fire-trails). Furthermore, the program is capable of accepting any spatial data layers so that distributions can be modeled across multiple surfaces instead of climate alone. *MaxEnt* has been used in studies of invasive species (Giovanelli *et al.* 2008; Rödder and Lötters 2009), distribution shifts under climate change (Fitzpatrick *et al.* 2008; Rodríguez-Sánchez and Arroyo 2008), predicting rare species distributions (Williams *et al.* 2009), species richness (Pineda and Lobo 2009), and diversity hotspots (Murray-Smith *et al.* 2009). The program has also been used to identify possible climate refugia (Marske *et al.* 2009), and to improve species management by optimizing reserve locations (Carroll *et al.* 2010).

3. Benefits of bioclimatic models

The main attractions of BMs include ease of use, flexibility of the data (use of small, incomplete datasets), and the availability of programs (e.g. *MaxEnt*), making them widely used in published literature. At the broadest scale, global biomes are to some degree

determined by climate. In Australia, for example, there are 89 bioregions defined, in part, by climate (IBRA, 2007). Therefore, it may be more relevant to consider the historical and future climate impacts on species distributions in the context of scale and specific variables (Fig. 1) (Austin and Van Niel 2011b). For example, Sykes *et al.* (1996) used bioclimatic variables to represent physiological mechanisms to predict the future distributions of several tree species across Europe. For two species, *Picea abies* and *Fagus sylvatica*, the authors noted that shifts in their distributions were associated with warmer temperatures during winter, thereby reducing the time required for chilling (Sykes *et al.* 1996). Furthermore, Bakkenes *et al.* (2002) used a climate model to assess the distribution changes to over 2000 European plant species. In particular, they reported 32% of the species that encompassed an area around 44% of the total modeled geographic extent will have disappeared by 2050.

A significant use of BMs lies in examining the risk to conservation areas. Predicting how future climate will impact reserves is of conservation importance, particularly when these areas are designed to protect fragile or vulnerable species. Several studies have examined the effectiveness of conservation networks around the world and have illustrated a decline in climatic suitability of these areas (Araújo *et al.* 2011; Hannah *et al.* 2007). Araújo *et al.* (2011) found that the potential of protected areas in Europe to be climatically better suited for species than non-protected area was mixed. This might be due to the varying topology of the protected areas, as the authors point out that mountainous areas will provide climate refugia for some species. Such studies provide evidence for the need to re-evaluate protected areas in order to conserve species in the future.

Some species do not have the capacity to shift their distribution beyond protected, or otherwise natural, areas due to physical or environmental barriers. This is the case for species which have poor dispersal or are habitat specialists. In these instances, species can be

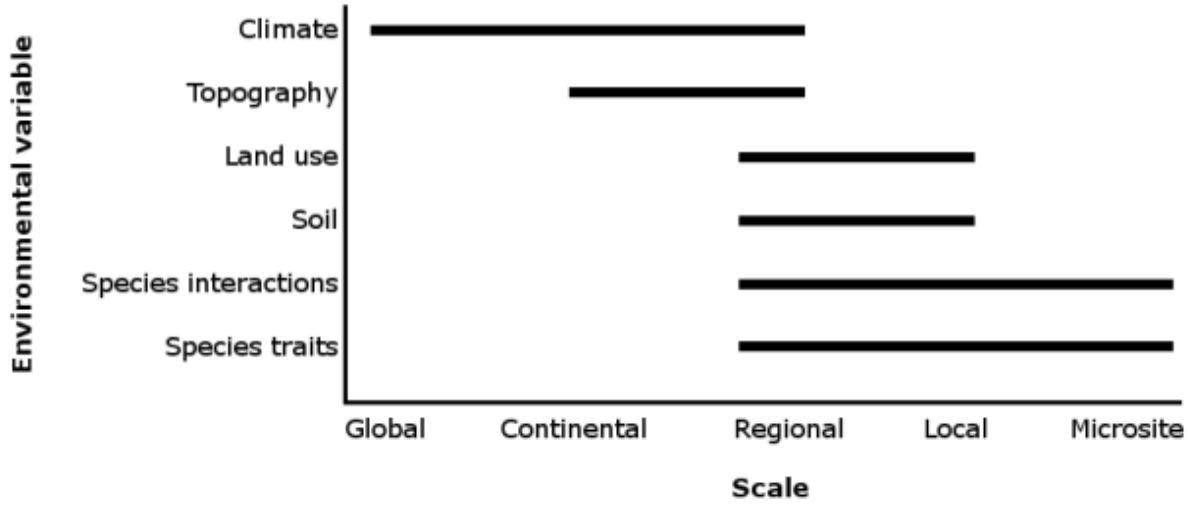

Fig. 1. Example showing how different environmental variables can influence species distributions at different scales. The schematic represents the relative scales at which a variable is likely to be most influential on a distribution. Adapted from Pearson and Dawson (2003).

conserved through translocation or reintroduction by assisted migration (Guisan *et al.* 2013). Since the premise of translocations is to determine the habitat in which the species could exist based on its current suite of climatic tolerances, BMs suit this purpose. For example, two butterfly species were introduced into areas on Britain which were beyond their current distribution, but were predicted to be climatically similar (Willis *et al.* 2009). The authors then noted the distribution expansion of the butterflies increased by 2.5- and 21-fold, respectively. Further, growth rates of introduced populations were similar to natural populations over a six year period (Willis *et al.* 2009), although any other effects on flora and fauna in the ecosystems were not examined closely. While the rate of colonization success will be dependent on the species and its interactions, it is strongly argued that the risk of action outweighs the risks of inaction (Gray *et al.* 2010). However, this may depend on any collateral effects of the translocated species in the new ecosystem.

4. Limitations of bioclimatic models

Apart from climate, there are other environmental factors that can influence species distributions. The selection of these predictors is dependent on the ecological processes that are thought to be influential to the particular species, as well as the availability of pertinent data (Austin 2007; Austin 1998; Franklin 2009). Climate change will produce unique, different environments which are not at equilibrium with the incumbent species (Kawecki and Ebert 2004). In this context, applying a correlative BM would be considered questionable without further supportive evidence (Kearney *et al.* 2010).

If we consider a correlation between a species and an environmental variable, we may be uncertain as to whether this correlation is due to a direct relationship, a biotic or abiotic interaction giving an indirect effect, or a response to another factor not included in the model (Kearney and Porter 2009; Mac Nally 2000). Therefore, since most BMs can only illustrate

spatial variation, mechanistic models are required to quantify temporal variation in a dynamic environment. These models can be performed separately or in tandem with correlative models, and are referred to as semi-mechanistic (Mokany and Ferrier 2011). Using two correlative and three mechanistic models, Buckley et al. (2010a) compared models to predict the distribution shifts of the skipper butterfly (*Atalopedes campestris*) and a fence lizard (*Sceloporus undulates*). While the authors noted many similarities between the results of each model, the correlative models generally over-predicted the distribution margins of the two species. This is probably because the model was restricted to thermal tolerance and did not include other limiting factors such as inter-species competition and interactions (Buckley *et al.* 2010a). The authors also found correlative models using temperature and precipitation to over-predict the range of *Sceloporus undulates*, further illustrating the need for improvements by expanding beyond simple climatic variables.

In the next section, I focus on several important plant-related factors that have been used by the modelling community. Because plants are sessile, they must possess the necessary conditions to be able to adapt or migrate to new suitable habitat when the current habitat becomes unsuitable or face local extinction. Indeed, the different adaptive capacity of populations is thought to be a major driving force of diversity within a species (Rutter and Fenster 2007). Therefore, plants provide an excellent study system to examine the importance of limiting factors. Specifically, I outline the importance of species traits, species interactions and environmental factors. Each of these factors is further exemplified by experimental testing in subsequent chapters of this thesis.

4.1. Species traits

An organism possesses the genetic capability to alter its development and physiology from exposure to current or historical environmental conditions. These specific environmental

response traits have evolved over time and are known to vary among genotypes, populations and species (Sultan 2000). It is suggested that an epigenetic response to natural selection and phenotypic plasticity are the two main mechanisms that enable a population to adapt to a changing environment (Charmantier *et al.* 2008). Several studies have demonstrated that phenotypic plasticity can also differ among populations of the same species (Byars *et al.* 2007; Galloway and Fenster 2000; Stewart Jr. and Nilsen 1995). This variation can be observed as a difference in one or more traits among individuals.

Morphological or physiological traits provide an indication of a plant's fitness. Phenotypic plasticity is the response of a plant genotype to alter a trait in response to spatial or temporal environmental heterogeneity. If one or more trait changes match the environmental conditions, then these traits can improve plant fitness by positively influencing survival and reproduction (Violle *et al.* 2007). Much of the current knowledge about phenotypic plasticity comes from plant studies. Plants often display dramatic differences in phenotype when exposed to different environments and these, more importantly, can be easily bred in alternative or artificial environments (Sultan 2000). For example, Moran, *et al.* (1981) demonstrated significant population-environment interactions in 11 out of the 15 morphological traits examined when they compared seven populations of the large cocklebur (*Xanthium strumarium*) in Australia. Basic studies of plant plasticity focus on quantifying morphological factors such as plant height, leaf number or flower number. Sultan (2000) noted that traits involved in resource acquisition can display functional, long-term differences. For example, plants will increase biomass ratio from shoots to roots when soils have low nutrient levels, such as nitrogen (Gedroc *et al.* 1996; Reynolds and D'Antonio 1996), or low soil moisture content (Bell and Sultan 1999).

Analyses of phenotypic plasticity and its impact on population adaptability provide an important insight into the role of plasticity in governing species distributions (Dorken and Barrett 2004). For example, in a large-scale study that included 88 *Leucadendron* species in South Africa's Cape Floristic region, Thuiller *et al.* (2004b) found individual leaf area to be correlated with the average position of the species across an aridity gradient. Likewise, first flowering date was significantly associated with a species position along the gradient (Thuiller *et al.* 2004b). Plasticity in flowering time is a direct response to environmental conditions, and it is thought that populations located in unfavourable or disturbed environments will reproduce earlier to ensure reproductive success (Sultan 2000). Albert *et al*. (2010a; 2010b) note that significant variation in traits among populations can explain up to 70% of the total variation. More importantly, the authors note that the strength of an individual trait response among populations will vary depending on the species, traits and environmental gradients (Albert *et al.* 2010b). Therefore, including traits such as those discussed here will permit enhanced precision when predicting whether plants will cope with temporally variable environmental stresses.

Plasticity of life-history traits can also improve the reproductive success of plants by changing their breeding system, reproductive allocation or sex expression (Midgley *et al.* 2010). The latter is particularly common in andromonoecious species (those which display hermaphroditic and staminate flowers). For example, the proportion of staminate flowers in *Solanum hirtum* was shown to vary among genotypes between 9.3% to 63.6%, most likely due to a plant's resource status (Diggle 1993). This confirmed the hypothesis that fruit set caused a dramatic decline in resources available for further growth (Diggle 1993). Indeed, some plants are able to alter their breeding system depending on pollen availability. For example, under certain circumstances, plants from several genotypes of *Solanum carolinense* were able to relax their self-incompatible system and produce self-pollinated seed in the

absence of out-cross pollen (Travers *et al.* 2004). *Campanula rapunculoides* plants exhibit a plastic response to floral age and lack of earlier fruit development by switching from self-incompatibility to self-pollination (Vogler *et al.* 1998). Such adaptive traits act as insurance for successful reproduction when out-crossing fails due to isolation or by chance.

Adaptive traits are also expressed in the earliest form of plant development. This is due to an organism's need to express a viable phenotype to survive, or germinate, before displaying an adaptive phenotype (Huang *et al.* 2010). Huang *et al*. (2010) discovered that when seeds were exposed to different field and experimental conditions, quantitative trait loci (QTLs) had a significant effect on germination and expression of adult traits. In other words, seed dormancy appears to be an important and early form of adaptation acting to inhibit germination until environmental conditions improve (Baskin and Baskin 1998; Huang *et al.* 2010). Differences in seed dormancy amongst populations of a species are well known (Andersson and Milberg 1998; Qaderi and Cavers 2002; Sultan 2000), and the life stage at which plasticity can be observed may vary between genotypes or populations (Sultan, 2000 and references within). For example, in two populations of *Lobelia siphilitica*, genotypes within populations were found to vary significantly in their bolting time, height and leaf growth rates, and fruit and leaf production when exposed to different levels of available light (Pigliucci and Schlichting 1995). The authors noted that these differences were due to variation between genotypes in their growth rate and growth period (Pigliucci and Schlichting 1995). Since dormancy is shaped by local environmental conditions, measures of germination and early survival in populations provide sound data on population level responses under changing environments.

4.2. Species interactions

Species interactions occur at different trophic levels, be antagonistic (i.e. plants and herbivores, or between plants competing for resources) or mutualistic (e.g. animal pollinators or seed dispersers, symbiotic fungi). In order to define a manageable project, the focus here is on the traits that influence species interactions at the reproductive stages of the angiosperm life cycle that represent the most sensitive component to changes in the environment. All plants have some capacity to tolerate a dynamic environment; however, studying the factors that can affect individual plant survival provides a unique opportunity to understand the limitations to the species distribution. Plants have two forms of dispersal; as pollen and/or as seeds. Self-incompatible species rely on abiotic or biotic factors to distribute pollen between genotypes for reproductive success. Several studies have examined the importance of including species interactions in species distributions (Heikkinen *et al.* 2007; Pellissier *et al.* 2010; Van der Putten *et al.* 2010). Pollination success, in particular, is expected to vary amongst different populations of a species due to differences in the isolation and abundance of plants (Mustajärvi *et al.* 2001). Furthermore, pollinator abundance can vary throughout a species' distribution and over time. Out-crossing plants dependent on biotic pollination are unable to extend their distribution beyond that of their pollinators (Van der Putten *et al.* 2010).

Davis *et al.* (1998b) pointed out that the omission of species interactions is the single greatest shortcoming of BMs. The authors illustrate this by showing that due to the competitive interactions between three fruitfly species and a parasitoid wasp, the inter-species experimental clines varied significantly from any single species clines (Davis *et al.* 1998b). A criticism of their conclusion is that on a macroecological scale, such interactions do not exert a dominant role when added to climate. Hodkinson (1999) contends that it is more important for studies to examine the link between species traits and environmental variation at the

micro-habitat level and not at the scale suggested by Davis *et al*. (1998). Araújo and Luoto (2007) found the Davis *et al*. (1998) hypothesis that a species range was a consequence of its physiological response to climate to be untrue when they examined the interaction between the Apollo butterfly (*Parnassius mnemosyne*) and three host plant species. The authors reported species interactions had a significant impact on the predicted distribution of the butterfly at a macroecological scale. However, the authors conceded that *P. mnemosyne* was purposely chosen as its distribution critically relied on the plant host species. Thus, it is important to examine the model scale and the dominant factors which govern a species distribution at each level (Fig. 1).

It is also expected that the abundance of individuals within a population will have an indirect effect on reproductive success due to the attractiveness to pollinators (Morgan *et al.* 2005). Therefore, in smaller populations the number of pollinator visits to individual flowers are likely to be lower, leading to insufficient pollen transfer and a decrease in seed set (Ågren 1996; Mustajärvi *et al.* 2001). However, such results can be equivocal, particularly when plant density is also considered. For example, a study on the interactions between pollinators and population size and density and its effect on the reproductive success of *Lychnis viscaria* found that rates of pollinator visits were higher in sparse populations, regardless of size (Mustajärvi *et al.* 2001). This was likely to be due to the larger inflorescences of plants in sparse populations thereby rendering them more visible to potential pollinators (Mustajärvi *et al.* 2001). Lamont *et al.* (1993) argue that pollen quality better explains differences in fertility among populations than plant abundance. It is possible, though, that pollen quality could be dependent on habitat quality or suitability, as well as temperature and moisture availability, which could also be linked with germination success and/or survival, thus giving rise to differences in population size.

Interactions between species have an important impact on a species distribution. The complexity of interactions between species means that a predicted geographic distribution of a species might have significant ramifications for other species distributions, or may not be extrapolated reliably at all. So BMs could lead to inaccurate predictions in some cases, although the effects of interactions could be insignificant in comparison to climatic factors when distributions are modeled at broad scales (Pearson and Dawson 2003).

4.3. Environmental factors

There are several environmental or landscape factors that can indirectly influence the climate available to a species. Species will exhibit significant variation across environmental gradient(s) (Byars *et al.* 2007; McIntyre and Lavorel 1994; Ohsawa and Ide 2008), therefore including indirect factors in models will help capture this variation and give a more accurate output. For example, altitude significantly affects the temperature and precipitation profile, making it one of the strongest influential drivers of variability in species morphology. It also indirectly influences the level of evapo-transpiration (Ohsawa and Ide 2008). For example, Byars *et al.* (2007) noted that populations of the common alpine grass, *Poa hiemata* are significantly smaller in size at higher altitudes than populations at lower altitudes – a trend commonly seen in other species (Clausen *et al.* 1941).

Associated with altitude is slope which affects the flow of water, causing variability in soil moisture content and soil depth. Furthermore, the slope aspect influences the amount of solar radiation which then indirectly affects soil moisture content and temperature (Austin 2007; Segurado and Araújo 2004). For example, Hanba *et al.* (2000) reported greater water use efficiency for evergreen tree species present on upper slopes in a Japanese temperate forest. McIntyre and Lavorel (1994) stated that slope and altitude (measured as topography)

explained 6.6% of the variance in species compositions in Australian grasslands, with most species being present on mid to upper slopes.

Seed dispersal distance can have a direct impact on plant fitness as it determines the environments where seeds and seedlings will survive or perish, influencing the rate of recruitment, invasion, distribution shifts and gene flow among populations (Nathan and Muller-Landau 2000). In addition, seed dispersal can occur over time through seed banks, permitting individuals to avoid unfavourable climates for both local and nonlocal sites (Howe and Smallwood 1982). More specifically, the advantage of seed dispersal, includes; (1) escaping from density- or distance-dependent mortality near the parent; (2) successful colonization of disturbed or otherwise new habitats, which is particularly beneficial when the current environment becomes unsuitable, and; (3) directed dispersal to sites with a high survival rate (Howe and Smallwood 1982; Wenny 2001). While these are interdependent, the importance of each will vary between populations (Howe and Smallwood 1982).

While the projection of a species' modeled envelope across the landscape provides an insight into where the species must disperse to in order to maintain its preferred climate niche, the ability for a species to migrate into different habitats will depend on several environmental conditions unrelated to climate. Landscape patterns are often delineated by vegetation types. Consequently, efforts have been made to predict species distributions using vegetation type (i.e. land cover). It has been documented in recent studies that including vegetation type in models has varying effects which are species-dependent (Heikkinen *et al.* 2006). As expected, the interacting effects of climate and vegetation are more relevant at fine scales of modeling (Heikkinen *et al.* 2006; Luoto *et al.* 2007).

In association with vegetation types and preferences of species, the suitability of an environment is also partly determined by other landscape processes. In particular, edaphic characteristics, such as soil moisture content and pH are known to vary at a fine scale and affect the reproductive niche of plants (Pickett and Bazzaz 1976; Pierce *et al.* 1999; Weaver and Hamill 1985). In turn, this limits the ability of plants to colonize and become established in new environments (Coudun and Gégout 2007; Coudun *et al.* 2006). Specifically, pH is reported to control the uptake of minerals by plants and has been correlated with several other edaphic variables (Schoenholtz *et al.* 2000).

5. Enhancing predictive models

I have argued that SDMs, and BMs in particular, can be a powerful tool to predict how a species might respond to a future environment. In addition, I have highlighted that how a species responds to external environmental factors will depend on its population attributes and their particular plant traits. The benefits of predictive correlative models stem from the notion that a species' current climate envelope can be directly related to specific climate variables, and that future changes in the distribution are also associated with the directional shift in climate. However, careful consideration for the choice of hypotheses, spatial extent and scale are required before the assumption associated with the effects of climate overriding environmental heterogeneity can be made. In addition, BMs are particularly beneficial when the cost of species surveying is high. The model can then be used to assess the habitat suitability between locations, which, in turn, permits surveys to be more efficient (Beaumont *et al.* 2005). Furthermore, in the case of many rare and endangered species, so little is known about their biology and ecology that BMs are the only method to provide an estimation of their current and future distribution.

Since the distribution of a species shifts as one population changes in some way, it seems appropriate that we use both correlative and mechanistic approaches to attempt to capture this variation and to enhance model accuracy. Correlative elements could help to alleviate the limitations of spatial distribution (if that is an aim of the study) and the traits that are important to a species and its populations. Incorporating a limiting mechanistic component will then illuminate how a population might change over time, particularly with an understanding of important population traits and the influential environmental factors. Due to the complexity in the response of populations and individuals to changing environments, it is unlikely that BMs will be able to include this information in the projections. However, this raises the question of how much local adaptation there is, under what circumstances, and how can we quantify it? If populations from the distribution centre and the edges are sampled, then this will encompass much of the adaptive variation. Surveyed populations should be sampled to quantify the population structure and to ascertain how the population currently responds to its local conditions. These population factors include, but are not limited to, abundance and area (estimated), plant height, number of flowers or inflorescences per plant, plant density, and seed viability and germination. The output from such a model would not only produce the spatially explicit response of populations over time, but also identify the key traits which enable a population to persist or move in the landscape. Ultimately, in order to answer these questions and to improve SDMs, it is important to provide some validation and address uncertainties by performing ecological experiments to determine whether climate influences species traits, and whether other environmental variables are influencing the distribution.

The key consideration when developing models is to define the most efficient method to balance correlative and mechanistic factors. This could be achieved if the focus shifts from species to populations, documenting and embracing the existing variability among dynamic

populations that occurs in response to the environment (Anderson *et al.* 2009). It is also likely that for increased reliability this would be performed on a per species basis to cover idiosyncratic properties of different species. A population response could be predicted based solely from population monitoring and surveying in the field coupled with the knowledge of the species life-history and application of the predictive model. From a single-species approach it is then possible for future models to be adapted from this framework to address other important ecological levels, such as the community- and ecosystem-level or species interactions as was recently proposed by Mokany and Ferrier (2011). However, community-level models may suffer from generality and would be unable to extrapolate detailed changes to individual species within. There is a fine balance between the depth and breadth of knowledge which should be considered when determining how best to model changes to species distributions. The conceptual utility of a single-species approach is evident in that results have the capacity to be extrapolated to other species with similar life-history traits. It is not enough to know what traits vary, but also how these will ultimately affect and subsequently define the species distribution in the future (Albert *et al.* 2011). Ideally, any model which uses climatic, environmental and/or mechanistic variables should be experimentally backed-up to illustrate the importance of the included variables. Additionally, genetic studies would be crucial to complement extensive ecological sampling, particularly to determine whether two putative distinct populations are the same population, separated by either a soil seedbank or a physical barrier.

When these data are available, the simplest model could predict population dynamics over time, similar to a population viability analysis (Huntley *et al.* 2010b). Often it is this temporal resolution that is lacking in models (Buckley *et al.* 2010b). If the model predicts a high proportion of the sampled populations to decrease in abundance or to become locally extinct, then there is some confidence that the overall distribution is likely to retract with

future climate change. Thus, the output from such a model would not only produce the spatially explicit response of populations over time, but it would also identify the key traits that enable a population to persist or to disperse in the landscape. Ideally, this approach would provide an accurate assessment of a population's 'health'. From this, populations of species can be better sampled, and management priority can be given to those species whose populations are predicted to decline in future environments.

6. *Actinotus*: a model genus

To demonstrate the importance of populations and their traits, *Actinotus helianthi* Labill. (Apiaceae) will be used as a model species to explore ways to enhance SDMs and BMs. The genus *Actinotus* is comprised of 20 species – 19 endemic to Australia and 1 endemic to New Zealand. Species are either perennial herbs or sub-shrubs, or annual fire ephemerals. Keighery (1982) characterised five Western Australian *Actinotus* to be out-crossers, whereas *A. glomeratus* Benth. was noted to be mainly inbreeding. Little research has been undertaken on the breeding systems of east Australian *Actinotus*. It is likely that species with a shruby habit, such as *A. helianthi* are out-crossers, but this needs to be confirmed. However, previous work on *A. helianthi* has been focused on establishing species in the horticultural industry as a cut flower and potted plant (von Richter and Offord 1997a; b).

In addition to their varying breeding systems and life-histories, Australian *Actinotus* occur in a range of environments. Thirteen species are found in mesic environments, three species are restricted to sub-alpine areas, and the remaining three species are found in semi-arid environments. More importantly, species vary in their predicted distributions within the same environment. For example, *A. gibbonsii* F.Muell. has a relatively large geographic distribution and is found in 15 bioregions between Cairns on the far north coast of Queensland (QLD), south of the New South Wales (NSW) and Victoria (VIC) border, and in

central NSW and QLD. By contrast, *A. schwarzii* is a vulnerable species restricted to several populations in the MacDonnell Ranges (Northern Territory) (Nano and Pavey 2008; White *et al.* 2000).

Several species, including *A. helianthi* and *A. leucocephalus* Benth. have significantly higher germination success after fire (Baker *et al.* 2005; Emery and Lacey 2010). This further highlights the need to include impacts of disturbance factors in distribution models as populations are likely to differ in their opportunistic responses to disturbances and stochastic events.

6.1. *Actinotus helianthi*

Actinotus helianthi (flannel flower) is an erect perennial sub-shrub occurring in eastern Australia in NSW and southern central QLD. The species is found on sandy or rocky, nutrient-poor soils along the coast of NSW as well as in an isolated populations in central NSW. The species is characterized by inconspicuous flowers aggregated in umbels, subtended by involucral bracts (Fig. 2). Umbels are andromonoecious, with peripheral staminate flowers surrounding fewer hermaphroditic flowers. Inflorescences are paniculate with the central branch terminated by the primary umbel. Populations are often at their most abundant two years after fire, and some plants may reach reproductive maturity within the first year (Benson 1985). Lee (1995) originally observed different growth habits among populations. These different growth habits were not examined by Lee (1995).

Actinotus helianthi was originally wild-harvested and cultivated as a cut flower crop by the local horticulture industry. Several issues, however, surrounded the capacity to propagate the species by seed and vegetative tissue resulting in limitations to mass production. While many of these issues have been overcome (Offord and Tyler 1993; von

Fig. 2. Umbels of *Actinotus helianthi* comprise a cluster of flowers subtended by white involucral bracts.

Richter and Offord 1997a; b), inconsistencies still occur when germinating seed from different source populations and years (Emery *et al.* 2011; Emery and Lacey 2010). It is likely, therefore, that germination is influenced by both spatial and temporal environmental variation. *A. helianthi* has also been documented to have increased vegetative growth when fertilizer was applied (von Richter and Offord 2006). This raises the question of whether the species can tolerate a variety of soil environments. Indeed, *A. helianthi* was reported to be successfully transplanted and grown at 19 sites around Australia, including Queensland, Victoria, South Australia and Western Australia (von Richter and Offord 2000). Specifically, the authors found the species grew best in areas which had well-drained soil. Furthermore, the application of fertilizer was shown to improve vegetative and reproductive growth. Little is known about the reproductive niche of *A. helianthi* and the capacity for numerous interactions with species that visit umbels during primary flowering.

In this thesis, I use *Actinotus helianthi* as a single species method of modeling to focus on using population-level factors to demonstrate several important limitations that should be considered when predicting species distributions. Traditionally, a single species approach involves the use of occurrence records only. However, this assumes that each occurrence will respond to environmental conditions similarly. Given that the number of populations of *A. helianthi* encompasses wide latitudinal and ecological gradients, we might expect the species to exhibit variation in traits and interactions, especially between geographically-distant populations. Alternatively, a multiple species approach could allow predictions to be compared across a number of phylogenies and life-histories, allowing a representation of how climate could impact biodiversity. However, the utility of assessing changes to biodiversity relies heavily on the availability of single species distribution data (Mokany and Ferrier 2011). Therefore, using a single species approach provides an important foundational first step. Furthermore, Keith *et al*. (2008) note that changes to the distribution

of a species are likely to be influenced by its life-history and disturbance regime. These variables could not be examined in detail if comparisons were used across multiple species. Mokany and Ferrier (2011) also point out that multiple species modelling less suitable for taxa that are poorly studied or a high amount of species richness, such as plant and invertebrates. Furthermore, comparing multiple species cannot account for differences among populations, despite evidence stressing the importance of population-level effects (Albert *et al.* 2011; Albert *et al.* 2010a; Albert *et al.* 2010b; Kattge *et al.* 2011; Wright *et al.* 2001). I chose not to use multiple *Actinotus* species as a single species examined in detail would provide a clearer representation of the likely population factors that could influence distribution. The next step would be to extrapolate the findings from this thesis and apply them to other species.

7. Conclusions

As environmental conditions change over time, selection should favour different phenotypes, and species persistence will rely on the ability of individuals within to adapt or migrate to new habitat. By quantifying the traits that make up a species, it is hoped that this will enhance and validate model outputs, thereby complementing current, well-established species modelling techniques. In order to determine how populations might respond to future environments, and thus understand the impacts to species distributions, we need to define several mechanistic population traits, including phenology, reproduction, and interactions. To best understand these factors requires a species-level approach. This then provides a method to model the relationship between these key population traits and the environmental conditions that influence these. This should provide an understanding of the physiological and environmental constraints on a population and their influences on survival. The challenge is to develop these models with the help of experimental evidence to increase our ability to accurately predict the effects of future environments on populations and validate *in silico*

predictions. These predictions would greatly benefit conservation management to prioritize and minimize the loss of diversity.

7.1. Thesis aims

The aim of this thesis is to test the assumptions and limitations of BMs outlined in this chapter by performing field and laboratory experiments, which incorporate individual and population level data to determine whether populations are equivalent in their response to current and future environments. Using *Actinotus helianthi* as a model species, I aim to illustrate the importance of collecting experimental evidence from several species and environmental factors, including early plant performance traits, species interactions and soil. Using this information, I intend to determine whether a species distribution model can then be subsequently improved on a species with some level of confidence of its output due to the collected experimental data.

Chapter II

POPULATIONS OF *ACTINOTUS HELIANTHI* ACROSS A WIDE GEOGRAPHIC RANGE EXHIBIT DIFFERENT CLIMATIC ENVELOPES AND COMPLEX RELATIONSHIPS WITH PLANT TRAITS

Abstract

Climate envelopes are generated by overlaying climate variables derived from temperature and rainfall data onto mapped geographic locations of occurrences. Typically the species data are amalgamated into a single climate envelope, missing the opportunity to account for the potential of different environments to independently shape the functional plant trait values within populations. Here we explore how climate envelopes vary among populations, and whether individuals with similar trait values are similarly matched to particular climate envelopes or to spatial layers of environmental classifications based on additional variables other than climate. We generated climate envelopes from 35 populations of the widely distributed plant species *Actinotus helianthi* Labill. (Apiaceae). Populations with at least 84% similarity in their local climate were grouped by hierarchical cluster analysis. We then tested whether the similar climate envelopes would co-vary with populations of plants with similar traits. The same method was used to examine whether populations with more individuals were better adapted to their local climate than populations with fewer individuals. We also compared whether the climate envelopes were representative of other environmental groupings, including the Interim Biogeographical Regionalisation of Australia (IBRA) and soil types. Plant trait values were significantly different among populations ($P \leq 0.001$) and soil types ($P \leq 0.003$). All traits, except *diam* and *condist*, were significantly different among bioregions. Seven climate envelopes were identified across sampled populations, and plant

trait values within climatically-similar populations were highly dissimilar (*Global R* = 0.09). Larger populations (with more individuals) within a climate envelope displayed greater similarity among traits (*Global R* = 0.19). IBRA regions and soil types showed greater similarity with plant traits (*Global R* = 0.27; *Global R* = 0.25, respectively). This study demonstrates how the collection of data on plant traits and other environmental factors beyond climate can improve models of species distributions. Consequently, studies that rely on climate-only data, or single broad climate envelopes, may be too general, or disconnected from the population-level processes that shape the persistence and distribution of species across the landscape.

Introduction

Bioclimatic models are commonly used to predict the effects of temperature and rainfall on the geographic distribution of species. Their utility has increased the knowledge of ecological and geographical tolerances of species by depicting the spatial extent of suitable habitat and by modeling expected changes over time in response to altered climates (Beaumont *et al.* 2005; Busby 1991; Gallego-Sala *et al.* 2010; Hijmans and Graham 2006). These models typically amalgamate species occurrence records into a single climate envelope (i.e. spatially-similar habitats) that depict where the species is currently distributed. This model, which is subject to multiple ecological assumptions, can then be used to predict how the envelope might change under future conditions (Araújo and Peterson 2012; Heikkinen *et al.* 2006; Luoto *et al.* 2005). More recently, these models have evolved to also incorporate non-climatic, mechanistic factors, including species traits (Hanspach *et al.* 2010; Pöyry *et al.* 2008), species interactions (Araújo and Luoto 2007; Heikkinen *et al.* 2007; Kissling *et al.* 2012), abundance (Huntley *et al.* 2010a; Van Der Wal *et al.* 2009) and landscape or topological factors (Harris *et al.* 2014; Slavich *et al.* 2014). These changes recognize that a climate envelope may not authentically represent the main environmental processes that

shape species traits because the impact of climate is most prominent at a coarse, macro-influential scale for continents and the globe (Austin and Van Niel 2011b; Davis *et al.* 1998a; Pearson and Dawson 2003). Additionally, combining species records into a single climate envelope generalizes across existing variation at both the population and individual. Alternatively, examining the relationship between climate and plant trait values among populations will help to identify the relative importance of climate for determining species traits at a local or regional scale, and thus inform our models of changing distributions.

If a species is present across multiple environments that differ in factors that can affect plant trait values (e.g. soil availability for plant growth and height) then it is likely that each population will contain individuals with different suites of traits suited to the local environment. Albert *et al.* (2010b) note that significant variation can be detected using a single trait approach. Furthermore, the variation reported had no spatial structure, indicating strong variation between individuals possibly due to fine-scale environmental heterogeneity. The authors also note that the majority of variation detected was between populations. This result was then confirmed in a subsequent study that estimated that multiple trait variation between and within populations, accounted for 70% and 30% of the variation, respectively (Albert *et al.* 2010a). Wright *et al*. (2001) describe a similar result for leaf traits, which exhibited more variation within a single habitat than between habitats. In a comparative review based on the global TRY plant trait database, Kattge *et al*. (2011) estimate around 40% of trait variation exist within-species. If traits are shaped more by their local climate than by other environmental factors, then it could be expected that certain phenotypic trait values should be similar amongst sites with similar local climatic envelopes. For these reasons, a climate envelope approach may be more effective if it represented this fine-scale variation.

Matching plant trait values across environmental gradients also provides evidence for how the broad and local scales interact to shape the extent to which traits are expressed, and may help in generating climate envelopes across gradients. Such gradients are generally a single dimension; focusing on a single variable such as precipitation, temperature or salinity (Díaz *et al.* 1999; Thuiller *et al.* 2004b). At a global scale, temperature has been reported to influence a greater number of plant traits than precipitation (Moles *et al.* 2014). A reduction in leaf width and surface leaf area have also been reported in several Australian plant species along decreasing precipitation and decreasing soil phosphorus gradients (Fonseca *et al.* 2000). In South Africa, Thuiller *et al.* (2004b) used a multivariate analysis to identify niches for 88 species of *Leucadendron*. The authors identified three main gradients – aridity, precipitation and temperature tolerance – and the position of species within these gradients defined their traits and, therefore, their niches (Thuiller *et al.* 2004b). Other environmental factors, such as topography or soils can also vary in ways that a directional gradient cannot. Incorporating these factors permits a continuous quantitative description of the landscape within a species distribution, improving the prediction of traits beyond climate alone (Lavorel *et al.* 2011).

Given the likelihood of variation to occur at a trait level, the spatial scale in which climate envelopes are produced will ultimately determine their usefulness. Pearson and Dawson (2003) suggest a heirarchical framework could be used to determine whether some of the limitations of climate envelopes can be addressed. Therefore, in order to understand how a species might respond to changes in climate envelopes, it is necessary to discover if individuals within and among populations with similar local climates are also similar in their trait values. Any recorded variation may be due to plasticity or be genetically-differentiated (Clausen *et al.* 1940; Petrů *et al.* 2006), both of which can be complimentary rather than exclusive (Albert *et al.* 2010b). To this end, we generated multiple climate envelopes by

grouping populations that had similar local climate. Our study focussed on a widespread species as we expected populations from geographically-distant areas to be more likely to differ in key trait values. Such a system allowed us to then explore the extent of variation in plant trait values within and among populations. Using a heirarchical cluster analysis technique allowed us to assess some of the biases and assumptions that are assoicated with correlative models by having more information for each species occurrence (Dormann *et al.* 2012). We can then determine whether the observed patterns in trait variation match different climate envelopes identified by objectively grouping individual plants based on the similarity of the climate envelopes generated.

This approach enabled us to address the following question: (i) do individuals within and among populations have similar trait values and do these patterns then match our generated climate envelopes? Since individual neighbors are expected to be more genetically-similar, we expect trait value variation to be highest among populations (Albert *et al.* 2010a). We used several trait values to provide a fuller understanding of the indicators of plant performance. We then refined our question by repeating our analysis on populations estimated to contain at least 1,000 individuals to ask (ii) do more abundant populations have plant trait values that are more closely matched to their environment than populations with fewer individuals? We predict that smaller populations will be less well-matched to their environment as they are more recently colonised. Reader (1998) reported up to 88% of interspecific variation in mean plant trait values across populations could be explained by abundance. Examining the effects of abundance also links spatial climate modelling with demongraphic models (Keith *et al.* 2008). We then built on our initial questions by asking (iii) do other spatial patterns that include non-climate variables better represent the spatial variation in trait values than climate envelopes? To address this, we used two sets of environmental groupings: the Atlas of Australian Soils classification (Isbell 2002) and the

Interim Biogeographic Regionalisation of Australia (IBRA). The former is a national hierarchical framework for classifying soils into varying scales while still incorporating a number of major soil properties. IBRA bioregions are 89 distinct geographic areas which are defined by topological and climate factors as well as species communities (Interim Biogeographic Regionalisation for Australia, 2007). Bioregions are then further divided into 419 sub-regions that represent variability in ecosystems at a finer scale. We used IBRA as a refinement of climate envelopes by testing whether the combination of climate as well as other environmental and landscape variables was more effective in predicting similar plant trait values than climate alone. In this regard, using bioregions provides a more complete representation of the potential selective forces of plant trait values.

Materials and Methods

Study species

Actinotus helianthi Labill. is a perennial sub-shrub capable of growing up to 2 m tall. Flowers are aggregated into pseudanthic capitula, characterized by large, white, petaloid involucral bracts (Lee 1995; Webb 1980). Inflorescences comprise a terminal (primary) capitulum subtended by at least one secondary branch, each of which is further subtended by tertiary branches. *Actinotus helianthi* is endemic to Australia and occurs on oligotrophic soils of eastern New South Wales and central Queensland. The recorded distribution of the species spans a latitudinal distance of approximately 1,255 km (between 35.61°S and 24.37°S) and a longitudinal distance of approximately 780 km (between 153.43°E and 147.18°E). Long-term average annual rainfall and temperature range from 1,200 mm and 22°C on the coast, 600 mm and 24°C in central New South Wales, and 700 mm and 29°C in central Queensland. Thus, the species experiences a wide range of climatic conditions throughout its distribution, making it a highly suitable species for this study.

Field data

Thirty-five populations of *A. helianthi* representing the known geographic range of this species and its associated climate gradients were sampled in 2011 (Fig. 1). All populations occurred in sclerophyll heathland or woodland. The following plant trait values were recorded for each of 30 randomly selected plants at each population (1,050 plants in total): (1) above-ground height (*height*), (2) leaf lamina length (*leaf*), (3) the number of inflorescences (*stem*), (4) the number of umbels (*umbel*), (5) diameter of the main stem at ground level (*diam*), (6) the distance to the closest conspecific (to the nearest cm) (*condist*), and (7) the number of umbels per inflorescence (*umbelstem* – derived from *stem* and *umbel*). All measurements, except (6), were made to the nearest mm. The traits selected represent soft traits and some are described in Cornelissen *et al.* (2003). These traits were selected as representative of plant growth and performance, and are more easily recorded across a large number of individuals than other functional traits (Cornelissen *et al.* 2003). Two population traits were also collected from each location. The number of individuals (*abundance*) was recorded for each population with < 300 plants, and was estimated for populations with > 300 individuals. The percentage of reproductively mature plants (*maturity*) within a population was also estimated (Table 1).

Environmental data

The relevant climate data were obtained from the WorldClim website (www.worldclim.org; Hijmans *et al*. 2014). These data are freely available, and are most commonly used to determine species bio-climatic envelopes (Hijmans *et al.* 2005). Fourteen climate variables were used at the highest resolution available (30 arc-seconds; *c.* 1km^2; Table 1). The climate at each population was extracted from the variables in ArcGIS v10.1 (ESRI, 2012). Spatial layers for IBRA bioregions and sub-bioregions, the Australian Soil Atlas, and digital elevation were obtained from the Australian Department of the Environment website

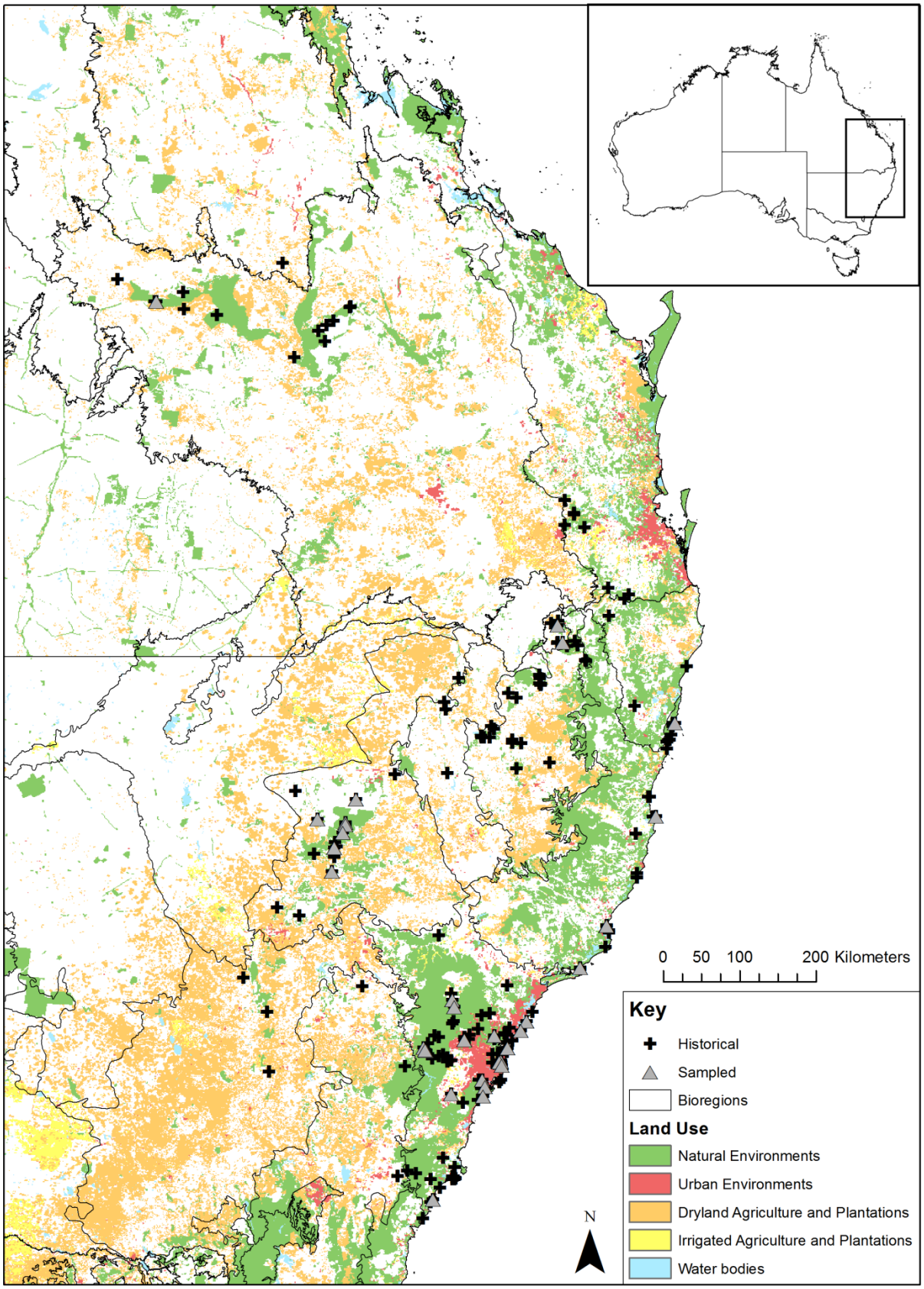

Fig. 1. Distribution map of the 35 *Actinotus helianthi* populations sampled. Historical records from Atlas of Living Australia (www.ala.org.au) accessed on August 16, 2010.

Table 1. Climate variables, plant and population traits and site variables used.

Data source	Variable name	Description
Worldclim	annrain[a]	Mean annual rainfall
	anntemp[ab]	Mean annual temperature
	coldT[a]	Mean temperature of coldest quarter
	dryrainM[ac]	Mean rainfall of driest month
	dryrain	Mean rainfall of driest quarter
	dryT	Mean temperature of driest quarter
	maxwarmT	Max. temperature of the warmest quarter
	mincoldT	Min. temperature of the coldest quarter
	warmrain	Mean rainfall of warmest quarter
	warmT[a]	Mean temperature of warmest quarter
	wetrainM	Mean rainfall of wettest month
	wetrain	Mean rainfall of wettest quarter
	wet[ab]	Mean temperature of wettest quarter
Plant traits	condist[a]	Distance to the closest conspecific (cm)
	diam[a]	Diameter of the main stem at ground level (mm)
	height[a]	Above-ground height (cm)
	leaf*	Leaf lamina length (mm)
	stem	Number of inflorescences
	umbel[a]	Number of umbels
	umbelstem[a]	Number of umbels per inflorescence
Population traits	abundance[a]	Estimated number of individuals in a population
	maturity[a]	Estimated proportion of individuals that are reproductively mature in a population
Site variable	elevation	elevation (m)

[a] variable was included for analyses.
[b] variable was included for analyses of large populations only.
[c] variable was excluded for analyses of large populations.

(http://www.environment.gov.au/). The relevant data from these layers were extracted using the same method as the climate variables.

Data analysis

To explore the relationship between local climate and plant trait values, we performed multivariate analyses on the climate and biological datasets. In order to avoid any problems associated with co-linearity (Dormann *et al.* 2013), Pearson's correlation coefficient analysis was performed on the climate data. A conservative value of $\geq \pm 0.85$ was used to identify highly correlated variables (Dormann *et al.* 2013; Elith *et al.* 2010). The following climate variables were retained, which included the most correlated variable (Appendix 2): (1) maximum mean temperature of the warmest quarter (*warmT*), (2) mean temperature of the coldest quarter (*coldT*), (3) mean rainfall of the driest month (*dryrainM*), and (4) annual precipitation (*annrain*).

To differentiate the data into climate envelopes, the four climate variables were converted into a resemblance matrix using the S15 Gower's similarity quantitative measure as implemented in PRIMER v6.1.16 (Clarke and Gorley 2006). Gower's coefficient is able to combine different types of descriptors and process, each one according to its own mathematical type (Gower 1971). A hierarchical cluster analysis (HCA) using the unweighted pair-groups method of arithmetic averages (UPGMA) was performed on the climate resemblance matrix and climate envelopes were generated from the output. A multi-dimensional scaling (MDS) ordination plot was used to visualize the climate envelopes in space. Each envelope was described according to its most important climate factor(s). Analyses of similarity (ANOSIMs), using one thousand permutations, were performed with the climate envelopes as the response factor to sub-bioregions nested within bioregions, as well as with soil types. ANOSIMs provide a distribution-free test to analyze multivariate data

for differences using permutations from a rank similarity matrix (Clarke 1993). A global R statistic is used in these analyses that scale between -1 and 1 with a 0 value indicating no differences between the datasets. If a global R statistic is significant (when $P \leq 0.05$), then an R statistic and a *P*-value are calculated for each pairwise comparison.

One population was removed from the trait data matrix as it contained fewer than 30 plants. A Pearson's correlation coefficient analysis was then performed on the trait data from the remaining 34 populations. The only highly positively correlated plant traits were *umbel* and *stem* ($R^2 = 0.86$), and the latter was removed from further analyses. The six plant traits and two population traits were then converted to a resemblance matrix using the Gower coefficient. A MDS ordination of the trait data, grouped by the climate envelopes, was performed to visualize patterns and where groups were identified, and an ANOSIM model was used to examine the similarities in the trait data among the climate envelopes.

To assess whether other environmental spatial aggregations were better able to depict the spatial patterns of plant trait values, we ran an additional ANOSIM using sub-bioregions nested within bioregions and an another using soil types. All ANOSIMs were run using 1,000 permutations. Multivariate general linear models (GLM) were run to determine the traits that varied significantly across populations, bioregions and soil types.

To compare all populations with large populations only, 19 populations estimated to contain more than 1,000 plants were retained in the dataset. Analyses were performed on these populations as described above except for the following differences. Two climate variables: mean annual temperature (*anntemp*) and mean temperature of the wettest quarter (*wetT*) were added, and *dryrainM* was omitted from a second Pearson's correlation coefficient analysis using the 19 populations due to be highly correlated with other variables

(Appendix 2). Five climate envelopes were generated by the HCA, with each envelope containing populations with ≥ 87% similar climate and an MDS plot was generated to examine separation of the groups. ANOSIMs and GLMs were performed as above using both the climate and trait data with bioregions and soil types. Correlation analyses and GLMs were performed in the SPSS statistical package (version 21; IBM software) whereas similarity and cluster analyses were performed in PRIMER (Clarke and Gorley 2006) & PERMANOVA+ (Anderson *et al.* 2008).

Results

Seven climate envelopes were generated by the hierarchical cluster analysis. Each envelope comprised populations which had ≥ 84% similarity in local climate. This value was subjectively chosen as it provided a good spatial grouping of populations (Fig. 2). The multi-dimensional scaling (MDS) ordination plot confirmed the separation of the envelopes with a two-dimensional stress of 0.04 (Fig. 3a). The relative differences between the seven envelopes are described as follows: (a) cooler average temperatures during summer (17.8 ± 0.2°C) and winter (6.4 ± 0.1°C); (b) warmer average temperatures during winter (14.1 ± 0°C); (c) higher average annual rainfall (1320 ± 17 mm) and higher average rainfall during the driest month (64 ± 1 mm); (d) highest temperatures during summer (26.2 ± 0°C) and lowest average annual rainfall (629 ± 0 mm); (e) lower average annual rainfall than group (c) (1006 ± 88 mm) and higher average temperatures during summer (22.3 ± 0.4°C); (f) cooler average temperatures during winter (8.8 ± 0.3°C) and low average annual rainfall (918 ± 27 mm), and; (g) very low annual rainfall (719 ± 19 mm) (Fig. 4). There was a strong longitudinal gradient in annual rainfall between the climate envelopes. Envelopes (a) – (c) were characterized by high annual rainfall at high altitude or coastal sites. Envelopes (d) – (g) were inland sites and had low annual rainfall compared to envelopes (a) – (c) (Fig. 4). The nested ANOSIM showed high similarity between the climates of the bioregions and their sub-

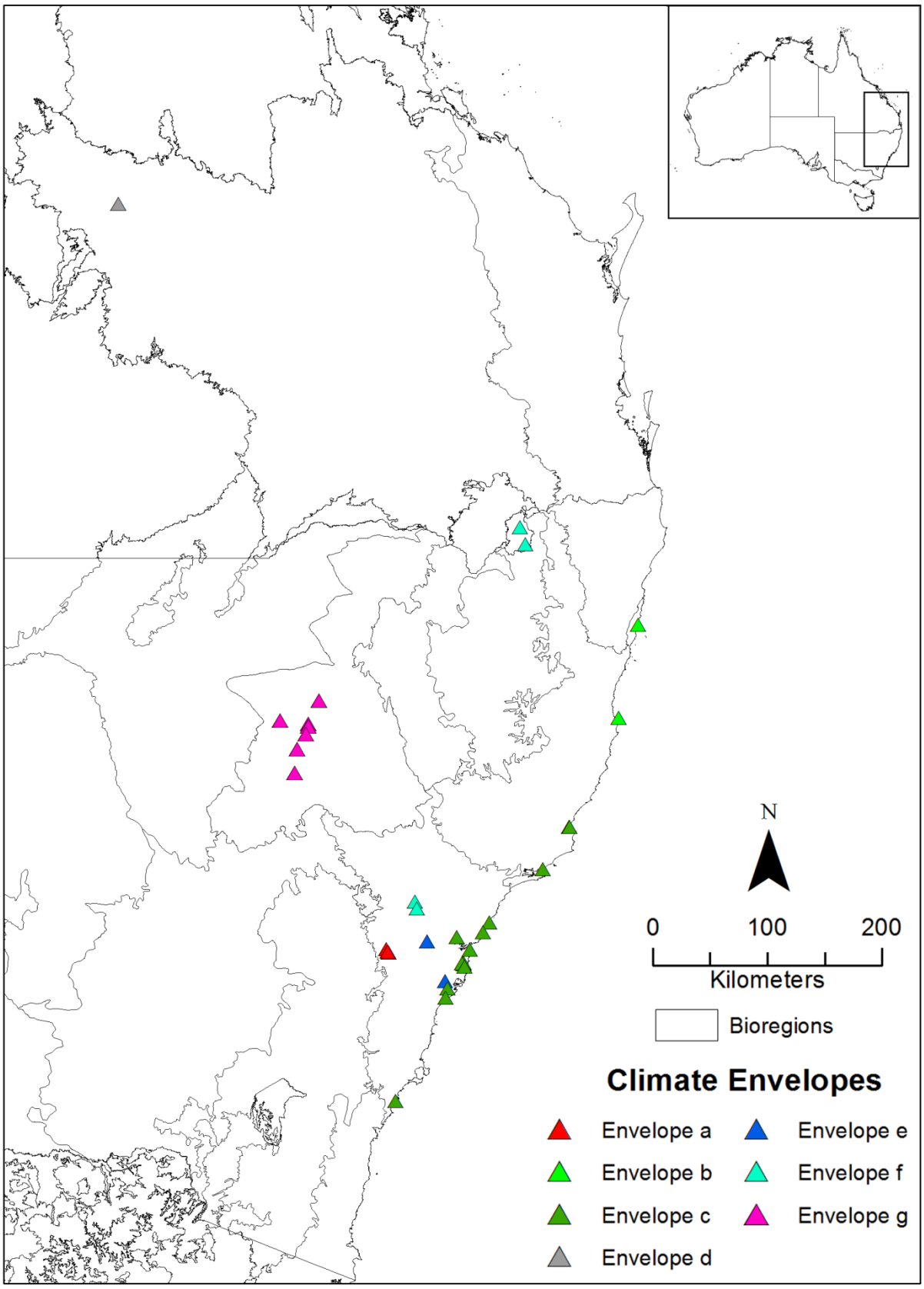

Fig. 2. Spatial locations of the seven climate envelopes identified from the Hierarchical Cluster Analysis dendrogram.

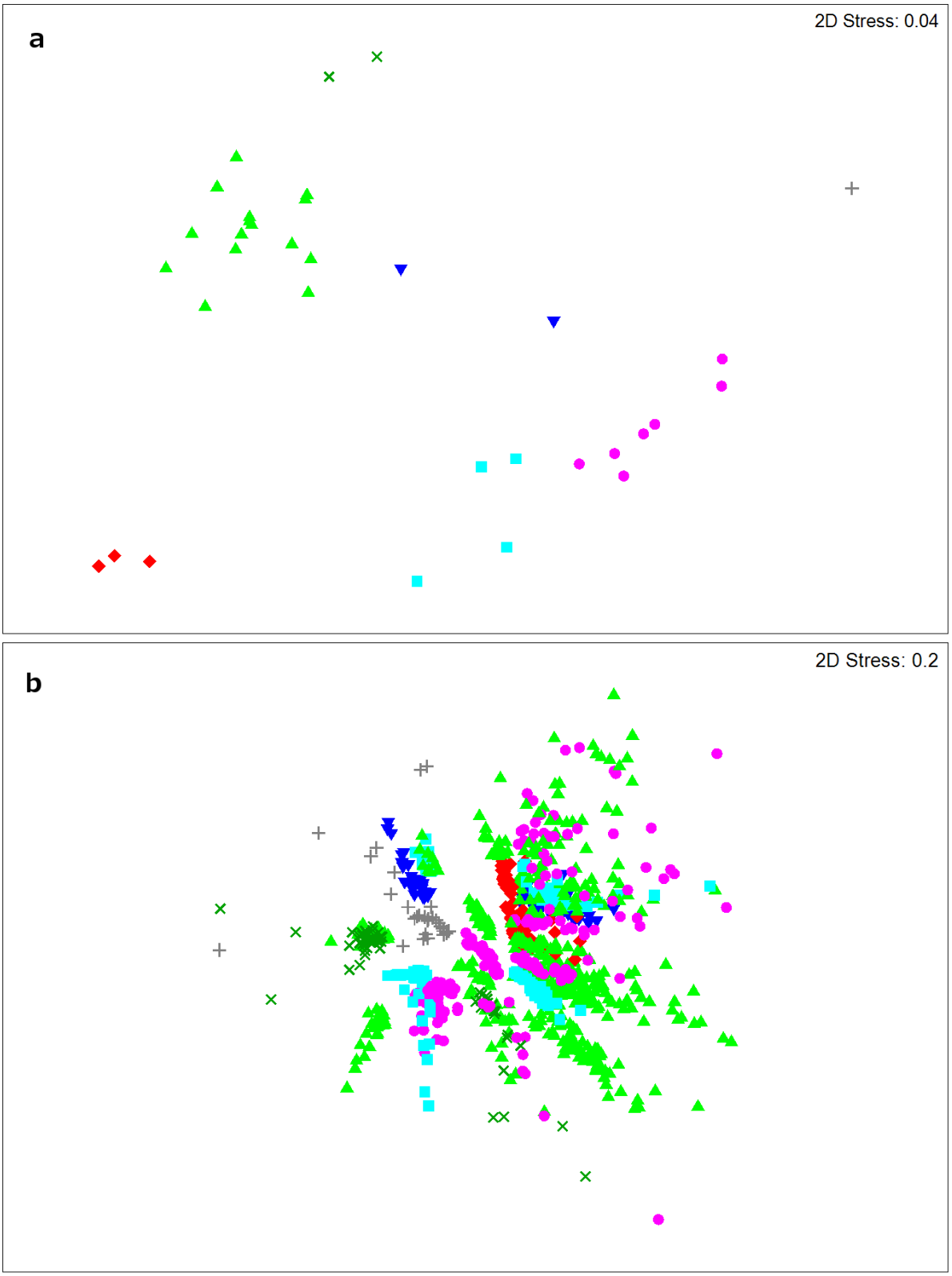

Fig. 3. Two-dimensional MDS ordinations illustrating groupings of the seven climate envelopes (a) by populations and (b) by plant traits. ♦: envelope-a; ×: envelope-b; ▲: envelope-c; +: envelope-d; ▼: envelope-e; ■: envelope-f, and; ●: envelope-g.

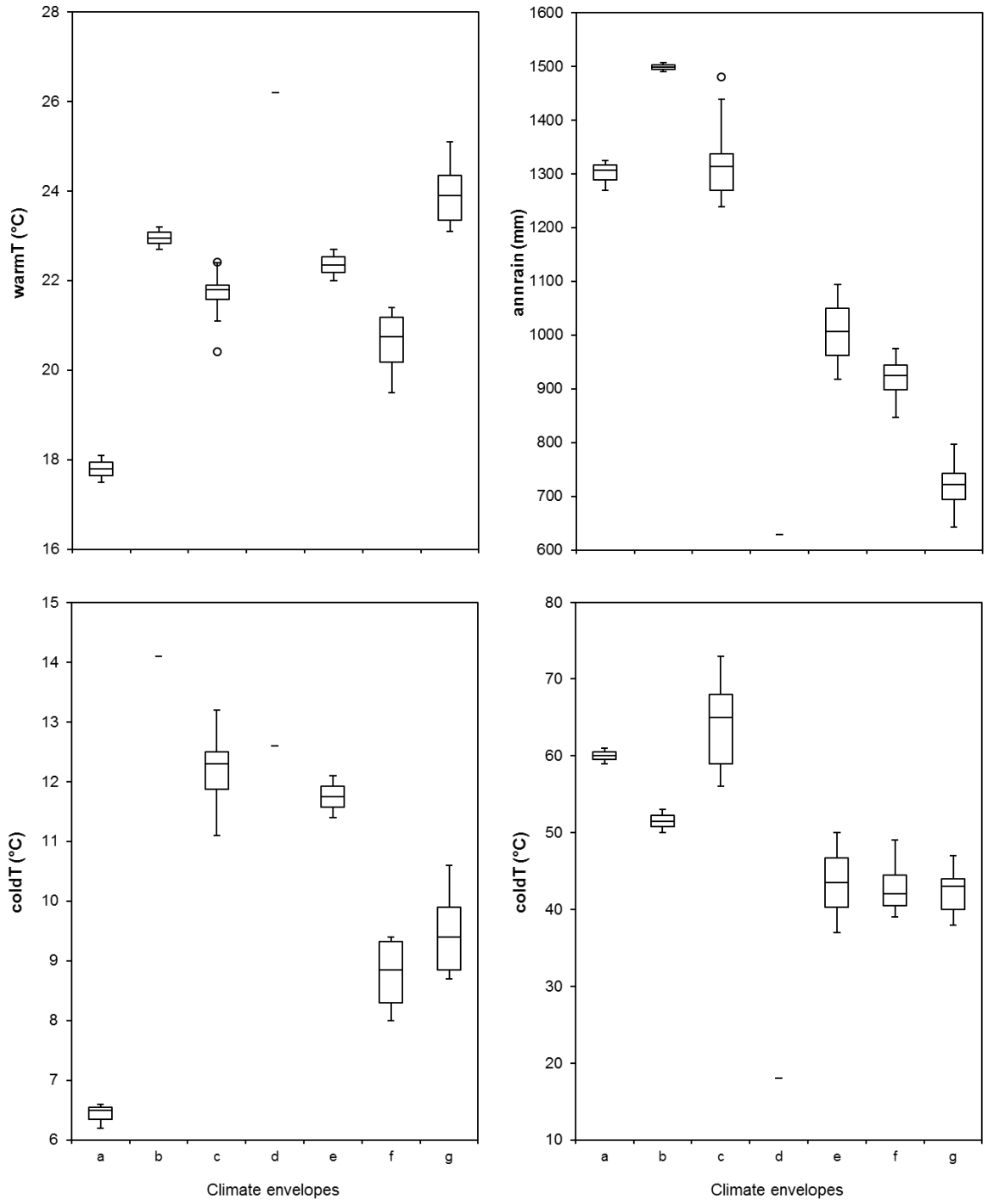

Fig. 4. Boxplots of the seven climate envelopes for each of the four contributing climate variables, including mean temperature of the warmest quester (*warmT*), mean temperature of the coldest quarter (*coldT*), mean annual rainfall (*annrain*), and mean rainfall of the driest quarter (*dryrain*).

bioregions (*Global R* = 0.61; *P* = 0.002 and *Global R* = 0.70; *P* = 0.001, respectively). The one-way ANOSIM with soil type showed a very low similarity with the climate envelopes (*Global R* = 0.14; *P* = 0.001).

All plant trait values were significantly different across populations ($P \leq 0.001$) and soil types ($P \leq 0.003$), and all trait values except *diam* (*P* = 0.251) and *condist* (*P* = 0.085) were significantly different across bioregions ($P < 0.001$). From the Pearson's correlation matrix, plants that were taller tended to have larger stem diameters and more umbels. Plant traits within the 34 analysed populations showed high disparity (stress = 0.2) within climate envelopes. The two-dimensional MDS ordination plot did not show any pattern or clusters of populations that shared similar trait values (Fig. 3b). This was confirmed by the ANOSIM with each climate envelope containing populations with significantly dissimilar plant trait values within (*Global R* = 0.09; *P* = 0.001; Fig. 5). When analysed with bioregions, trait values showed significant divergence among populations from within the same bioregion (*Global R* = 0.27; *P* = 0.03) and sub-bioregions (*Global R* = 0.46; *P* = 0.001). Plant trait values within populations from the same soil type also displayed a weak similarity (*Global R* = 0.25; *P* = 0.001).

Four of the seven climate envelopes were retained when the 19 large populations (> 1,000 plants) were analysed. Envelopes (a), (c), (f) and (g) remained while a fifth envelope (h) was recovered and is defined by high annual rainfall. The five climate envelopes showed good separation (Stress = 0.01) from the MDS plot (Fig. 6a). Large populations showed significant dissimilarity between the plant trait values and climate envelopes (*Global R* = 0.19; *P* = 0.001; Fig. 6b), as well as sub-bioregions (*Global R* = 0.53; *P* = 0.001) and soil types (*Global R* = 0.31; *P* = 0.001). Plant trait values showed some congruence with bioregions (*Global R* = 0.17; *P* = 0.45).

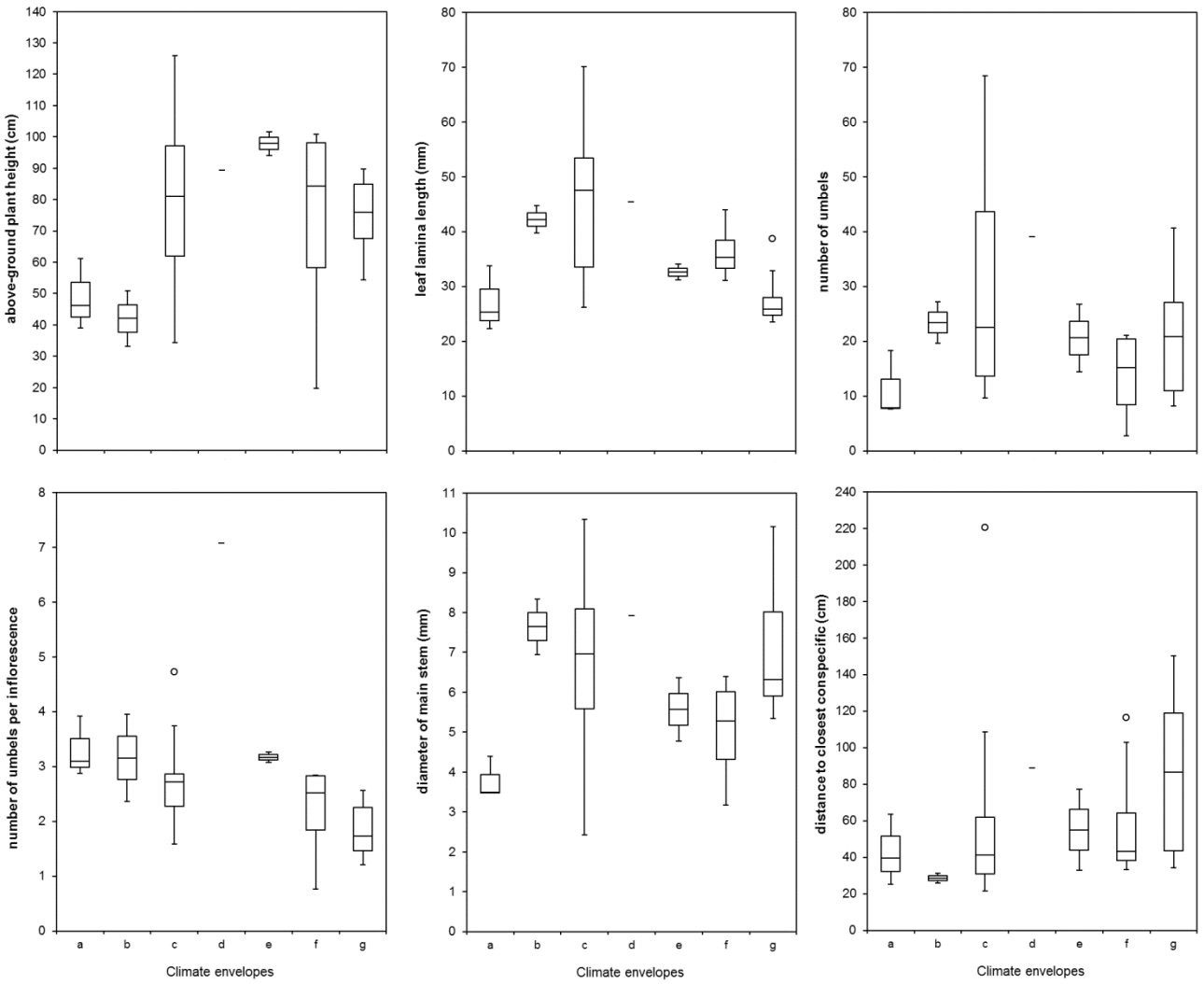

Fig. 5. Boxplots of the seven climate envelopes for each of the six plant traits used in the analyses, including above-ground plant height, leaf lamina length, the number of umbels, diameter of the main stem at ground level, the number of umbels per inflorescence, and the distance to the closest conspecific.

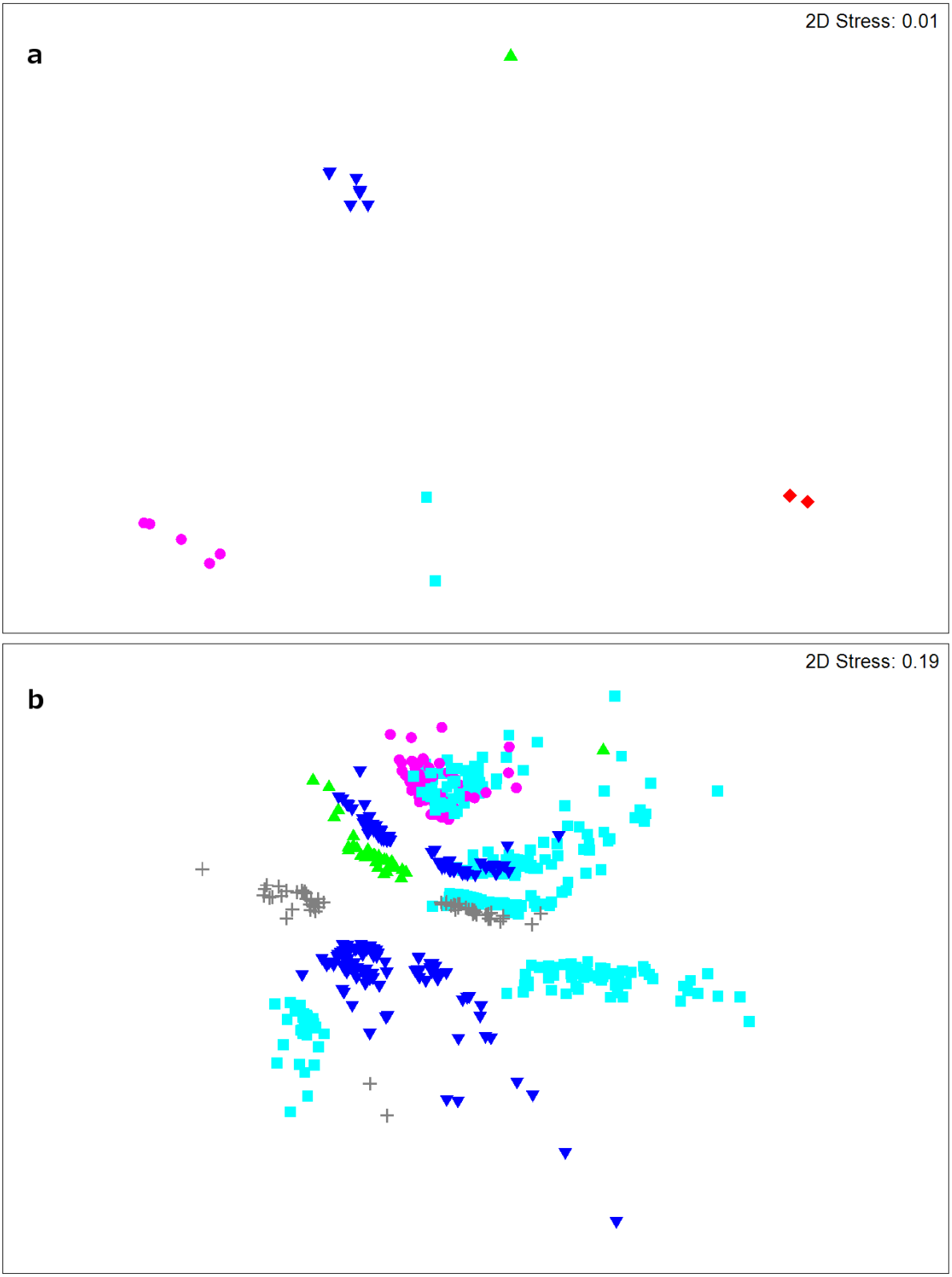

Fig. 6. Two-dimensional MDS ordination of the 19 populations with at least 1,000 individuals separated into five climate envelopes by (a) populations, and (b) plant traits: ♦: envelope-a; ▼: envelope-c; ■: envelope-f; ●: envelope-g, and; ▲: envelope-h.

Discussion

The aim of this study was to generate different climate envelopes across the geographic range of a species and to determine the importance of climate and non-climate variables in explaining plant trait variance. We found that the relationships between climate envelopes and observed plant trait values were spatially complex and dependent on scale. *Actinotus helianthi* has a wide distribution and its populations experience a range of climatic conditions identified in this study. We demonstrated that for a given year, plants within a population have dissimilar trait-values to plants in geographically disjunct populations with a similar climate envelope. Our results are indicative of the differential response of plants to climate being either a tolerance of, or adaptation to, local conditions (Davis and Shaw 2001). Our results agree with other studies that examine plant trait variation across populations (Albert *et al.* 2010a; Albert *et al.* 2010b). This variation observed in plant trait values was not able to reflect our population-level climate envelopes.

The impact of climate at a local scale does not correspond with its impact at coarser scales (Araújo *et al.* 2011). However, population-level climate envelopes do provide a foundation for assessing the level of predictability among complex plant traits within populations, thereby providing a valuable means to examine the relationship between species and their surrounding environment. In this study, we have demonstrated the importance of incorporating population-level data into a species distribution model. Taking this further, a demographic distribution model (DDM) (Merow *et al.* 2014) uses demographic data on survival, growth and reproduction to develop a mechanistic understanding of population-level responses based on forecasts of environmental changes. These models link vital rates directly to ecological processes and can predict where within the species distribution may expand or contract based on population growth rates. We also recommend using DDMs to evaluate and

test different assumptions associated with climate envelopes as they include measures of uncertainty associated with limited data or model assumptions.

The IBRA bioregions and sub-bioregions is a system developed where a proportion of areas within each 'environmental envelope' are listed as protected in order to minimize the impact of climate change on the environment (Natural Resource Policies and Program Committee, 2009). Although, there is some disparity of opinion regarding whether the classification provides an appropriate system (Marchant *et al.* 2000; Peters and Thackway 1998), the system still represents the best regionalization of Australia for conservation and ecological studies. For example, a study on crayfish identified two bioregions in Tasmania that should be given top priority for conserving the highest level of diversity (Whiting *et al.* 2000). The author's results differed from previous reports that suggested high priority areas for conservation based on taxonomy alone. Here, we have utilised these spatial 'envelopes' in a novel way to explore their relationship with *A. helianthi* trait values. We found several trait values to significant differ among bioregions. Furthermore, the climate envelopes identified in this study showed some connection with the Australian bioregions and sub-bioregions groupings. This result is not surprising given that the bioregions are, in part, defined by climate as well as a combination of other factors, including vegetation, geology and topography. Our results confirm that bioregions are a further refinement of the climate envelopes identified in this study, and may present a useful means to assess population differences across the species distribution.

In contrast to the reasonable match to bioregions, the climate envelopes generated were not readily aligned to the distribution of different soil types. Soils vary at a local scale, and specific factors, such as pH, can elicit a different physiological response from plants to the environment. For example, *Populus augustifolia* seedlings were twice as likely to survive

in their local soil, raising the possibility of epigenetic effects determined by soil chemistry or soil biota in conveying local adaptations (Smith *et al.* 2012). The authors also reported that height, leaf area and the number of leaves were 15% to 20% greater when grown in their local soil. In support of the effects of soil, the availability of nitrogen and carbon, as well as pH were reported to vary between soils at the different sites. In a global study, soil nutrient factors explained more of the variation observed in leaf traits, such as leaf area (Ordoñez *et al.* 2009). The authors also point out that soil factors also interacted with climate factors in explaining leaf area. Phosphorus content has also been shown to be correlated with some traits in Australian plant species (McDonald *et al.* 2003). Although the classification of Isbell (2002) is geographically broad, *A. helianthi* is found across a wide geographic range, thus it could be expected for some trait values to show similarity between the different soil types. Whether finer scale soil classification systems show greater similarity with plant trait values warrants further investigation.

Local adaptation is important for individual plant survival, and, therefore, population survival. The degree of local adaptation in plants can be higher in larger populations (> 1,000 flowering plants) than smaller ones (Leimu and Fischer 2008). Furthermore, plant abundance is a more influential factor than life-history and spatial or temporal variation (Leimu and Fischer 2008). We might expect this since plants in larger populations may have a longer period of time to better match their traits to the local environment. While not always a good predictive factor on its own, when coupled with other species trait values, abundance can also be shown to influence overall ecosystem function (Hooper *et al.* 2005). Climate and topography can also have an interactive effect on population size (Reader 1998). For example, in New Zealand, higher mean daily temperatures during winter resulted in larger populations of *Agrostis capillaris* L. whereas higher mean daily spring temperatures led to larger populations of *Lolium perenne* L. (Wan *et al.* 2009). Topography can indirectly affect

plant abundance by influencing soil biota and moisture content, and limiting the number of growing plants (Radcliffe and Lefever 1981). While sampling larger populations might be more beneficial for a study such as ours where we are interested in the conformity and predictability of plant trait values with local climate, including smaller populations better represents the diversity of phenotypes and, therefore, the overall adaptability and potential survival of the species throughout its geographic range. However, caution is warranted when using a single estimate of abundance. Abundance of *A. helianthi*, for example, can be strongly influenced by the time since last fire. In addition, our estimate did not include the soil seedbank, which is an important parameter for calculating population persistence (Wardle 2003). Incorporating both above- and below-ground abundance estimates into the model could provide a better understanding of how populations interact with their environment.

Exploring plant trait values across individuals and populations is pivotal, in an ecological context, to classifying species into functional groups (Albert *et al.* 2011; Díaz *et al.* 1998). Differences in trait values could be associated with species competitiveness (Goldberg 1996) or as a response to species interactions (Díaz *et al.* 2001). The fact that other environmental patterns showed some congruence across *A. helianthi* plant trait values indicates that climate is not the sole selective force, and that different ecosystems are driven by different interactions. This suggests that when climate changes, each population will respond based on the composition of individual phenotypes with the potential for divergent outcomes across the geographic range. Only if individuals possess similar phenotypes so that the mean trait value of neighboring populations are similar can we expect these populations to respond similarly, and thus for the climate envelope to meaningfully represent the aggregated outcome. If individual plants do not possess similar phenotypes then it is likely that an array of responses will occur, even within the same climate envelope. Subsequent

studies could then test population genetic structure across the landscape, identify whether the capacity of species traits to respond to local climate is constrained due to genetically-fixed traits associated with local environmental factors (Storfer *et al.* 2007).

Complex interactions between populations, traits and climate have been documented for other biota. For example, a study of European birds found populations to increase or remain stable when changing their migration period, whereas declining populations did not (Møller *et al.* 2008). Interestingly, migratory period was the only significant factor associated with population size for the decade 1990-2000. Prior to this, habitat type and other latitudinal factors were more important (Møller *et al.* 2008). Furthermore, it has also been shown that in marine crustaceans, populations exhibit strong local adaptation and cannot change their thermal tolerance after 10 generations of selection (Kelly *et al.* 2012). Such results give support to the notion that climate-only studies underestimate the probability of species distribution changes. Whether *A. helianthi* is locally adapted to its environment requires populations to be tested under common conditions, which is currently under investigation.

Recent studies highlight the need to include plant traits, as well as environmental factors, in addition to climate variables for modelling patterns in species distributions. Several studies have produced models that perform better with the addition of environmental factors, including soil (Coudun *et al.* 2006; Dubuis *et al.* 2013) and elevation (Hof *et al.* 2012). Plant traits and population size are also being promoted as important for species predictions (Douma *et al.* 2012; Duff *et al.* 2012). This study supports these recommendations by demonstrating that for widely distributed species, such as *A. helianthi*, climate alone does not provide sufficient precision to explain variation in functional plant trait values throughout the geographic range. Predicting species response to future climate change requires a multifaceted and integrated trait-based approach to understand how

populations differ across space. Combined experimental and field-based approaches are required in order to gather the necessary data to properly develop the algorithms for this framework. In addition, genetic landscape studies that examine the relationship between genotypes and environmental interactions would further complement our work. Other functional traits not examined in this study, such as those at the earliest and most sensitive developmental stages (i.e. reproductive and seed) warrant future investigation to strengthen the results from this study and to ultimately determine how best to classify the existing variation. Given that all species are undergoing a period of environmental change it is important to effectively predict changes in species distributions.

Acknowledgements

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Chapter III

REPRODUCTIVE ECOLOGY OF THE PERENNIAL FLANNEL FLOWER, *ACTINOTUS HELIANTHI* (APIACEAE - MACKINLAYOIDEAE)

Abstract

Actinotus helianthi Labill. (flannel flower) is a perennial sub-shrub endemic to the east coast of Australia. Despite its value as a cut-flower crop and potted plant, the floral phenology and breeding system of *A. helianthi* have not been fully described. Understanding the reproductive system of *A. helianthi* will help improve industry and conservation value by optimising the production of viable seed. Here we characterise the reproductive biology and determine if the source of pollen affects seed set and germination. Umbels of *A. helianthi* comprise a central aggregation of numerous hermaphroditic flowers, surrounded by peripheral, unisexual (functionally male) flowers. Hermaphroditic flowers are protandrous. Peripheral male-only flowers have pollen present during the male phase of the hermaphroditic flowers, but not during their female phase. There is some temporal overlap between sexual phases among umbels of different ages. We document within-umbel floral phenology and pollen counts to quantify the potential for geitonogamous pollination to occur between primary and secondary umbels. We tested four pollination types in two populations of *A. helianthi* – within-umbel (intra-umbel geitonogamy), within-plant (geitonogamy), between-plant (xenogamy) and open-control. Within-umbel pollinated flowers produced very low viable seed set in each population (1 % and 6 %, respectively), and were excluded from analyses. By comparison, seed set and germination percentage did not differ significantly among the other three manipulated treatments. Populations differed significantly in the percentage seed set (31 % and 68 %), but not in the germination of fresh seeds (25 % and 21

%). The difference in seed set between populations could be due to pollination service, but once flowers are pollinated, the seeds produced are of similar quality in terms of germination. These findings may apply to other Apiaceae or other self-compatible species with similar temporal gender expression.

Introduction

The showy flowering heads of the flannel flower, *Actinotus helianthi* Labill. (Apiaceae) has led to its popularity as a horticultural species, but the basic reproductive biology of this native angiosperm remains unknown. *A. helianthi* is grown particularly for the cut-flower trade due to the long, straight stems that terminate with a flower head (von Richter and Offord 1997a). Despite its desirability to the horticulture industry, the flannel flower suffers from a number of cultivation difficulties, including root disease (Offord and Bullock 2009) and erratic propagation (Lee 1995; Offord and Tyler 1993). Much of the research to date has focused on optimising the method of cultivation from cuttings or from seed (Emery and Lacey 2010; Lee 1995; von Richter and Offord 1997a; b). The next step is to investigate whether the reproductive ecology of *A. helianthi*, specifically the mode of pollination, affects seed quality and, subsequently, propagation.

Understanding the reproductive system of any species is a complex task in plant population biology (Devaux *et al.* 2014), and such estimations have required various combinations of field trials and laboratory manipulations (Brys *et al.* 2008; Cohen 1966; Davila and Wardle 2002; Offord 2004; Rees 1994; Willi *et al.* 2007). Reproductive success in out-crossing plants is expected to vary most prominently among populations due to variability in pollen availability (Aizen and Harder 2007; Davila *et al.* 2012), as well as changes in the abundance of plants and external environmental factors such as pollen vectors (Ågren 1996; Brys *et al.* 2008; Kunin 1997; Mustajärvi *et al.* 2001).

Apiaceae is a species-rich family of flowering plants found in numerous habitats, including deserts, marshes, subalpine tundra and woodlands (Lawrence 1970). Much of the research on seed set and quality in Apiaceae has been focused on wild sources of economically-important crop species (Hendrix 1984; Hendrix and Sun 1989; Koul *et al.* 1986; Robinson 1954), with a number of studies examining plant and insect interactions (Berenbaum 1990; Lindsey 1984; Niemirski and Zych 2011; Zych 2007). Webb (1981) lists several broad features of Apiaceae floral phenology: (1) andromonoecism, (2) highest proportions of co-sexual flowers occur in outer umbels and in primary umbels, (3) anthesis occurs centripetally within umbels, and (4) self-compatibility. Most species are protandrous, however some North American Apiaceae are protogynous (Schlessman and Graceffa 2002; Webb 1984). Most Apiaceae are characterised by simple or compound umbels comprising co-sexual flowers. Despite the relative uniformity of floral morphology, a number of different breeding systems have been documented for the family (Keighery 1982; Koul *et al.* 1986; Koul *et al.* 1993; Lindsey and Bell 1985; Webb 1981). Many Apiaceae are thought to be xenogamous and/or geitonogamous, particularly for those which have numerous, conspicuous pseudanthia within an inflorescence (Keighery 1982). Most species also have a generalist pollination system (Proctor *et al.* 1996), although some specialist behaviour has been documented (Zych 2007). In addition, because of the often complex, structured inflorescence morphology, and the temporal expression of gender, geitonogamy is likely in many Apiaceae.

The occurrence of geitonogamy in Apiaceae can be comparable with xenogamous pollination rates (Gaudeul and Till-Bottraud 2003; Marcinko and Randall 2008). Geitonogamous pollination often occurs in hermaphroditic species, and if the species is also self-incompatible, there is no fitness benefit of pollen remaining within the plant (de Jong *et al.* 1993). Several studies have estimated the consequences for reproductive success using

pollen from the same plant. For example, artificial geitonogamous pollination resulted in twice as many aborted fruits than in the naturally-pollinated flowers of the milkweed, *Asclepias speciosa* Torr. (Finer and Morgan 2003). Karron *et al*. (1995) noted that geitonogamous pollination was more likely to occur in low density populations of *Mimulus ringens* L.. Seed set in *Ipomopsis aggregata* Pursh. was 42% lower when self pollen was combined with pollen from a xenogamous source than just the latter alone (Waser and Price 1991). Therefore, it is important for studies that evaluate the reproductive ecology of species with co-sexual flowers to include the effects of geitonogamy on seed set, as well as the possibility of inbreeding depression in future generations.

Actinotus comprises 20 species – 19 in Australia and 1 in New Zealand. Species are perennial (either sub-shrubs or herbs) or annual herbs (some of which are fire-ephemerals). Keighery (1982) considered five species of Western Australian *Actinotus* to be out-crossing, but did not describe their floral phenology. Relatively little is known about the breeding systems of the other 14 Australian *Actinotus*. An exception is *A. helianthi*, which has been used by the Australian horticulture industry as a cut flower crop and potted plant. A recent study by Emery *et al.* (2011) confirmed that germination success differs substantially among populations even when a germination stimulant (smoked water) was used. However, the degree to which different pollen sources have an effect on seed set and germination of *A. helianthi* remains unknown. The aims of our study are three-fold: firstly, to document the floral phenology for *A. helianthi* to determine the potential for geitonogamous pollination; secondly, to investigate whether the source of pollen affects seed set and germination in *A. helianthi*; and finally, to determine how seed set and germination varies between populations. These studies are important for understanding potential interactions between plants and pollinators and the effects on the reproductive system. If our manipulations illustrate that geitonogamy is inevitable (due to the phenology), this then has important implications for *ex*

situ propagation. It would then be recommended for secondary umbels to be removed from plants grown for seed production. If there are no differences in seed germination, then we suggest that the species is used to selfing and plants can still be grown for seed production with few deleterious effects of inbreeding. Furthermore, the results from this study will contribute towards our understanding of the plant attributes that might determine persistence and geographic distribution of this species.

Materials and methods

Study species

Actinotus helianthi is a geographically widespread sub-shrub that grows to 2 m tall, and occurs in dry sclerophyll woodland and heath vegetation along the east coast of Australia (Fig. 1). Plants grow in oligotrophic soils from Ulladulla on the New South Wales (NSW) south coast to Salvator Rosa National Park in central Queensland (QLD). The species is characterised by umbels comprising a central aggregation of co-sexual flowers surrounded by morphologically unisexual male flowers. Each umbel is subtended by several large, white, petaloid involucral bracts (Webb 1980; Lee 1995). The paniculate inflorescence is terminated by a primary umbel with each of several higher order branches also terminated by an umbel (Fig. 2). Flowering commences in the primary umbel in September (early spring) and progresses sequentially to the cessation of tertiary flowering in March. The morphology of co-sexual flowers is typical for the family, comprising an androecium of five stamens and a gynoecium of two styles. However, the corolla is reduced to five, trichome-like structures, and the ovary comprises a single locule with one functional ovule. Germination significantly increases when the seeds are exposed to smoke, and following a fire, thousands of seeds germinate and form a dense understorey (Emery and Lacey 2010).

Fig. 1. *Actinotus helianthi* population along the edge of a walking track at Manly Dam in September, 2010. Photo by N. Emery.

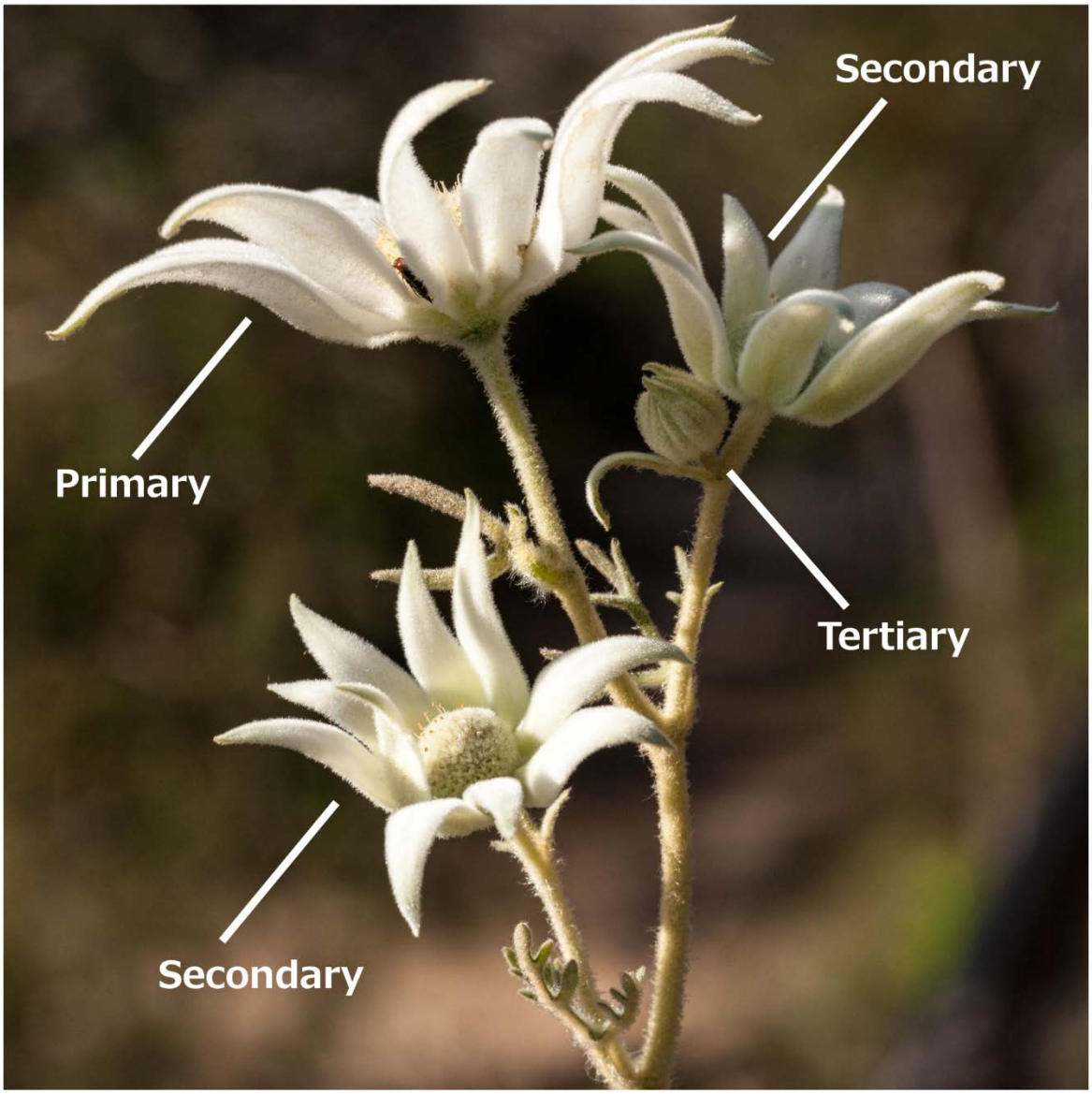

Fig. 2. A typical inflorescence of *Actinotus helianthi* from Manly Dam. A primary umbel is subtended by multiple secondary branches, each of which can carry one or more tertiary branches. All branches are terminated by an umbel. Photo by N. Emery.

Study sites

A natural (field-based) population and a cultivated population were studied during the period of flowering of the primary and secondary umbels in October and November, 2011. The cultivated population was established from fruit collected from the corner of Putty Road in Yengo National Park, where *A. helianthi* was the dominant under-storey species in open sclerophyll woodland, 330 m above sea level (32° 58’ 9” S, 150° 41’ 5” E). Seed was collected from this population in January, 2008 and stored at the Australian PlantBank since. 54 plants were then grown from this seed source in pots at the nursery at The Australian Botanic Garden, Mount Annan in the Greater Western Sydney region of NSW (32° 58’ 50” S, 150° 41’ 11” E, hereafter referred to as Mt Annan). The plants were watered on a daily basis via automatic sprinklers. The second, natural population was located on the north-east section of Manly-Warringah War Memorial Park (33° 46’ 03” S, 151° 14’ 50” E; hereafter referred to as Manly Dam). The vegetation at Manly Dam was predominately open heath, 125 m above sea level. The Manly Dam population comprised about 200 plants over an area of approximately 250 m^2. Both populations experience different climatic conditions. Mean monthly temperatures range from 17.3 °C to 29.5 °C (43 year average) at Mt Annan, and 16 °C to 26.5 °C (10 year average) at Manly Dam. Annual rainfall in 2011 was 757 mm (61-year average: 788 mm) and 1,647 mm (56-year average: 1331 mm) at Mt Annan and Manly Dam, respectively (Bureau of Meteorology 2014).

Floral phenology

Stages of floral phenology were initially identified by monitoring an inflorescence on a single plant from Mt Annan. Tagged umbels were examined daily over a 38 day period to determine the relative timing of anther dehiscence and stigma receptivity. Male phase was judged to commence when the first anther began to dehisce. The onset of stigmatic receptivity was recognised by the presence of a glistening tip on each of the two, fully erect styles. Stigmatic

receptivity was considered to have ceased when the styles incurved, and the stigmas dried and discoloured. Fruit development was assumed to have initiated once the stigmas were unreceptive, and to have completed when fruits were brown.

The number and the proportion of co-sexual and staminate flowers per umbel were recorded from two primary, two secondary and two tertiary umbels haphazardly selected from each of five plants at the Manly Dam population (a total of 30 umbels: ten umbels per order). The number of viable and non-viable pollen grains per anther was estimated by mounting 24 unopened anthers in a drop of glycerine. A pollen grain was recorded as viable if cytoplasm filled the grain when viewed with an Olympus CH-2 microscope at 100× magnification. Anthers of four co-sexual and four unisexual male-phase flowers were sampled from two primary, two secondary and two tertiary umbels haphazardly selected from the Manly Dam population.

Breeding system

At each population, a total of 40 umbels were selected across 30 plants were haphazardly tagged and randomly assigned to one of four pollination treatments to give ten replicate umbels per treatment. Replicates were subsequently adjusted to $n = 8$ per population because of damage to experimental inflorescences. Umbels were bagged prior to pollination for the first three treatments (below):

1- Intra-umbel geitonogamy: stigmas of hermaphrodite flowers in female phase were brushed 10 times with 5-10 excised unisexual male flowers from the same umbel. Umbels were re-bagged after treatment;

2- Geitonogamy: when a primary umbel had commenced the female phase, a secondary male-phase umbel with pollen present from the same plant was excised and brushed over the stigmas 10 times. Umbels were re-bagged after treatment;

3- Xenogamy: when an umbel had entered female phase, a male-phase umbel from another plant at least 5 m away was excised and brushed over the stigmas 10 times. Umbels were re-bagged after treatment;

4- Open control: umbels were tagged and left open to pollinators for the duration of stigma receptivity. Umbels were bagged when stigmas were no longer receptive.

Mature fruit were collected and stored in a controlled environment dry room (16 °C; 16% rH) for two weeks so that all fruit would equilibrate to similar moisture content. For each pollination treatment, seed set per umbel was determined as the proportion of seeds containing endosperm. Seed germination and viability were assessed by placing fruit containing mature seeds on water agar petri dishes made by heating 7 g of powdered agar per 1 L of deionised water to 200 °C for 10 minutes. The plates were sealed with plastic wrap and transferred to a germination incubator at a constant 15 °C with a 12 h light and 12 h dark regime. These conditions have been previously reported to induce the germination of *A. helianthi* (Emery *et al*. 2011). Smoke-water was not used in this study since we wanted to mimic germination conditions in the absence of fire to avoid having additional pre-germination treatments that might inhibit our ability to elucidate the impact of pollen source. This is a reasonable expectation as *A. helianthi* can persist in the landscape without being dependent on fire to stimulate germination. Agar plates were monitored for seed germination every 2-3 days for a 60 d period. Germination was considered to have commenced when a radicle protruded at least 2 mm from a seed. A cut-test was performed on non-germinated seeds to identify the presence of an embryo. Final seed germination percentages were subsequently adjusted from the viability results.

Data analysis

Differences between umbel orders in the number of flowers, the number of pollen grains per anther, and the proportion of male-only to co-sexual flowers were tested using univariate general linear models (GLMs). Bonferroni post-hoc tests were performed where appropriate. Mean seed set for each pollination treatment was calculated as the seed set proportion multiplied by the viability. Mean adjusted germination was then calculated by the effective seed set multiplied by the germination proportion adjusted for viability. The within-umbel treatment was excluded from statistical analyses (only one fruit germinated) to avoid unbalanced data. Mean seed set was modelled as a response to pollination treatment (fixed) and population (fixed) using a univariate GLM. Mean germination was also analysed using a similar model. Differences were considered significant where $P \leq 0.05$. All analyses were performed in SPSS Statistics 21 (IBM, New York).

Results

Floral phenology

Morphologically co-sexual flowers of *A. helianthi* are protandrous. In co-sexual flowers the onset of the male phase of individual flowers within an umbel was spread over two days, and was synchronous with anther dehiscence in the peripheral morphologically male flowers within umbels. The sex phases of co-sexual flowers were separated by a quiescent (non-sexual) phase of 2 to 3 days, during which styles were raised centripetally in the umbel. Umbels within an inflorescence overlapped in male and female sex phases to some degree. Flowering began in primary umbels with anther dehiscence occurring first in the central co-sexual flowers and progressing centrifugally towards the peripheral morphologically-male flowers. This was followed by stigma receptivity in the co-sexual flowers. Pollen was not apparent on the dehisced anthers of the flowers during the female phase. Primary and secondary umbels of the same inflorescence overlapped in anthesis. Male phase commenced

in secondary umbels when primary umbels had commenced their short quiescent phase (prior to the onset of stigmatic receptivity). The pollen presentation in secondary umbels overlapped with at least half of the duration of stigmatic receptivity phase in the primary umbel (Fig. 3; Fig. 4). Male phase in tertiary umbels commenced when stigmatic receptivity had ceased in secondary umbels. Tertiary umbels had a longer male phase (9 days) than secondary and primary umbels (approximately six and seven days, respectively; Fig. 4).

Staminal filaments of co-sexual flowers elongated and straightened, lifting the anthers above the incurved styles before the involucral bracts have fully recurved. Anther dehiscence occurred 24 hours after the staminal filaments reached maximum elongation. Anther dehiscence in peripheral, morphologically male flowers commenced 72 hours after the onset of anther dehiscence in the central, co-sexual flowers. Nectar was visible on two black nectaries during anther dehiscence. The male phase of each umbel lasted between four and nine days. The male phase lasted for 1 to 2 days before filaments and anthers began to senesce, which signaled the start of the quiescent phase. Following this short phase, stigmatic receptivity then commenced centripetally in co-sexual flowers. The two nectaries remained raised in each co-sexual flower, and styles were fully erect by 2 to 3 days. At this time, the terminal stigmas were glossy and appeared to be wet. After 3 to 5 days, the stigmas dried and discoloured, and the tips of the styles incurved. The involucral bracts then started to close over the umbel. Fruit matured on the umbels for 14 to 17 days before the involucral bracts were shed (Fig. 4).

The number of flowers (co-sexual and unisexual male) in each umbel was significantly different between umbel orders ($F_{2,27} = 10.192$; $P = 0.001$). Primary umbels comprised 263 ± 11 (mean ± SE) flowers (Table 1). The number of flowers in primary umbels was statistically similar to secondary umbels (247 ± 15 flowers). The number of

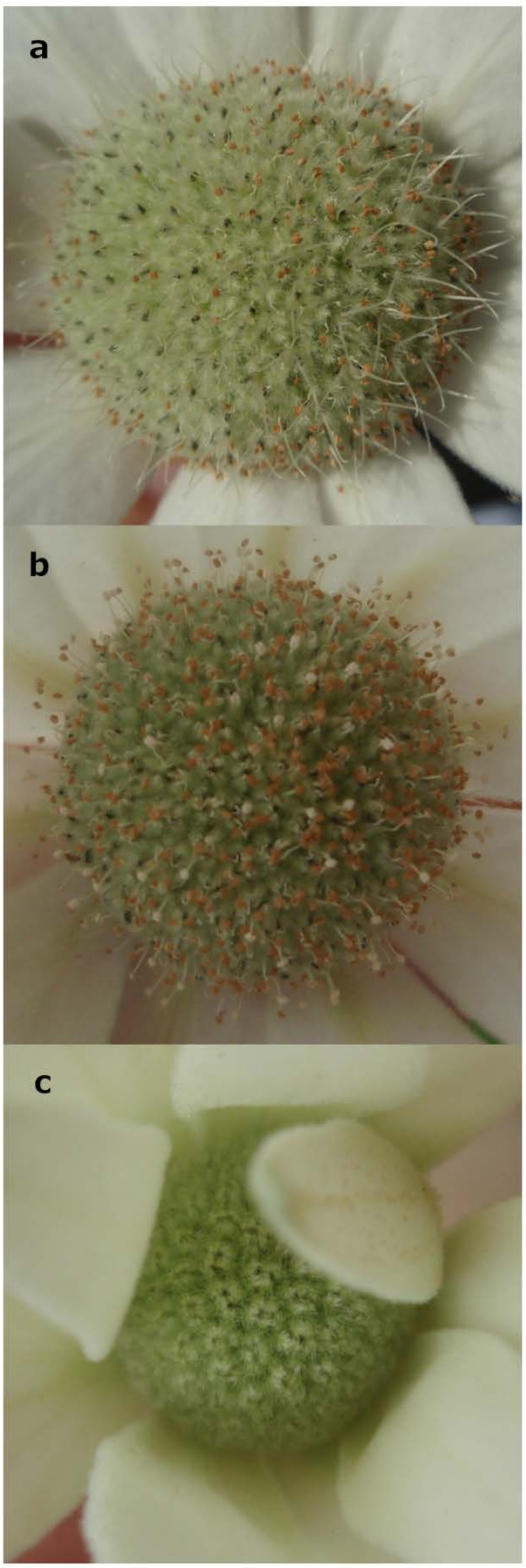

Fig. 3. Umbels of an *Actinotus helianthi* inflorescence twelve days from the start of the observation period. Primary umbel (a) was in female stage with erect styles, while the secondary umbel (b) was in male phase with pollen present in anthers, and the tertiary umbel was in bud (c). Photos by N. Emery.

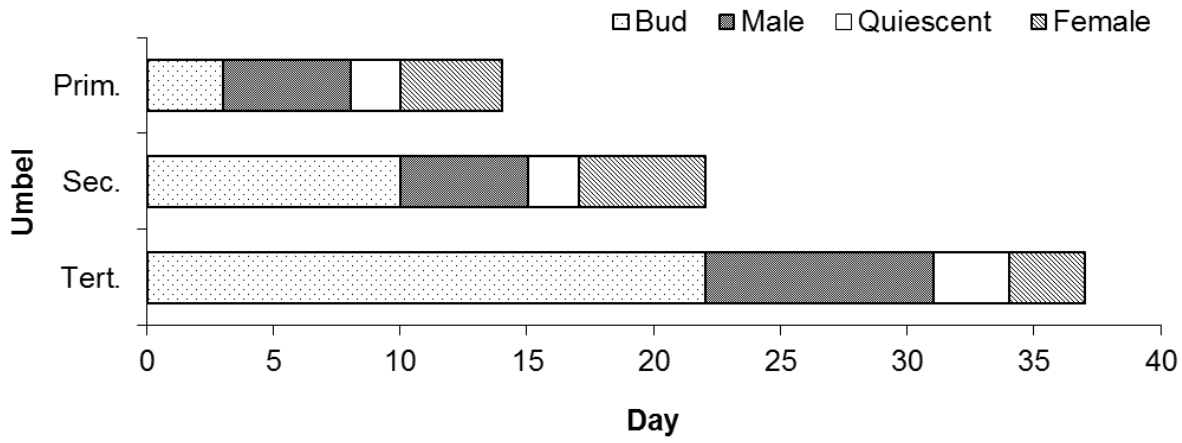

Fig. 4. Floral phenology of single umbels of an inflorescence on a plant from the Mt Annan population. Primary umbel (Prim.), secondary umbel (Sec.) and tertiary umbel (Tert.).

Table 1. Mean morphological differences among different umbel orders and flower types in *Actinotus helianthi*. Pollen grains were counted from $n = 4$ anthers for each anther-umbel combination. Flowers were counted using $n = 10$ per umbel order.

Umbel	*Flower morphology*	*Mean Viable pollen grains per anther*	*Mean Number of flowers*	*Proportion of total flowers per umbel*
Primary	Unisexual male	248 ± 22	149 ± 9	0.57 ± 0.04
	Co-sexual	292 ± 12	114 ± 12	0.43 ± 0.04
	All	**270 ± 14**	**263 ± 11**	**1**
Secondary	Unisexual male	189 ± 18	158 ± 9	0.64 ± 0.02
	Co-sexual	295 ± 8	90 ± 8	0.36 ± 0.02
	All	**242 ± 19**	**248 ± 15**	**1**
Tertiary	Unisexual male	233 ± 9	149 ± 11	0.80 ± 0.03
	Co-sexual	151 ± 20	37 ± 5	0.20 ± 0.03
	All	**192 ± 19**	**186 ± 12**	**1**

flowers in tertiary umbels (186 ± 12) was significantly different from primary ($P = 0.001$) and secondary ($P = 0.006$) umbels (Table 1). The proportion of unisexual male flowers was lowest in primary umbels (0.57 ± 0.04) and increased throughout the higher orders. In secondary and tertiary umbels, the proportion of unisexual male flowers to co-sexual flowers was 0.64 ± 0.02 and 0.80 ± 0.03, respectively (Table 1). The proportion of unisexual male flowers was significantly different between umbel orders ($F_{2,27} = 14.032$; $P < 0.001$). Bonferroni post-hoc tests showed a significant difference between primary and tertiary umbels ($P < 0.001$), but not primary and secondary umbels ($P = 0.129$).

The number of pollen grains per anther varied significantly among umbel orders and flower types ($F_{2,22} = 18.351$; $P < 0.001$). Flowers of primary umbels contained more pollen grains per anther than flowers of either secondary or tertiary umbels (Table 1). Bonferroni post-hoc tests revealed tertiary umbels to be significantly different to primary ($P < 0.001$) and secondary ($P = 0.012$) umbels. The number of pollen grains in anthers of unisexual male flowers was higher in primary umbels (292 ± 12) than unisexual flowers of secondary (189 ± 18) or tertiary umbels (233 ± 9; Table 1). There was no significant difference in pollen grains per anther of unisexual flowers between umbel orders ($F_{2,11} = 3.124$; $P = 0.084$). By contrast, the number of pollen grains in anthers of co-sexual flowers was significantly different between umbel orders ($F_{2,11} = 36.207$; $P < 0.001$). Post-hoc tests showed co-sexual flowers in tertiary umbels have significantly fewer pollen grains (151 ± 20; $P < 0.001$) than co-sexual flowers of primary and secondary umbels (292 ± 12 and 295 ± 8, respectively; Table 1). Pollen was not presented in the anthers of primary co-sexual flowers or morphologically male flowers when the former were in female phase.

Breeding system

Across the four pollination treatments, the number of fruit per umbel ranged from 31 to 120 (n = 32) and 34 to 245 (n = 32) for Manly Dam and Mt Annan, respectively. Flowers that received the intra-umbel geitonogamy pollination treatment produced few mature fruit in both populations (Fig. 5). No interaction between population and pollination treatment was detected ($F_{2,42}$ = 0.155; P = 0.86). Mean seed set for the remaining three pollination treatments in the Mt Annan population was consistently low, ranging from 26 ± 6 % of geitonogamously-pollinated fruit (within plant) to 38 ± 6 % of open (control) pollinated flowers (Fig. 5). In contrast, mean seed set of the Manly Dam population was significantly higher (P < 0.001), ranging from 65 ± 7 % for fruit produced with pollen from a different plant to 71 ± 6 % for fruit open pollinated flowers (Fig. 5). Seed viability ranged between 60 % and 72.5 % across the pollination treatments. Despite the highest proportion of viable fruit being produced when flowers were open pollinated, the difference was not significant (P = 0.32).

The open pollination (control) treatment had the highest mean germination in both populations (32 ± 5 % in Mt Annan and 24 ± 3% in Manly Dam). Mean germination for the xenogamous pollinations (treatment 3) and geitonogamous pollinations (treatment 2) were 24 ± 7 % and 19 ± 5 % in the Mt Annan population, and 22 ± 4 % and 16 ± 4 % respectively in the Manly Dam population (Fig. 5). There was no significant interaction in mean germination between population and pollination treatment ($F_{2,42}$ = 0.193; P = 0.82). Neither population nor pollination treatments were significantly different (P = 0.31 and P = 0.82, respectively).

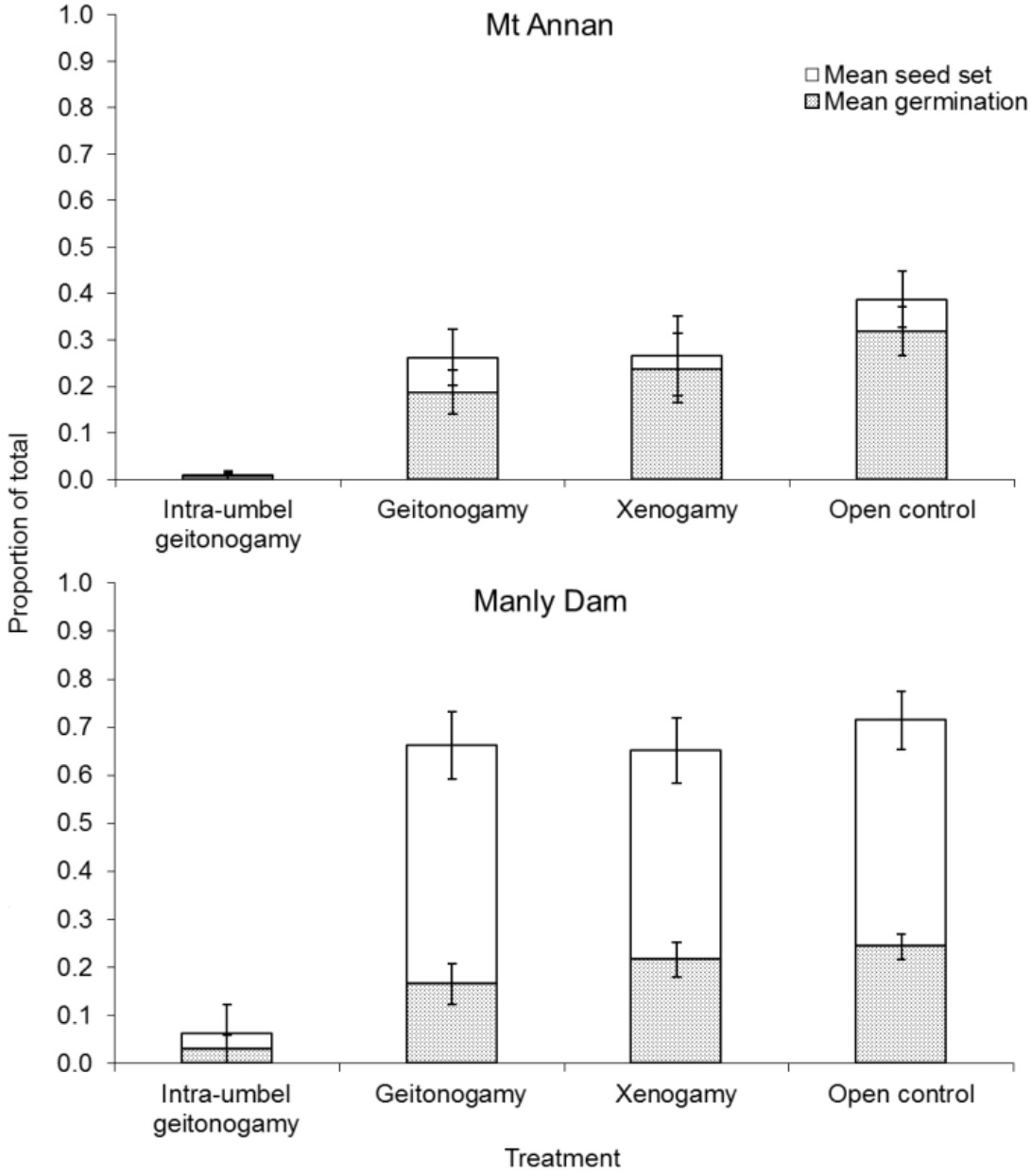

Fig. 5. Mean seed set for each pollination treatment (n = 8) is represented as the clustered dark and open bars. Mean germination was calculated as the proportion of seeds from the mean seed set that germinated and adjusted for viability, represented by the grey bars. Mean seed set was calculated as percentage seed set multiplied by the viability percentage, represented by the white bars.

Discussion

The breeding system of *Actinotus helianthi* comprises xenogamy and geitonogamy (i.e. different umbels on the same plant), the latter being due to the species temporal flowering structure. Intra-umbel geitonogamy produces little (if any) viable fruit. Our results indicate that propagation difficulties might not be caused by the reproductive system of *A. helianthi*, but rather how seeds are treated *ex situ* that determines the proportion of successfully germinating seeds. Co-sexual and unisexual flowers of each order of umbel present pollen simultaneously, and the presentation of pollen within an umbel concluded prior to the stigmas of co-sexual flowers becoming receptive. Thus, individual co-sexual flowers of each umbel of *Actinotus helianthi* are protandrous, thereby reducing the potential for pollen transfer within co-sexual flowers and individual umbels (Webb 1981). Our results demonstrate the potential for geitonogamous pollination to occur between separate umbels on the same plant is relaxed between successively higher orders of umbels, and flowering is not synchronous among same order umbels or inflorescences (Fig. 3). Thus, co-sexual flowers in primary umbels of plants with at least one inflorescence have a higher probability of being pollinated by male-phase flowers from a secondary umbel. A similar result was recorded in *Daucus carota* plants (Koul *et al*. 1993).

These results suggest that a mixed-mating strategy could be possible in *A. helianthi*. While autogamy may not occur, the capacity for geitonogamy provides insurance against low pollinator diversity and/or pollinators that move small distances (Goodwillie *et al.* 2005; Kalisz *et al.* 2004; Vogler and Kalisz 2001). Furthermore, nectar was available during both sex phases of co-sexual flowers as well as in unisexual male flowers, a feature exhibited in other Apiaceae, (e.g. *Angelica sylvestris* L.; Stpiczyńska *et al.* 2014). Langenberger and Davis (2002) reported the volume of nectar secretion to be around 5.7 times higher when co-

sexual flowers were in female phase than when in male phase. Whether nectar secretion influences pollinator behaviour in *A. helianthi* requires further investigation.

Examining the breeding system by manipulating the source of pollen provided a detailed understanding of the reproductive system of *A. helianthi*. While there could also be a genetic, maternal mechanism that prevents autogamy within an individual co-sexual flower (i.e. partial self-incompatibility in the sense of Gaudeul and Till-Bottraud 2003), this was not examined in the current study. Autogamy can have negative impacts on within-population genetic variation, which can adversely affect the fecundity and survival of individuals (Charlesworth and Charlesworth 1995). Thus, these negative impacts may outweigh any positive potential genetic advantage of selfing when xenogamy is less likely to occur (Gaudeul and Till-Bottraud 2003). However, as the co-sexual flowers of *A. helianthi* can be geitonogamously pollinated, this might negate the need for autogamy. It should be noted that we cannot specifically comment on the potential rates of geitonogamy *in situ* without examining the genetic diversity of the viable seeds produced (de Jong *et al*. 1993; Gaudeul and Till-Bottraud 2003). Interestingly, while viable seed production and germination did not vary among three of the pollination treatments (excluding the within-umbel geitonogamy), overall seed set was significantly higher at the Manly Dam population (Fig. 5). These results may explain the variation in germination success among populations that has been reported previously for *A. helianthi* (Offord and Tyler 1993) and for other angiosperms (Ågren 1996; Menges 1991). This may also be linked to the interaction between the attractiveness of flowers to pollinators, pollinator availability and pollinator diversity, and population size (Kunin 1997; Mustajärvi *et al.* 2001). These factors must be considered to determine the cause of variability when propagating *A. helianthi*.

Our results indicate that intra-umbel geitonogamy is not possible in the co-sexual flowers of *A. helianthi*. This means that pollen must come from another umbel from either the same or a different inflorescence for seed set to occur. Our results agree with the observations made by Keighery (1982), who observed five West Australian *Actinotus* species were xenogamously pollinated. Much of the potential for reproductive success occurs in primary umbels where pollen counts, and, therefore, the pollen ovule ratio (Cruden 1977), as well as the proportion of co-sexual to unisexual male flowers are at their highest. The low pollen count of the unisexual male flowers compared to the co-sexual flowers might be a strategy to encourage pollinators to forage on the latter (Manicacci and Despres 2001; Stanton *et al.* 1986; Willson and Rathcke 1974). Comparatively, the increase in the number of pollen grains in tertiary unisexual male flowers may be to increase the opportunity for achieving pollen export and thus fitness via male function. It is also noteworthy that pollen abundance can be influenced by environmental conditions, such as nutrient availability and herbivory (Cruden 2000; Young and Stanton 1990), which may further result in variation in the production of viable seeds between populations.

The production of viable seed from three of the four pollination treatments demonstrated the tendency for a generalist breeding system of *A. helianthi*. Moreover, seed set from geitonogamous (treatment 1 and 2) and xenogamous pollination (treatment 3), was comparable with natural (open) pollination (treatment 4). Autogamy in *A. helianthi* is prevented by the temporal separation of male- and female-phases within co-sexual flowers, and other examples of low autogamy have been reported in other Apiales. For example, seed set in the long-lived *Eryngium alpinum* L. was significantly higher in xenogamous pollen treatments, (18 % to 61 %) than in geitenogamous and intra-umbel geitonogamous treatments, (8 % to 38 %; Gaudeul and Till-Bottraud 2003). The proportion of viable seed set as a result of intra-umbel geitonogamous pollination peaked around 2 % for *D. carota* subsp.

sativus Hoffm. Thell., compared to a range of 35 to 68 % for open (control) pollination (Koul *et al.* 1989). In *Trachymene incisa* subsp. *incisa* Maiden & Betche, seed set was also significantly lower in autogamous (ca. 15%) compared to out-crossed (ca. 45%) treatments (Davila and Wardle 2002).

Keighery (1982) recorded six Western Australian *Actinotus* species to be protandrous and self-compatible, with five of the six species being predominately pollinated by native bees. The pollinators of *A. helianthi* are currently unknown, although bees, beetles and ants were regularly observed visiting umbels. Empirical studies into the pollinators of *A. helianthi* and other *Actinotus* would complement our results and help to elucidate the likelihood for geitonogamy to occur naturally *in situ*.

No pollen was available in the anthers of any co-sexual flowers when a primary umbel was in female phase. Furthermore, anthers from male-only flowers had dehisced pollen that was not available for transfer to stigmas This explains why seed set in *A. helianthi* was low following intra-umbel geitenogamous treatment. It is possible that the few seeds that developed as a result of this treatment were due to pollen contamination during the manipulative pollination treatments.

With the exception of intra-umbel geitonogamy, the effective germination percentage was consistent among pollination treatments and populations. The proportion of viable seeds that germinated, however, varied significantly between populations. *Actinotus helianthi* recruitment is higher following fire and, like many Australian angiosperms, germination success often increases when seeds are artificially exposed to smoke (Nelson *et al.* 2012). Furthermore, the population at Manly Dam and the source population of Mt Annan from Yengo National Park are known to be smoke-responsive (unpublished data). The lack of

variation in percent germination observed in this study is consistent with the germination of seed produced by artificial autogamy and geitonogamy pollination of *T. incisa* subsp. *incisa* (Davila and Wardle 2002). The difference in the proportion of viable non-germinated seeds between the two populations suggests that the degree of seed dormancy may be an important factor for propagating *A. helianthi*. As seed dormancy is, in part, influenced by the maternal environment as opposed to source of pollen (Roach and Wulff 1987), similar levels of germination would be expected across the different treatments.

The seeds of *A. helianthi* are morphophysiologically dormant (Lee 1995), and have two dormancy mechanisms. The first is a morphological dormancy that requires the growth of the immature embryo in order to be broken. Following the development of the embryo is the second physiological dormancy, which requires appropriate external environmental conditions in order to be relaxed (Baskin and Baskin 1998). Since the level of dormancy can vary among populations, so too can the period required for after-ripening for relaxing the physiological component (Andersson and Milberg 1998). The number of seeds germinated from Manly Dam might have increased if the seeds were stored for longer before being subjected to germination conditions. Therefore, we suggest that number of viable seeds is a sufficient measure of reproductive success, and successfully captures the variation among populations.

Actinotus helianthi provides an ideal model for examining how the mode of pollination affects seed set and germination. This may be particularly important for plants with geographically widespread distributions where pollinators might occur across all locations. We have demonstrated *A. helianthi* to have no capacity for autogamy. The sex-phase overlap within and between inflorescences of an individual, coupled with the statistically similar seed set between two pollination treatments indicate that this species can

set seed by geitonogamous pollination (i.e. from another umbel on the same plant). In addition, our study indicates that pollen source does not adversely affect seed set in *A. helianthi*. However, the level of seed set does vary between populations. The results presented here provide the first evidence of the reproductive system of *A. helianthi*. In addition, we have provided further support for the need to account for variability between populations when considering how a species might respond under future environmental conditions.

Acknowledgements

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Chapter IV

RIGHT HERE, RIGHT NOW: CURRENT POPULATIONS DIFFER IN THEIR EARLY PERFORMANCE TRAITS AND SPECIES INTERACTIONS

Abstract

Variation in local environments helps to drive the dynamic response of populations over space and time, and therefore, to shape the distribution and abundance of the species. Population differences in early performance traits are also influenced by ecological interactions with insect visitors, yet few studies combine the two. We use experiments and field observations to explore how population differences might co-vary with geographic location. In a common garden experiment we quantified the early life-history components of fitness across 17 populations of the understorey sub-shrub, *Actinotus helianthi* Labill.. Across several geographically disjunct populations, variation in localized species interactions were examined in the field by quantifying the diversity of insect visitors to *A. helianthi* and the impact of visitation rates on seed set (a proxy for reproductive success). We found populations to vary in germination success between 0.2 ± 0.1 % to 64.2 ± 2.3 %. Seedling growth and early survival varied between populations by as much as two and 44 orders of magnitude, respectively. Specifically, we identified that variation existed at multiple levels from the maternal plant to biogeographic regions. The abundance and diversity of insect visitors also varied among populations and seed set was found to be site specific. There was a trend for populations with taller plants and larger floral display sizes to be more frequently visited by pollinators. We also identified a positive linear relationship between the intensity of visits by flies and seed set success. Our results indicate that *A. helianthi* populations exhibit local adaptation across the studied environments. This could have negative

consequences for populations that migrate to new suitable habitats under changing environments.

Introduction

As environments change, organisms must evolve new characteristics and broader tolerances, or shift into more suitable habitats. These differential responses to changing environments by populations will be, in part, dependent on the existing level of local adaptation of the plants within. Local adaptation is a well-documented phenomenon in plant populations (Ågren and Schemske 2012; Bennington *et al.* 2012). However, in the context of shifting distributions of the species, a fixed match of phenotype to the local conditions within populations would likely hinder migration to different environments (Pigliucci and Schlichting 1996; Via and Lande 1985). Plants can express different phenotypes among populations in different environments or across climatic gradients (Byars *et al.* 2007; Qaderi and Cavers 2002) but their potential or capacity to alter this response also varies, and may itself be influenced by the local environment (Breza *et al.* 2012).

It has been recently questioned whether populations have a greater capacity to survive in their local environment compared to populations from other environments (Hancock and Hughes 2014; Hancock *et al.* 2013). In order to quantify any local vs. non-local advantage, plants traits are often recorded and related to survival as a measure of plant performance. There are specific plant traits that are likely to be more effective at demonstrating the importance of local adaptation. Often, differences in morphological traits such as plant height, leaf number and flower number within populations are reported (Byars *et al.* 2007; Galloway and Fenster 2000; Stewart Jr. and Nilsen 1995). However, there are other plant traits, such as those expressed in early development which might be more sensitive to environmental change.

Traits of individuals within a species have also only been recently examined in the context of species distributions (Hanspach *et al.* 2010; Pollock *et al.* 2012; Thuiller *et al.* 2004b). However, most of these traits are not reported at a population level, but rather an aggregated species level. Flowering, fruiting, germination and young seedlings are the most sensitive stages for growth and reproductive success (forming a plant's 'reproductive niche') and likely to affect individual plants and their distribution if they are compromised by environmental processes (Billings 1952). While all plants have some level of tolerance for dynamically-changing environmental conditions they are exposed to, determining the extent of this tolerance would provide a more detailed representation of the survivorship of species across distributions. Therefore, working at the population level provides a unique opportunity to understand the factors that can limit individual plant survival, and, in turn, the species distribution.

Early performance traits

Early performance traits, such as dormancy, for example, are likely to be under strong selection as plants must be able to express a viable phenotype for juvenile survival before expressing an adaptive adult phenotype (Huang *et al.* 2010). A dormant seed is the earliest stage at which adaptation to the local environment can occur (Huang *et al.* 2010), with the degree of dormancy determined by the maternal genotype and influenced in part by the maternal environment (Baskin and Baskin 1998; Roach and Wulff 1987). Furthermore, since dormancy is also shaped by local environmental conditions, measures of germination and early survival in populations provide sound data on species level responses under changing environments. Therefore, the challenge is to determine the degree of adaptation to local environmental conditions during such early developmental stages. There are several methods

to quantify local adaptation with common garden and reciprocal transplant experiments being most frequently used.

Reciprocal transplant experiments that compare phenotypes in the 'home' environment with an 'away' environment provide the strongest test for local adaptation (Ågren and Schemske 2012), but present methodological and ethical difficulties. For example, Petrů and Tielbörger (2008) point out that a number of plant community variables, such as soil resource availability and degree of biotic interactions, are likely to differ between transplant sites. Common garden experiments, on the other hand, are used to assess the relative contribution of genetic and environmental influences on intraspecific variation by comparing phenotypes of individuals from different populations, when grown together in the same environment (Clausen *et al.* 1940; Colautti *et al.* 2009). Phenotypic differences might reflect either environmental gradients or responses to different ecological habitats. For example, differences in time of emergence, time to flowering and reproductive output (number of flowers and fruit) were observed among and within five populations of *Campanula americana* from a wide latitudinal gradient when grown in a common glasshouse environment (Kalisz and Wardle 1994). Such differences expressed in the common environment provide appropriate means to report evidence for local adaptation (Colautti *et al.* 2009) and broad sense genetic variation (Kalisz and Wardle 1994). Linking variation in early life-history traits with local environments will be important to elucidate how a species' populations might respond to future conditions.

Species interactions

Species range shifts give way to changes in the timing of flowering phenology (Chambers and Keatley 2010; Fitter and Fitter 2002). The wide variety of breeding systems and plant mating strategies (Barrett and Harder 1996; Charlesworth 2006; Lloyd 1980) coupled with

the need for biotic pollen vectors (Willmer 2011), add to the variability exhibited in early life-history traits (Ågren 1996; Olsson and Ågren 2002). These changes can then affect the functioning of a species depending on whether any interactions with other species are subsequently influenced. Therefore, species interactions underly differences in plant performance and are expected to vary across populations.

Species interactions are also important in predicting population persistence (Heikkinen *et al.* 2007; Van der Putten *et al.* 2010). Pollination, in particular, is sensitive to habitat changes (Aguilar *et al.* 2006; Vergeer *et al.* 2003) and is expected to vary prominently between populations of a species due to isolation and changes in the abundance of plants (Kunin 1997; Mustajärvi *et al.* 2001). This is also pertinent as biotically pollinated plants cannot extend their range beyond their pollinators (Van der Putten *et al.* 2010). Insect pollinators are also not evenly distributed across environmental gradients. For example, Devoto *et al.* (2005) examined pollinator diversity across a rainfall gradient in Argentina, noting that bees dominate flower visits over flies in dryer areas (43% and 28%, respectively), whereas the converse was true for wetter areas (25% bees and 43% flies). Thus, studies which examine the effects of pollinator diversity on seed set across multiple populations are useful for estimating persistence of populations across the landscape.

In this study, to better understand the factors that influence species distributions population differences in plant traits were examined in two experiments. Firstly, we used a common garden environment to address two main questions:

1. Are seeds from the same plant (i.e. collected from different umbels on the same plant) more variable in terms of germination, seedling growth and survival than among populations across the geographic distribution?

2. Do populations from different bioregions exhibit more pronounced differences in germination success and early survival than nearby populations?

Secondly, the role of insect visits in influencing seed set among plant populations was compared within and between populations. Several traits were measured that can influence pollinator visits, including plant abundance, plant height and floral display size to address the following:

3. Does insect diversity vary between populations and is this variation also reflected in overall seed set?
4. Do plant traits within populations influence visits, and are there specific insect groups which are associated with higher seed set?

This study will contribute mechanistically to more accurate models for predicting species distributions by determining how individuals within populations vary in early life-history fitness traits, as well as the interactions which influence such traits. Practical outcomes will include documenting how patterns in germination, growth and survival of plants among populations are influenced by the environment. Identifying species interactions will also inform decisions about how best to manage and conserve native plant populations in the face of changing climates.

Materials and methods

Study species

Actinotus helianthi Labill. (flannel flower) is a common sub-shrub of sclerophyll vegetation in eastern Australia. Plants grow up to two metres tall in nutrient-poor sandstone soil from Ulladulla on the New South Wales (NSW) south coast to Salvator Rosa National Park in central Queensland (QLD) and in the Pilliga region of central-west NSW. Flowers are aggregated in umbels surrounded by whitish-cream bracts covered in dense velvet-like hairs. Umbels are andromonoecious with peripheral staminate flowers and inflorescences are

paniculate (order three) with the central branch terminated by an umbel. Anthesis proceeds sequentially within an inflorescence from the primary umbel through to the tertiary umbels. Flowering begins in the primary umbels in September (mid spring) and concludes in the tertiary umbels, usually by March (early autumn). *Actinotus helianthi* is insect-pollinated, and may outcross or be geitonogamously (between umbels) pollinated (Chapter 3). Fruit of *Actinotus* are pseudomonocarpic.

Actinotus helianthi is an ideal species with which to investigate how population differences might vary geographically for the following reasons. Firstly, the species is known to vary in its growth habits among populations (Lee 1995). Secondly, cultivation and conservation of the species is severely hampered due to erratic germination success, particularly among populations and between years (Emery *et al.* 2011; Lee 1995). While the addition of smoke improves the overall germination response, results are known to vary by population (Emery and Lacey 2010). Seed germination is therefore influenced by both spatial and temporal environmental variation. Thirdly, little is known about the species reproductive niche and the species that visit umbels during primary flowering. Finally, the geographic distribution spans wide latitudinal and ecological gradients and thus environmental conditions and interactions can vary significantly among geographically distant populations.

Experiment 1: early performance traits

Seventeen wild populations were sampled across NSW (Fig. 1; Table 1). Populations were chosen to span a wide latitudinal and rainfall gradient of mesic coastal sites from 35.39°S to 32.66°S, and to include more arid inland habitats (149.29°E to 149.42°E; Fig. 1). The sampled populations covered around 42% of the latitudinal and 52% of the longitudinal geographic range of the species. Although not the full distribution, this geographic range includes multiple spatial scales relevant for comparative studies. The populations represented

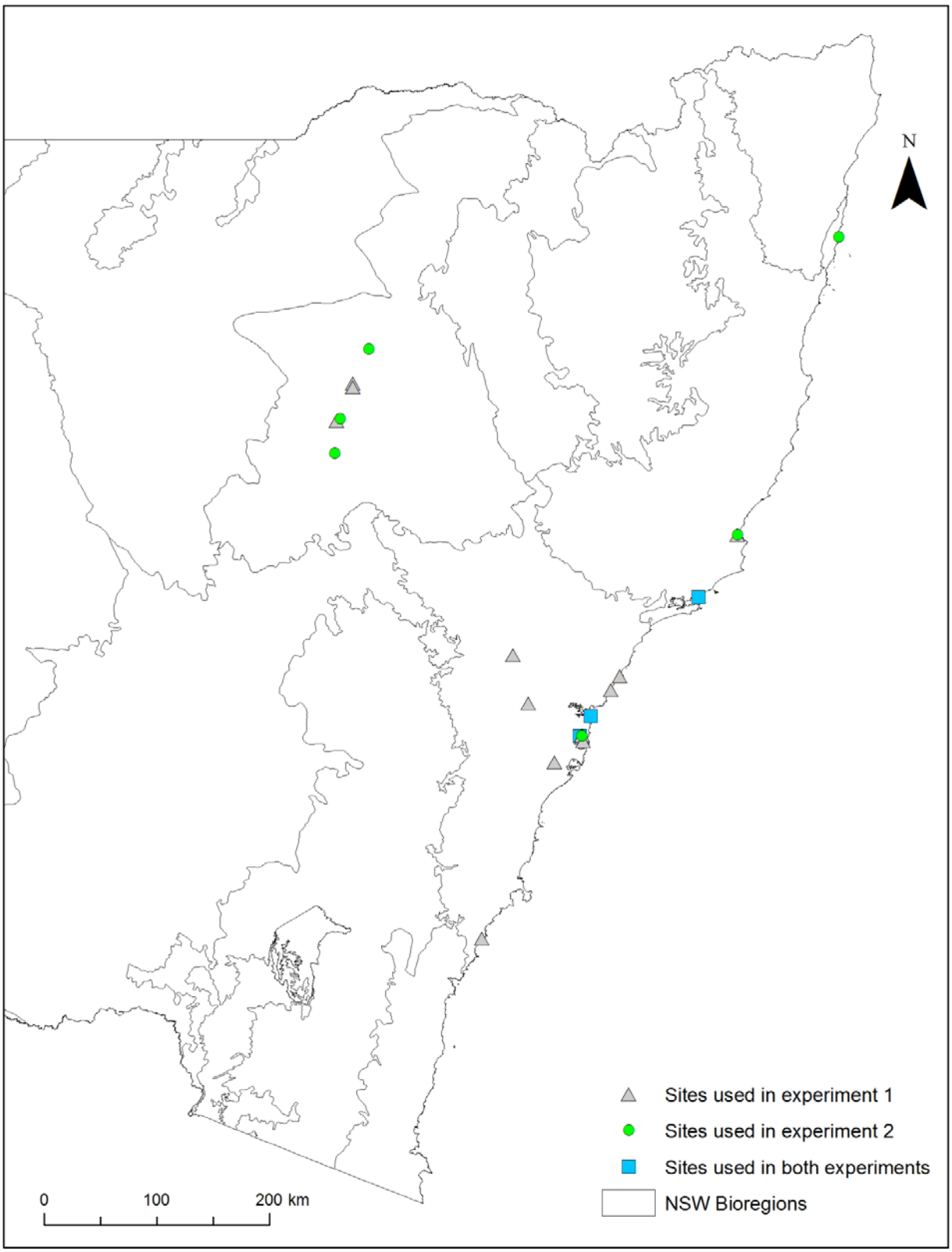

Fig. 1. Map of New South Wales (NSW), Australia showing the locations of all populations sampled for this study.

Table 1. Description of the codes for populations of *Actinotus helianthi* used in both experiments.

Population code	Population location	IBRA bioregion
C1[1]	Pilliga Nature Reserve, Coonabarabran	Brigalow Belt South
C2[1]	Pilliga Nature Reserve, Coonabarabran	Brigalow Belt South
C3[1]	Coonabarabran	Brigalow Belt South
C4[2]	Pilliga Nature Reserve, Coonabarabran	Brigalow Belt South
C5[2]	Coonabarabran	Brigalow Belt South
GR[1]	Georges River National Park	Sydney Basin
HN[1 2]	Hawks Nest	NSW North Coast
MD1[1]	Manly-Warringah War Memorial Reserve	Sydney Basin
MD2[1]	Manly-Warringah War Memorial Reserve	Sydney Basin
MD3[1]	Manly-Warringah War Memorial Reserve	Sydney Basin
MD4[1 2]	Manly-Warringah War Memorial Reserve	Sydney Basin
MD5[2]	Manly-Warringah War Memorial Reserve	Sydney Basin
MEN[1]	Mendooran Road, Mendooran	Brigalow Belt South
MW[2]	Grevillea Avenue, Minnie Water	NSW North Coast
PB[1 2]	McKay Reserve, Palm Beach	Sydney Basin
SH[1]	Sydney Harbour National Park	Sydney Basin
TUN[1]	Tuncurry	NSW North Coast
TUN2[2]	Tuncurry	NSW North Coast
UL[1]	Ulladulla	Sydney Basin
W1[1]	Wyrrabalong National Park, Norah Head	Sydney Basin
W2[1]	Wyrrabalong National Park, Bateau Bay	Sydney Basin
WOL[1]	Wollemi National Park	Sydney Basin
YEN[1]	Yengo National Park	Sydney Basin
StarB[1]	'Starbright' cultivar	n/a

[1] fruit sourced from this population was used in Experiment 1.
[2] fruit sourced from this population was used in Experiment 2.

three biogeographic regions: Sydney Basin, NSW North Coast and Brigalow Belt South. Each biogeographic region is further divided into a number of sub-bioregions. Bioregions act as a surrogate for complex climate, edaphic and biotic patterns as defined by the Interim Biogeographic Regionalisation for Australia, and are derived using distribution information of climatic, geological, geomorphological and biological elements (Dunlop and Brown 2008).

Field collections were made between 13 November 2010 and 12 February 2011. Mature fruits were collected from primary umbels across ten randomly selected plants (with at least 200 fruits collected from each plant) within each population. Fruit were stored and equilibrated in a dry room (15% rH; 15°C) in the NSW Seedbank at the Australian Botanic Garden, Mount Annan until the experiment commenced in April 2011.

The cultivar 'Starbright' was included in this study as it is propagated by tissue culture and, therefore, used as a genetically consistent 'control' comparison with wild collected populations. Fruit of 'Starbright' was obtained from a bulk collection across multiple plants made in November 2010. To avoid any potential performance bias when grown in the common environment, 'Starbright' was not included in any analyses.

Two-hundred fruit from each of the ten plants from the 18 populations were tested for germination using the following protocol. For each plant-population combination, four replicates of 25 fruit were sown into 9 cm sterilised plastic Petri dishes containing a water agar medium (7 g L^{-1}), and a further four replicates of 25 fruit were sown onto a 1% smoke-water (Seed Starter, Kings Park and Botanic Garden, Perth) agar medium made according to the procedure outlined in Emery and Lacey (2010), giving a total of 1,140 dishes. Petri dishes were sealed using plastic wrap and subjected to a constant temperature of 15°C with a 12 h/12 h light and dark photoperiod, according to protocols developed by Emery *et al*. (2011).

Fruit were checked for germination every 2-3 days until day 45, following an initial two week incubation period for embryo elongation, a requirement for morphophysiologically dormant species (Baskin and Baskin 1998). Due to the large number of dishes, non-germinated seeds were not checked for viability at the end of the trial.

Germinated seeds with a radicle < 5 mm long were removed from the agar using fine forceps and transplanted into individual 25 × 50 mm plugs in 105-plug seedling trays. A total of 20 germinated seeds from the smoke-water treatment for each plant-population combination were transplanted to the trays, giving a total of 3,600 seedlings. Seedling trays were Papertec Plug Packs (Highsun Express, Brisbane, Australia) containing a 1:1 soil mix of peat and perlite and pH balanced to 5.5. The soil mix was encased in a sheet of biodegradable and root-penetrable paper, which reduces water use and allows the plugs to be removed from the tray without touching and/or damaging the plants.

The trays were placed on benches with a thermal mat in a glasshouse at the Australian Botanic Garden, Mount Annan and mist-watered for the first four weeks and lightly hand-watered there-after. The plants were fertilized with a foliar spray-application of the soluble fertiliser Aquasol™ (Hortico®, Australia) from the time of leaf emergence. After 75 days, the seedling trays were removed from the glasshouse and transferred into a polyhouse and were watered every second day. Individual plant height was recorded at 90 days after transplanting as a measure of performance. Plant survival was monitored fortnightly until the experiment was concluded at 153 days.

SPSS Statistics 21 software package (IBM, New York) was used for statistical analysis. We used univariate linear mixed models with Type III Sum of Squares to analyze variability in three early fitness components. For germination and growth at day 90, we tested

for differences within and between populations with plants nested within populations. To examine germination and growth at a regional scale, we tested for differences within and between bioregions and sub-bioregions with sub-bioregions nested within bioregions. Four bioclimatic variables (mean annual temperature, mean maximum temperature of the warmest month, mean temperature of the wettest quarter, and mean temperature of the warmest quarter) and estimated plant abundance were included as covariates in the germination and growth models. Abundance was excluded as a covariate from the latter, as it was deemed to be unlikely to influence growth of the F_1 generation. Climate covariates were determined by running a Pearson's correlation coefficient analysis on 19 climate factors (Hijmans *et al.* 2005). A value of $\geq \pm 0.75$ was employed to identify highly correlated variables (Dormann *et al.* 2013).

We used an estimated performance measure for survival which was calculated as follows for each population: $g_{sm} \times j_i$. Where g_{sm} is the proportion of germinated seeds in the smoke-water treatment and j_i is the proportion of surviving plants at the conclusion of the experiment. Performance over time was analyzed as a repeated measure across populations and ranged between 1 (100% population survival) to 0 (0% population survival).

Experiment 2: insect abundance and seed set

Nine *A. helianthi* populations from three bioregions were sampled in NSW (Fig. 1; Table 1). Field observations were made between October and November, 2012 to coincide with primary (peak) flowering. At each population, six randomly selected plants were tagged and all umbels at anthesis – or which had stigmas present – were tagged. Plant height (*height*), number of umbels (*umbels*), and the number of flowering umbels (*flower*) were recorded for each tagged plant. Each tagged plant was observed for ten minutes by two individuals and any insect visits to tagged umbels were recorded. After visiting a tagged plant for the first

time, an insect was tracked and the number of umbels subsequently visited on the same plant was recorded. In order to account for any temporal variation, the six tagged plants were monitored throughout the day in a randomized order at 1000, 1200, 1400, and 1600. Three populations (MD4, MD5, and PB) were surveyed for three consecutive days. It was then determined that only Coleoptera and Thysanoptera differed significantly between survey days within a population. Therefore, it was deemed that one day of surveying for subsequent populations was sufficient to encompass the diversity of floral visitors. Individuals of the most common insects were opportunistically collected from *A. helianthi* plants at each site in between surveys for identification in the lab.

Following the final survey, tagged umbels which had pre-receptive stigmas were protected from further insect visits by encasing the umbels with organza bags. This allowed fruit to develop unhindered while all fruit are captured inside the bag when they detach from the maternal plant. Fruit were collected in January, 2013. Seed set was recorded as the proportion of viable seed per umbel. A viable seed was identified as dark brown in colour and hard when a small amount of force was applied with fine forceps. Seeds that could be bent, squashed or were pale in colour were recorded as non-viable.

Data matrices for plant traits within populations, insect floral visitor abundance and seed set were summed by plant (six replicates per population) and analyzed in PRIMER (Clarke and Gorley 2006) and PERMANOVA+ (Anderson *et al.* 2008). The plant trait data were normalized and converted to a resemblance matrix using the Euclidean distance measure. The insect abundance data were square-root transformed to limit bias from highly-abundant groups and converted to resemblance matrices using the Bray-Curtis similarity measure. Seed set data were also square-root transformed and a resemblance matrix was created using the Euclidean distance measure.

To test for among population-differences in relation to the three response variables; insect diversity, plant traits and seed set, separate one-way Analysis of Similarity (ANOSIM) models were run, each using a thousand permutations. We used distance-based redundancy analysis (dbRDA) to identify plant traits which were correlated with insect abundances, and to determine correlations between seed set and insect groups. Significant correlations were determined when $P \leq 0.05$.

Results

Experiment 1

Mean percent germination between populations ranged from 0.2 ± 0.1 % (MD4) to 64.2 ± 2.3 % (PB; Fig. 2). Germination increased with the smoke-water stimulus for all 18 populations, with final germination ranging from 59.3 ± 4.1 % to 97.5 ± 0.6 % (Fig. 2). Germination tested on water agar varied by individual plant from 1 % in MD4 to 81 % in GR, and between 5 % in TUN to 82 % in C1 on smoke-water agar. Germination exhibited significant interactions between agar type and the 17 wild populations ($P < 0.001$) and between agar type and plants within populations ($P < 0.001$). Plant height ranged from 19.9 ± 1.0 mm (MD2) to 46.3 ± 4.1 mm (W1; Fig. 3). Populations, and plants within populations, had significantly different levels of growth at 90 days ($P < 0.001$). At the end of the experiment on Day 153, 'Starbright' had the highest performance of 0.519 relative to the other populations. By comparison, final performance of the 17 field-collected populations at Day 153 ranged from 0.008 to 0.355 (Fig. 4). A significant interaction between time and population also affected performance ($P < 0.001$).

Germination differed significantly between bioregions and between sub-bioregions within ($P < 0.001$). Climate and abundance covariates were also significant in this model ($P <$

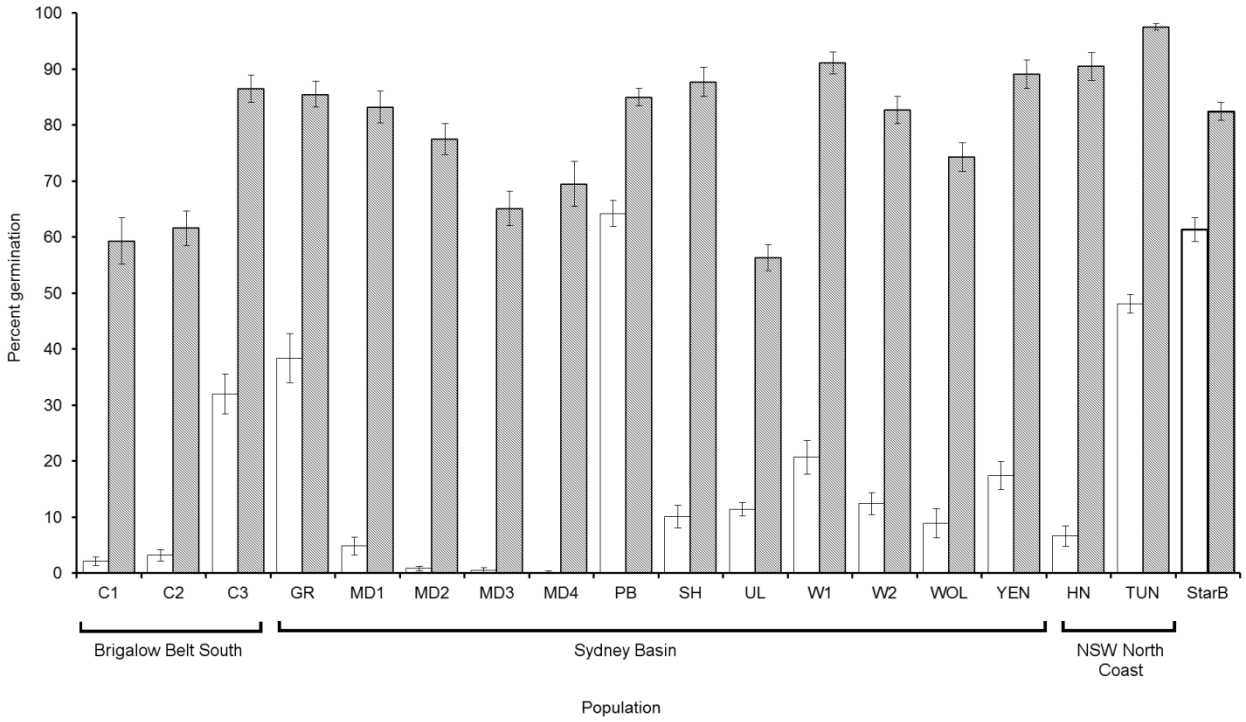

Fig. 2. Final mean percent germination for population of *Actinotus helianthi*. Populations are grouped by bioregion. Grey bars represent a 7 g L^{-1} agar with 10 ml L^{-1} smoke-water concentrate germination medium, and open bars represent a 7 g L^{-1} water agar germination medium. Bars are mean ± s.e.

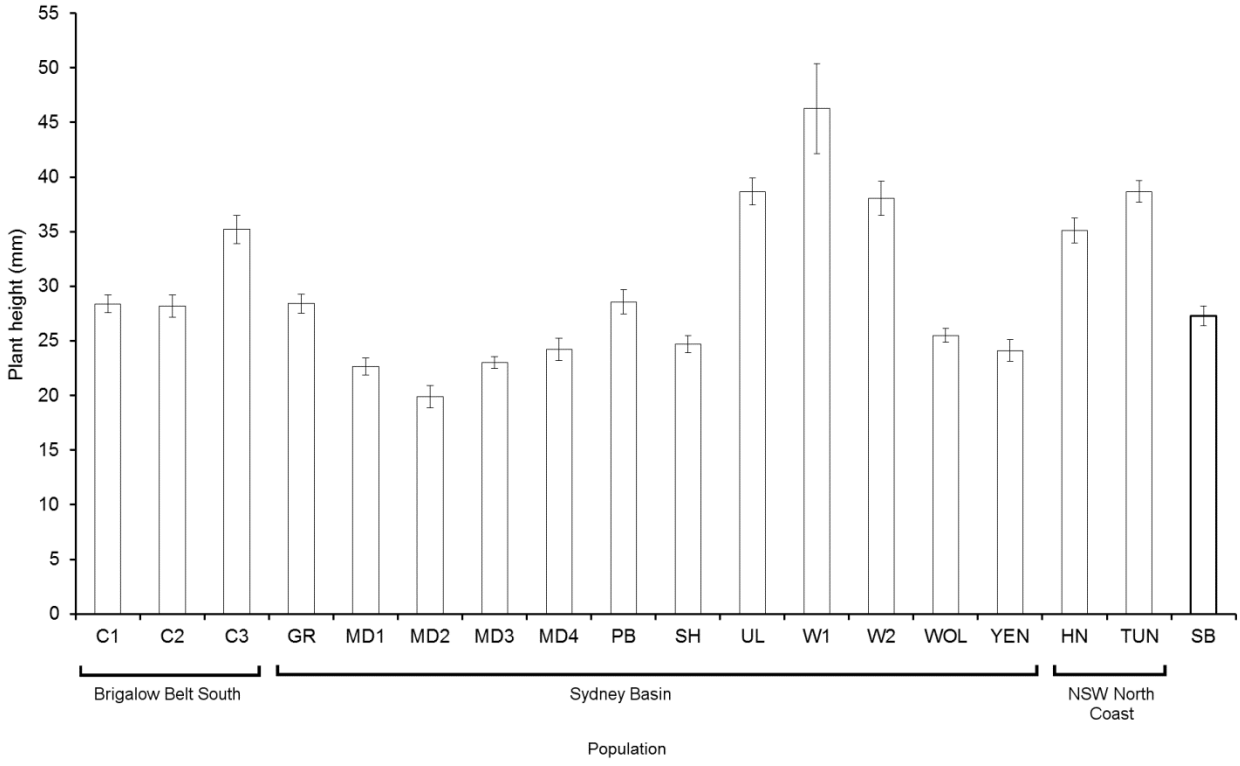

Fig. 3. Mean plant height per population of *Actinotus helianthi*. Populations are grouped by bioregion. Bold bar represents the 'Starbright' cultivar. Bars are mean ± s.e.

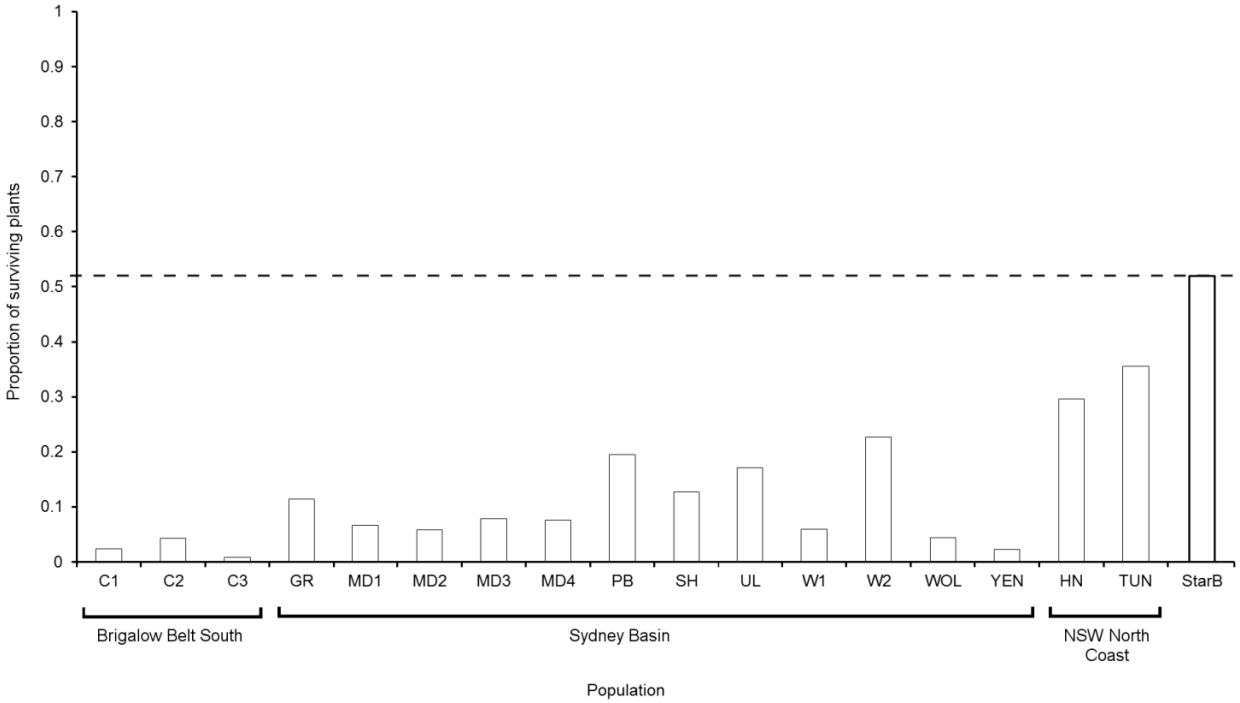

Fig. 4. Proportion of surviving plants per population at day 153. Populations are grouped by bioregion. Dashed line and bold bar represent the highest performance of the ‘Starbright’ cultivar (0.519).

0.001). Plant growth at day 90 was significantly different among sub-bioregions within bioregions ($P < 0.001$), but not between bioregions ($P = 0.063$). All four climate covariates were significant in the model ($P < 0.001$).

Experiment 2

The total number of recorded visits per day ranged between 93 and 460 (181 ± 23), and the total number of visits per population ranged between 93 and 565 (301 ± 57). Apiodea, Coleoptera, Curculionoidae, Diptera, Formicidae, and Thysanoptera were the major insect groups and comprised an average of 96.6 ± 1.6% of the total abundance of visitors at each population. Some of the most common insect visitors to *A. helianthi* included *Apis mellifera*, *Exoneura bicolor*, *Ischiodan scutellaris*, *Mordella leucosticte* and *Tomoxioda aterrima*. Bees visited around three times more umbels per plant (3.19 ± 0.29) than all other groups. Populations varied significantly in their species diversity of insect visitors (Global $R = 0.55$, $P = 0.001$). From the pairwise tests, only three population pairings were not significantly different (C5 and MEN, HN and TUN2, and MEN and TUN2). Average seed set of a population ranged from 62 ± 10% to 90 ± 2% (Fig. 5), and was significantly different among the surveyed populations (Global $R = 0.19$, $P = 0.001$). Diptera were the only insect group which displayed a positive correlation with average seed set, accounting for 11% of the variation in this value.

The nine populations exhibited significant variation in their plant traits between sites (Global $R = 0.32$, $P = 0.001$). Average plant height in a population ranged from 60.83 ± 12.47 cm to 133.50 ± 8.22 cm. The average number of umbels per population ranged from 15 ± 2.14 to 76.33 ± 18.9, and the average number of umbels in flower per population ranged between 5.67 ± 1.64 to 37.50 ± 8.79 (Fig. 6). All three plant traits were significantly associated with the insect abundances ($R^2 = 0.17$, $P = 0.023$). Plant height was the most

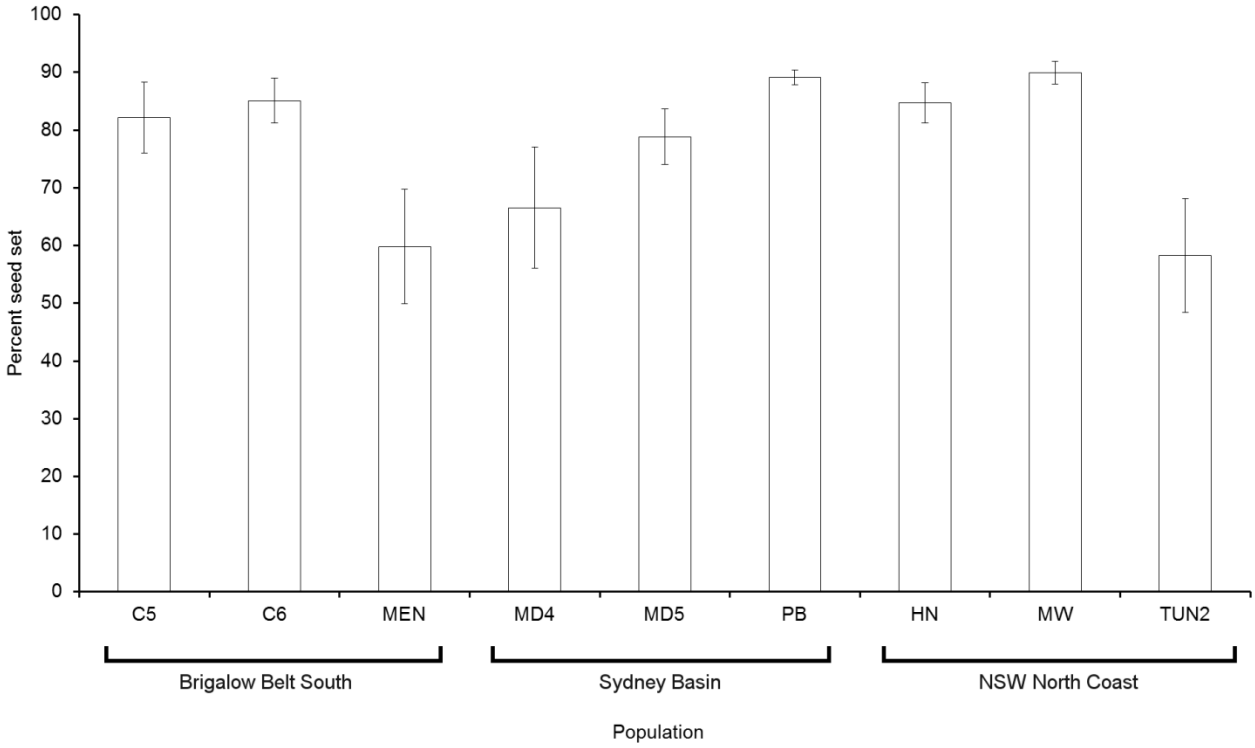

Fig. 5. Mean seed set of nine *Actinotus helianthi* populations. Bars are mean ± s.e.

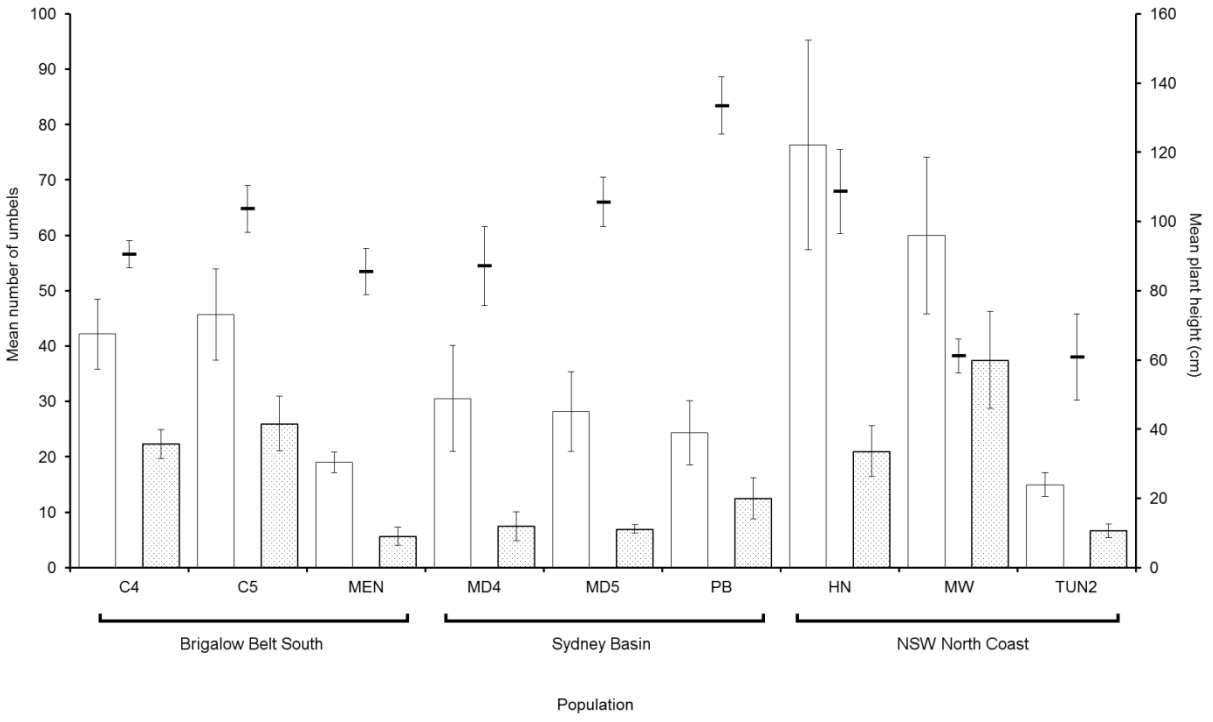

Fig. 6. Mean and standard error values for three plant traits at nine populations of *Actinotus helianthi*. Populations are grouped by bioregion. Back lines: mean above-ground plant height; open bars: mean number of umbels per plant; stippled bars: mean number of umbels in flower per plant.

influential of the three traits, accounting for 11.6% of the variation (Fig. 7).

Discussion

Early performance traits

We have shown that plants from populations of *A. helianthi* grown in a common environment differed in early life-history traits of germination, growth and seedling survival. These early-expressed traits are important as they will determine which individuals persist and, therefore, influence the capacity of a population to respond to changes in the local environment.

The differences between populations we recorded from our common environment indicate that genetically-fixed variation could cause differential survival rates when seeds are dispersed to novel environments. The grouping of populations within bioregions and sub-bioregions was not a simple predictor for performance in the common environment, but there was evidence of population-dependent differences in growth at the small spatial scale of sub-bioregions. There was also an indication of early plant growth rates converging across bioregions ($P = 0.063$). This could then translate into differences among populations in adult reproductive traits. For example, a comparative study of the difference in fitness between provenances of *Themeda triandra* (previously *T. australis*) demonstrated that locally-adapted plants had superior reproductive traits than non-local plants, in terms of their percent flowering, flowering time, above-ground biomass and culm weight ($P < 0.001$) (Hancock and Hughes 2014). It should be noted, however, that initial differences in reproductive output could converge after multiple years, although the degree of convergence is likely to be species-dependent (Hancock *et al.* 2013). Therefore, it would be beneficial to follow growth over several years, or ideally the entire life-time to determine how the early differences in performance recognized at the finer scale of sub-bioregions, manifested later in life and if it

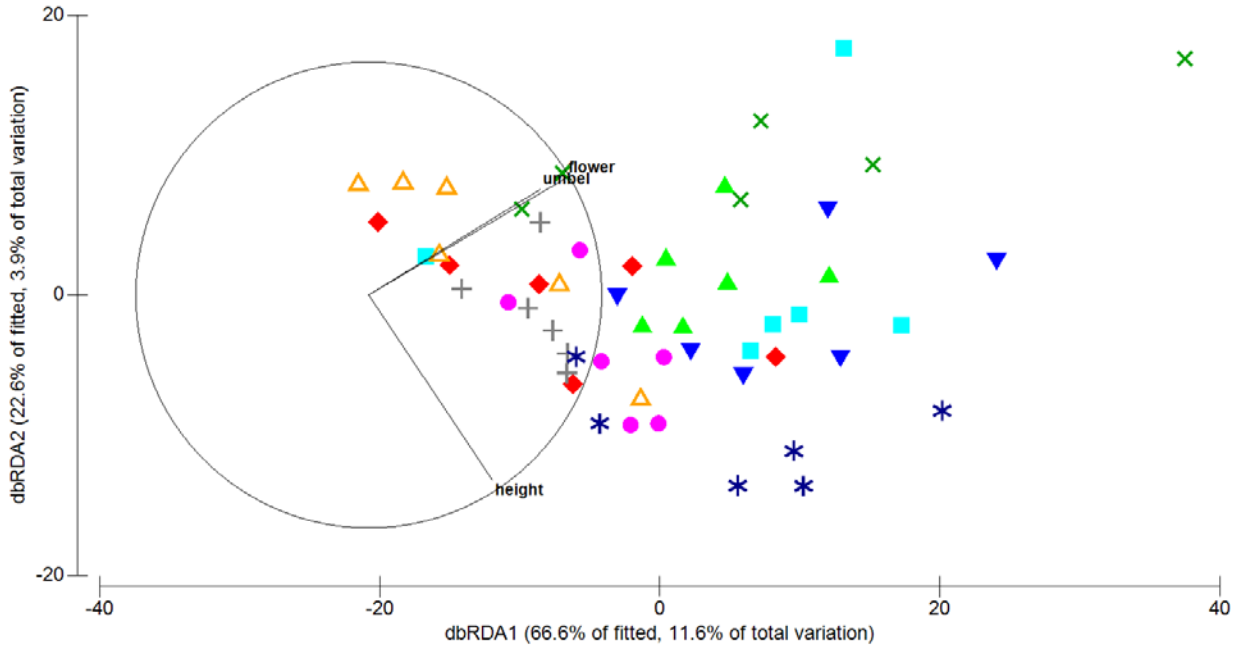

Fig. 7. Distance-based redundancy analysis (dbRDA) ordination and visualization of insect abundances fitted with plant traits as vectors for nine populations. Plant height (x-axis) explains 11.6% of the total variation, and number of umbels, plotted on the y-axis, explains 3.9% of the variation. C5 (triangle), C6 (inverted triangle), HN (square), MD4 (diamond), MD5 (circle), MEN2 (plus), MW (cross), PB (asterisk); TUN2 (outline triangle).

was correlated with similar differences in reproductive traits.

Above-ground height is a plant architectural trait which can vary in response to the environment (Sultan 2000), often displaying a plastic response when examined in experimental conditions (Pigliucci *et al.* 1997; Pigliucci and Schlichting 1996). Plant height and overall growth rate increase the plant's competitive advantage, particularly when seeds are dispersed into established ecosystems. In our study, mean plant height varied significantly between sub-bioregions and among the populations within, despite the readily available light and lack of inter-plant competition. Sultan (2000) noted that the timing of a developmental plastic trait such as height can also be plastic. In other words, plants might display plasticity for height in the early stages of growth, or variability in time of onset can exist between different genotypes or populations (Bell and Sultan 1999). Vile *et al*. (2006) showed that for 34 Mediterranean herbaceous species, the timing of anthesis for 50% of a population was dependent on the maximum plant height. A more recent study of six northern hemisphere grass and forb species found an overall positive correlation between the maximum height of plants, time to 90% of maximum height and the time of onset of flowering (Sun and Frelich 2011). Specifically, plants which grew faster earlier also tended to reach their maximum height and reproductive capability earlier. In our study, there was some indication that differences in early growth rates began to disappear at a regional scale. However, following this into the reproductive stage was not possible. While we could not examine the impact of plant growth on the reproductive output of *A. helianthi*, such data would complete the generational process and are important for predicting plant performance under prolonged disturbance or increased competition. This is especially pertinent since our results indicate that differences in early growth may not be apparent at coarser regional scales.

Germination success varied between populations and between plants within populations. Moreover, we found that while the addition of smoke-water stimulated seed germination, it was unable to eliminate the variation observed between and within populations. Population differences in germination success have previously been recorded in both annual and perennial plant species, including, *Saxifraga hirculus, Biscutella didyma, Bromus fasciculatus* and *Hymenocarpos circinnatus* (Ohlson 1989; Petrů and Tielbörger 2008). Seed dormancy and germination are heavily reliant on climatic variables, thus some degree of regional differences might be anticipated (Baskin and Baskin 1998; Petrů and Tielbörger 2008). The differences between populations expressed in this common environment indicate that genetic variation could account for variable survival rates when seeds are dispersed to novel environments. As seeds used for this study were collected from the wild, it is also possible that these differences reflect genetic variation among populations and/or maternal effects of different genotypes within populations (Donohue *et al.* 2005).

In addition to population factors, our results also indicate that the local climate of the maternal environment plays a role in both germination and early growth. Specifically, intimate relationships between the seed and climatic variables, such as temperature, have been widely documented across species (Probert 2000). This was also supported by the significance of the climate covariates included in our model. A study by Qaderi and Cavers (2002) reported consistent and significant variation in germination among one natural and two transplanted populations of *Onopordum acanthium* sampled repeatedly over four years. Germination success was positively correlated with mean daytime temperature during seed maturation on the maternal plant (Qaderi and Cavers 2002). By comparison, rainfall often displays a more unpredictable relationship with plants (Noe and Zedler 2001). For example, Petrů and Tielbörger (2008) found no obvious relationship between the first rainfall event and germination in three annual species. Therefore, a combination of factors exists that can

override climatic gradients, underlining the importance of including both climatic and local environmental factors (population traits) to better define the role of local adaptation in the species' response. That *A. helianthi* populations express differing germination responses to fire suggests that other factors in the maternal environment (e.g. temperature and precipitation) have a strong impact on germination of the progeny. Such variation is important when attempting to predict temporal changes to populations throughout the landscape.

Apart from the 'Starbright' cultivar, overall survival of juvenile plants was poor in the sampled populations. Survival is often overlooked in common garden studies despite being an important plant fitness trait (McCarragher *et al.* 2011). Germination alone does not give an accurate portrayal of fitness, as seeds will germinate when conditions meet dormancy relaxation requirements. However, the duration of suitable conditions varies, making growth and/or survival difficult if an individual cannot adapt. Low survival could reflect the quality of the common garden environment for *A. helianthi*. Indeed, the superior performance of 'Starbright' seedlings provide a relevant comparison as it has been bred from a maternal wild population as a vigorous and disease-resistant 'cultivar' for commercial sale (Offord and Bullock 2009), and is a 'gold standard' to test against wild populations in managed experimental conditions such as in this study. While seedling survival is likely to vary between years, the overall population differences observed in this study warrant further investigation using a reciprocal transplant experiment to examine survival under field conditions among and within regions to examine the impact of local climate. This would complement our findings.

Insect abundance and seed set

We have shown that average seed set success differs significantly between populations. Additionally, despite showing around 50% similarity among populations, insect abundance was site-specific. Six insect groups comprised between 84% and 99% of the total insect abundances across sampled populations. We are confident that our results are representative of main visitors to *A. helianthi*. Therefore, differences in visitor numbers might be due to variation in the local climate. The populations surveyed occur in localities which vary by around 4°C in average daily temperature during summer. The development and activity of insects are highly sensitive to climate, particularly temperature. This relationship between insects and climate is exemplified in bees and butterflies. In California, for example, butterflies were appearing earlier in the season in association with both higher winter maximum temperatures and less winter rainfall (Forister and Shapiro 2003). Specifically, a faster growth rate of larvae was associated with increasing temperature. While this pattern was not significantly correlated, the trend was positive over three decades (Forister and Shapiro 2003). The emergence of the small white butterfly (*Pieris rapae*) and the honey bee (*Apis mellifera*) in Spain were influenced by mean spring temperatures; both species were appearing up to six days earlier per decade (Gordo and Sanz 2006). While it is likely that the response of an insect species to temperature is dependent on its life-cycle development, even small changes in the local climate will influence the interaction between plant and visitor (Forister and Shapiro 2003; Gordo and Sanz 2006; Hegland *et al.* 2009).

Insect visits to *A. helianthi* plants were influenced by plant traits, and there was an indication from the results that taller plants with larger primary floral displays were more attractive to insects. Similar results were reported in waratahs by Pyke (1981), who found inflorescence height and the number of flowers per inflorescence to have a positive linear relationship with fruit set, regardless of the total number of inflorescences. Plants with a

larger number of flowers are well-documented to attract insects at a greater rate than plants which produce a smaller number (Brys *et al.* 2008). This results in higher plant fitness due to the increased number of visits by potential pollinators. Conner and Rush (1996) identified a positive relationship between flower number in *Raphanus raphanistrum* (wild radish) and the number of visits of syrphid flies over a three year period. Similarly, visits by small bees increased by 63% with a one standard deviation increase in flower number (Conner and Rush 1996). In the perennial herb, *Wurmbea dioica* the number of visits per plant had a significant positive correlation with flower size (Vaughton and Ramsey 1998). The authors note that this association was largely due to bees and butterflies ($P < 0.01$), however there was also an indication that flies might also favour larger floral displays or associated plant odours ($P = 0.08$). We postulate that plant height and floral display size are unlikely to be the most important plant traits for attracting visitors to *A. helianthi*.

The plant traits recorded in our study only accounted for 17.4% of the total variation in insect visitors. While this could be due to a short sampling period, it could also be due to other (unmeasured) plant or population attributes. For example, higher plant density has been reported to be associated with increased visits by pollinators and subsequent seed set (Kunin 1997; Mustajärvi *et al.* 2001). In *Brassica kaber*, seed-set was influenced by plant density, with sparse populations recording poorer seed set, possibly mediated by self-incompatibility (Kunin 1993). Plant density specifically affects visits by solitary and social bees ($P < 0.05$ and $P < 0.01$, respectively), and, to a lesser extent, flies (Kunin 1997). Interestingly, Mustajärvi *et al*. (2001) found bumblebees were more likely to visit sparse populations of *Lychnis viscaria* than dense populations. While this could be due to plants within low density populations having larger flowers (Klinkhamer and de Jong 1990), it might, also, be specific to the type of insects which are visiting. Regardless of the type of effect, studies have clearly

demonstrated that plant density is an important factor that should be included in reproductive studies.

The relationship between Diptera and seed set suggests that for *A. helianthi* seed set could be, in part, predicted by the abundance of flies visiting plants. Diptera abundance was also positively correlated with Apiodea abundance. On average, bees visited three times more umbels per *A. helianthi* plant than any other group of floral visitor, and are well known to be one of the most effective pollinators of plants. Whether the abundance of flies is a proxy for bee abundance and, subsequently, reproductive success, warrants further study, and might prove vital for the future persistence of this species. However, we note that different pollinators may vary substantially in how effective they are in transferring pollen, with flow on effects to seed set and seed quality. This means that caution is warranted when directly comparing visitation with pollination. Visitation provides a useful indication of potential reproductive difference, and future studies examining the pollen presence on visitors compliment and build on the hypotheses from this study.

Conclusions

Traits associated with the seed stage represent the most sensitive part of the plant life-cycle that can have an impact on its overall life-history (Ågren and Schemske 2012; Donohue *et al.* 2005). The results from this study suggest that the progeny of *A. helianthi* populations could be adapted to their local environment. This constraint might negatively affect populations that need to migrate to new suitable habitats in order to survive under changing climate. In addition, variation in plant traits for *A. helianthi* can occur at multiple scales. We have illustrated that the populations of a species are interacting in different ways with their local environments. Identifying the relationships between early performance traits and the environment are required to unravel and identify the mechanisms that permit populations to

migrate and/or adapt under changing environments. Future data on species with varying life histories will enable us to understand what common plant traits are important for species persistence, and whether there are any trends among these traits. We expect that significant variation observed in early performance traits and the reproductive stage of *A. helianthi* and other species will have significant consequences for its ability to disperse and colonize novel environments.

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Chapter V

EXPERIMENTAL EVIDENCE CONFIRMS THE INTERACTIVE EFFECT OF SOIL AND CLIMATE ON PREDICTED PLANT DISTRIBUTIONS

Abstract

Predictive climate models are useful for quantifying changes to geographic distributions by correlating species presence with their associated climatic variables. However, models that use only climatic data fail to fully account for other non-climatic attributes that might significantly influence future geographic occurrence. Caution is warranted, therefore, when interpreting bioclimatic models that do not incorporate a broader set of ecological factors. To further understand the impact of including non-climatic factors, such as edaphic attributes in predictive models, we used a series of iterative experimental data sets to compare and assess the predictive precision of our models. We used contemporary and historical geographic records of the plant species *Actinotus helianthi* coupled with bioclimatic data, to generate predicted habitat suitability using four IPCC AR5 climate scenarios in *MaxEnt*. Using these projections, seedling emergence and growth was tested in soil in two common garden environments from four local sites, and twelve predicted sites, where suitable climate was calculated to occur in 2070. Seedling emergence was significantly influenced by population and origin of soil. Multivariate analyses produced a model which identified pH, sodium (Na – sodicity), salinity and phosphate levels (PO_4) as best explaining the patterns in seedling growth. Consequently, we added soil pH and soil sodicity as spatial layers to the initial bioclimatic model to produce a new (climate and soil) model. The climate-only model over-predicted geographic areas with high suitability (60-100% suitability) in three of the four climate scenarios. These results illustrate the capacity to improve the precision and validity of

predictive bioclimatic models by incorporating experimental and/or field evidence to better represent the ecological preferences of species.

Introduction

Spatial variation in temperature and rainfall are altering species fitness, forcing some to migrate to higher altitudes or south to cooler temperatures, while others will become restricted to their current climatic tolerance or suffer from local extinctions (Bakkenes *et al.* 2004; Stocker *et al.* 2013). Species distribution models (SDMs) are common practice for quantifying and predicting this variation (Elith *et al.* 2006; Hijmans and Graham 2006; Thuiller *et al.* 2008). Traditionally, these predictive studies have correlated distribution shifts with climatic envelopes (also referred to as bioclimatic models). An accepted assumption of these models is that they treat the species as a single entity, irrespective of any population or environmental variability (Araújo and Peterson 2012; Hampe 2004; Heikkinen *et al.* 2006). Regardless of assumptions, models and their programs (such as the very popular *MaxEnt*) are an important tool for improving management and conservation strategies for species by providing a means to explore the relationship between a species and its climate envelope (Chapter 2). However, it is important that model predictions are subsequently validated and improved with experimental evidence which demonstrate the impact of other limiting environmental factors.

The ecological preferences of plant species can impact their successful establishment and persistence. In particular, environmental factors which influence the reproductive niche of a species are arguably the most important to understand. Such factors are often neglected in predictive studies due to a lack of available data. Consequently, studies examining the response of plant species to limiting environmental factors have only recently received attention (Bertrand *et al.* 2012; Condit *et al.* 2013; Dubuis *et al.* 2013; Thuiller 2013). In

particular, edaphic characteristics, such as soil pH, moisture and content are known to vary and affect the reproductive niche of plants, and in turn, limit the ability to colonize and become established in new environments (Coudun and Gégout 2007; Coudun *et al.* 2006; Pickett and Bazzaz 1976; Pierce *et al.* 1999). Specifically, pH is reported to control the uptake of minerals by plants and has been correlated with several other edaphic variables (Schoenholtz *et al.* 2000). For example, Pakeman *et al.* (2012) used a suite of 12 flowering species with differing seed sizes and dormancy types to demonstrate that the 'optimal' model for seedling survival and viability was found to be in soils with high pH, coupled with low moisture-content and C:N ratio. The distribution of tree species in the Panama forests have also been reported to be regulated by a combination of soil moisture and phosphorus, with many species having a specific tolerance for concentrations of the latter (Condit *et al.* 2013). The level of phosphorus in soil is also an important limiting factor for the establishment of many Western Australian plant species (Lambers *et al.* 2010).

As populations move, they have the potential to follow their current climate envelope or shift into new niches, with the fitness of a population dependent on whether the incumbent plants have a capacity to grow and reproduce in novel sites. A continuum of responses would be expected across individuals and among populations. These responses would depend on a tolerance for persisting in areas where nutrients are not limiting or whether the fundamental traits of the plant allow it store nutrients in structures, to survive in nutrient-poor environments (Dubuis *et al.* 2013; Pellissier *et al.* 2010). Therefore, while the effects of soil may not be apparent in shaping plant distributions, it can instead impact at the population level by imposing a selection pressure which influences population abundance.

In this context, the soil environment is crucial to the successful dispersal of populations in new locations and presents a good opportunity to validate model predictions.

Soil spatial data are also now readily available and can be included as layers in predictive models. However, while the soil environment is now accepted as an important factor in shaping species distributions, the degree to which edaphic variables drive a species' distribution is unknown.

Actinotus helianthi is a perennial sub-shrub native to the eastern states of Australia between the New South Wales (NSW) South Coast and central Queensland (QLD). The species predominantly grows on sandstone-derived soils in sclerophyll ecosystems, and is one of the dominant species following fire. *Actinotus helianthi* populations exhibit different growth forms throughout its distribution, which is not driven by the local climate (Chapter 2). Furthermore, differences in germination and early growth and survival among populations in a common environment suggest that the species may have fixed genetic ecotypes (Chapter 4). However, it is not known whether *A. helianthi* plants have the capacity to grow and colonize non-local sites. In this study, we assessed the precision of bioclimatic models by comparing climate-only models with climate and soil models developed using experimental evidence. We used contemporary and historical geographic records of the plant species *A. helianthi*, coupled with bioclimatic data, to generate predicted habitat suitability using four IPCC AR5 climate scenarios in *MaxEnt*. We experimentally tested the intraspecific variation in response to different edaphic environments in a widely-distributed plant species by growing seed in soil samples collected from four local sites, and twelve non-local sites where suitable climate was predicted to occur in 2070. We then used the results from our experiment to include two limiting soil factors within the bioclimatic models.

Therefore, the aims of this study were iterative: (1) to predict and quantify the spatial extent of suitable habitats in the future using an optimized bioclimatic (climate-only) model, (2) to use the predicted suitability index coupled with land-use maps to determine an

appropriate direction for soil sampling transects from four current population sites, (3) to examine seedling performance across populations between local and novel soils and determine whether any differences can be related to edaphic variables, and (4) to assess the precision of the climate-only model with a climate and soil model.

Materials and methods

Climate-only models – issues related to the quality and collection bias of herbaria data in predictive models are well-documented in the current literature (Hijmans *et al.* 2000; Kadmon *et al.* 2004; Loiselle *et al.* 2007). However, sampling bias can be reduced by using a larger subset of records (Kadmon *et al.* 2003). We cross-referenced a total of 539 herbarium records collected from the *Australian Virtual Herbarium* (AVH; http://avh.ala.org.au/) in August, 2010. We 'cleaned' the data by eliminating all duplicate records as well as compiler records whose location coordinates did not match the description after cross-checking. Compiler records are generated as a 'best estimate' of GPS coordinates and so may be less accurate than records logged by the collector. A new database of 137 *A. helianthi* AVH records was combined with 40 field records made between 2011 and 2013 giving a total of 177 presence records.

Habitat suitability models were generated using *MaxEnt* v3.3.3k (http://www.cs.princeton.edu/~schapire/maxent/). *MaxEn*t is a machine-learning environment which estimates the likelihood of occurrence using the principle of maximum entropy. The program uses a number of limitations to match an environmental variable with its empirical average under the overarching distribution (Loiselle *et al.* 2007; Phillips *et al.* 2006). We used *MaxEnt* because this method does not require absence records, and it has a proven track record for large comparative studies (Elith *et al.* 2006; Hernandez *et al.* 2006). To investigate the effect of climate on the distribution of *A. helianthi*, we used bioclimatic variables derived

from the WorldClim database v1.4 (www.worldclim.org). Each variable is a global climate layer set out in a 2.5 arc-minute (5 km × 5 km) spatial resolution grid (Hijmans *et al.* 2005). These layers were clipped to Australia for the background landscape and prepared in ArcGIS v10.1 (ESRI, 2012). Seven bioclimatic variables were included in the final model as they were hypothesized to influence the distribution of the spring and summer flowering *A. helianthi*, and each had a Pearson's correlation value of $\leq \pm 0.75$ with other climate variables (Dormann *et al.* 2013; Elith *et al.* 2010). The variables retained were: temperature isothermality, mean temperature of the driest quarter, mean temperature of the warmest quarter, mean temperature of the coldest quarter, mean annual rainfall, mean rainfall of the driest month, and rainfall seasonality (Appendix 3). The model was run using the cross-validation data method in *MaxEnt* to train the model. We allowed the model to have enough time for convergence by using a maximum of 5000 iterations. The model was replicated 25 times. Area under the ROC curve (AUC) was used as a measure of model performance (Phillips *et al.* 2006). Raes (2012) noted that the maximum AUC value is not 1, but rather 1-*a*/2; where *a* represents the species realized niche. Even though the use of AUC as an indicator of performance in presence-only models is not accurate, the aim of our study was neither to compare model performance nor to produce an 'optimal' model. Because AUC is the standard measure in *MaxEnt*, coupled with its widespread use in the previous studies, it was suitable for this study to assist with determining subsequent field collection sites.

We then predicted the future distribution of *A. helianthi* at 2030, 2050 and 2070. Bioclimatic layers have been derived for four future climate scenarios from the Intergovernmental Panel on Climate Change (IPCC) 5th generation report (AR5). The four scenarios are referred to as Representative Concentration Pathways (RCP). These scenarios represent the predicted levels of radiative forcing due to atmospheric constituents, such as CO_2, and measured in Watts per metre squared (Wm^{-2}) by 2100. These forcings take into

account the vast range of plausible scenarios that include socioeconomic and technological developments (Moss *et al.* 2010). For example, RCP 2.6 represents a low-level scenario that results in 2.6 Wm^{-2} forcing. RCP 4.5 and 6.0 are medium-level scenarios that stabilize by 2100, and RCP 8.5 is a high-level forcing that is predicted to continually rise throughout the 21st century (Van Vuuren *et al.* 2011). Future bioclimatic data were downloaded from the Climate Change, Agriculture and Food Security website (CCAFS; www.ccafs-climate.org). The future data used in this study were derived from the CSIRO's Mk3.6 climate model. This model has been built from the Mk3.0 model and is most relevant to Australia and adds new ocean, sea-ice and soil-canopy models as well as including an interactive aerosol scheme. (Gordon *et al.* 2002; Jeffrey *et al.* 2013). The data were also clipped to the same extent as the current bioclimatic layers.

Field collections – Seed was collected from four *A. helianthi* populations in January, 2013. Two populations (C4 and C6) occurred in the central-west Pilliga region of New South Wales (NSW), and the remaining two were situated in the NSW north coast (TUN and HN) (Fig. 1). The two regions represent the maximum longitudinal extent of the species' distribution. Populations within each region were approximately 65 km apart. Seed collections were stored in paper bags in a dry room (15 °C, 15 % r.h.) until sowing in June, 2013.

Soil sites – Non-local sites for soil collection were determined by overlaying model projections and land use maps in ArcGIS. For each population, a 'line of best estimate' was drawn from the current population location (i.e. local site) to the closest area with the highest suitability prediction for 2070 as according to the RCP2.6 ('best case') scenario, and was in an area of 'natural vegetation' (Fig. 1). Sites were labeled at 1 km, 10 km and 40 km from the local site, giving a total of 16 sites. A 40 km distance was deemed appropriate as it represents, on average, around a 500 m migration/dispersal event each year. A non-local site

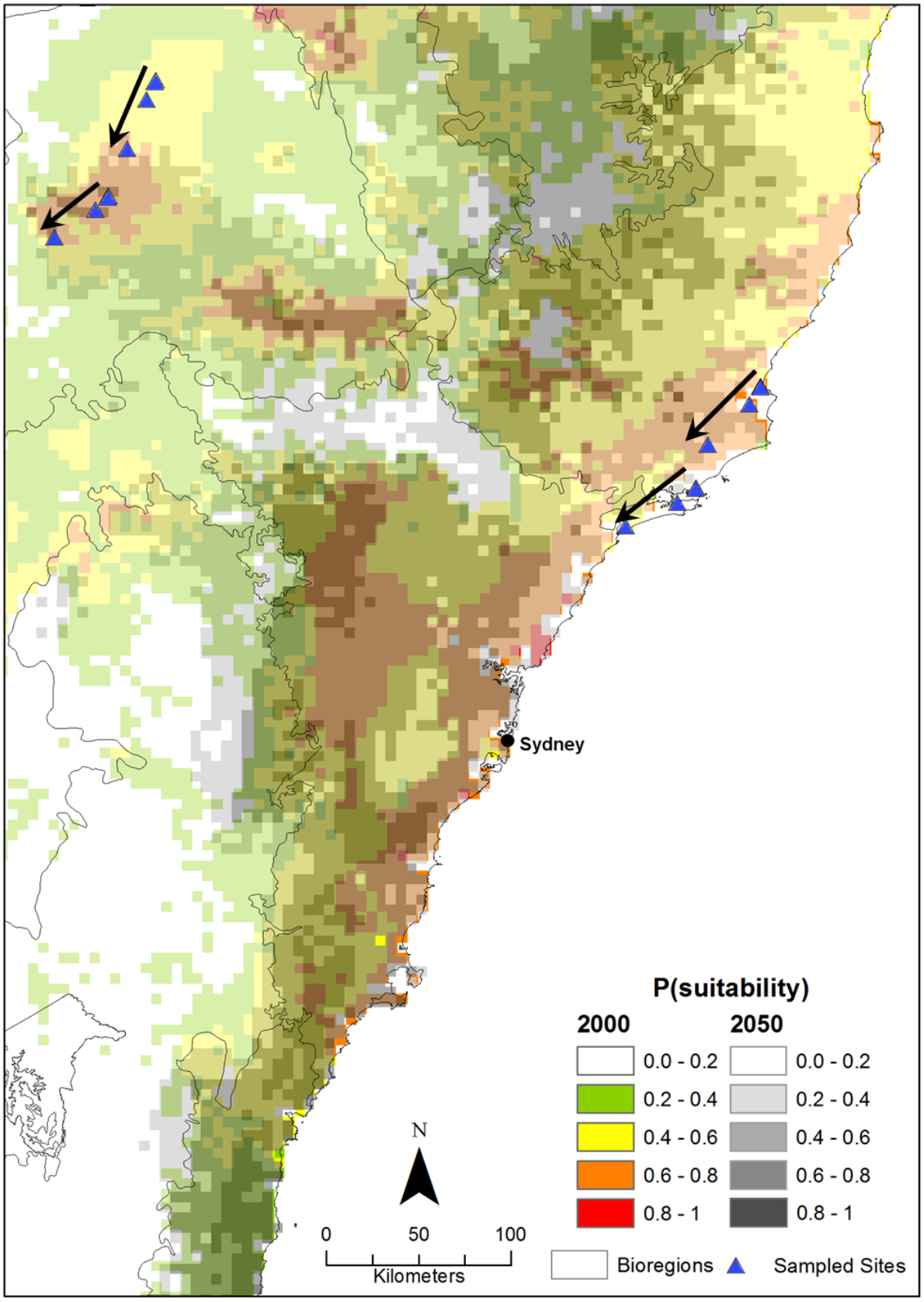


Fig. 1. Map of locations where soil was collected. Arrows indicate the direction of sampling from home site following the trend in suitable climate from 2000 to 2070 using the 'best case' RCP2.6 scenario.

was deemed suitable for potential colonization if it satisfied two criteria: (1) an area of natural sclerophyll vegetation with minimal disturbance, and (2) accessible by car or on foot. When a site did not meet the criteria, the next closest suitable site was selected within a 2 km radius.

A 30 metre transect was set up at each of the four local and twelve non-local sites running parallel to any visible gradient. The use of a transect permits bulk soil from novel locations to be collected across a suitable site, rather than just a single point. In addition, *A. helianthi* populations can cover a large area, so a single soil block collection might not be representative of the range of the local edaphic environment. Approximately 200g of soil was collected at two metre intervals along the transect starting at zero. At each collection point, any leaf litter was removed and the top 5cm of soil was then excavated using a metal container and shovel. Bulk soil from a site was stored in sealed plastic bags.

Soil experiment – a sub-sample of soil from each field site was sent to Sydney Environment & Soil Laboratory (SESL) at Thornleigh, Australia for complete chemical and physical analysis. A dataset of 23 edaphic variables was created from the results of the external soil analysis, and is summarized in Table 1. A potting mix currently used at The Australian Botanic Garden, Mount Annan to grow *A. helianthi* was also included in the design as a standard for 'optimal' soil conditions. Seed from a population was sown in its local soil (sL), soil 1 km away (s1), soil 10 km away (s10), soil 40 km away (s40), and the common potting mix (sC). We used a factorial design to examine the effects of soil on seedling emergence. There were five replicate pots (120 mm × 50 mm forestry tubes) labeled for each population-soil combination, giving a total of 100 pots. Each pot contained nine seeds sown in a 3×3 grid. Since the plants were only grown to an early seedling stage, any detrimental effects from density and, therefore, intra-specific competition was deemed to be minimal. The pots

Table 1. Summary of the 23 soil variables used in the study. C1: Coonabarabran 1; C2: Coonabarabran 2; HN: Hawks Nest; TUN: Tuncurry.

	pH (H_2O)	pH (CACl)	Salinity	Na	Cl	Mg	K	Ca	Al%	H%	eCEC	NO3	PO4	SO4	Fe	Mn	Zn	B	Clay Content	Particle Size	EC	OC%	OM%
C1 (Local)	5.9	4.6	0.02	10	30.1	24.5	22.7	75.6	1.4	50.1	6.9	0.8	1.7	0.6	16	4.2	0.4	0	25	1	0.19	3	5.1
C1 (1km)	7.1	7	0.13	10.9	24.1	48.3	21.3	303.4	0	0	9.9	1.2	2.9	0.6	25.4	10.2	0.2	0	25	1	1.24	2.6	4.4
C1 (10km)	5.8	4.6	0.02	6.4	44.9	14.9	16.9	31.7	3.9	62.2	5.1	0.9	0.8	0.6	14.7	3.2	0.1	0	25	2	0.19	2	3.5
C1 (40km)	5.6	4.4	0.02	3.3	33.5	15.1	17.8	34.9	3.2	59.1	4.7	0.3	0.5	0.6	15.1	4.2	0.1	0	25	1	0.19	2.2	3.8
C2 (Local)	4.8	3.8	0.03	5.3	28.9	13.6	25.9	25.7	17.3	64.3	8.8	0	1.3	0.6	49	3	0.5	0	15	3	0.42	6.1	10.4
C2 (1km)	5.7	4.8	0.02	2.8	19.3	16.9	14.6	94	0.7	45.4	6.1	0.6	6.6	0.6	21.8	10.8	0.3	0	15	1	0.28	2.4	4.1
C2 (10km)	50.6	4.9	0.07	9.5	30.7	322	253	1248	0.3	33.2	14.5	18	26.1	3.6	122.5	42	1.3	0	25	2	0.67	4.6	7.9
C2 (40km)	6.1	5.5	0.05	5.4	19.1	58.1	70.8	257.8	0	0	9.8	1.9	1.7	0.6	24.5	72.8	1.7	0	30	2	0.43	2.8	4.8
HN (Local)	5.3	3.9	0.02	23.7	16.4	27.5	8.3	81.2	0.4	50	6.8	0	0.2	0.6	7.1	0.8	1.2	0	15	1	0.28	3.9	6.6
HN (1km)	5	3.6	0.02	14.2	9.9	12.2	5.1	49.5	0.8	62.9	5.2	0.3	0.1	0.6	6.9	0.5	0.4	0	15	1	0.28	3.5	5.9
HN (10km)	5	3.5	0.02	8.2	9.6	4.9	2.9	18.4	2.8	72.4	2.9	0.3	0	0.6	7.5	0.1	0.3	0	15	3	0.14	1.2	2.1
HN (40km)	5.5	4.2	0.02	3.8	10.1	6.1	3.2	21.1	1.4	38.6	1.4	0	0	0.6	6.3	2.4	0.5	0	5	1	0.23	2.6	4.3
TUN (Local)	5.5	4.2	0.02	2.8	10.6	9	10.9	23	6.7	13.3	0.3	0.9	0	3.2	25.1	1.1	0.7	0	5	1	0.23	0.2	0.4
TUN (1km)	5.6	3.9	0.01	5.7	11	6.5	3.3	20.5	1.4	34.3	1.4	0.3	0	0.6	3.4	0.3	0.1	0	15	1	0.14	1.2	2.1
TUN (10km)	4.7	3.8	0.08	94.2	61.1	67	21.3	29.1	15.9	49.1	12	0.3	0.6	1.7	98	1.7	0.2	0	30	2	0.69	7	12
TUN (40km)	5.5	4.7	0.07	94.4	52.9	106.5	35.9	134.5	1.9	38.8	14.7	0.3	1	1.9	69	4.8	2.4	0	30	2	0.6	6.3	10.7

were transferred to a growth chamber at the University of Sydney (USyd) set to a 17 °C/24 °C cycle. This reflects average temperatures in March (early autumn) for the two coastal populations. The light regime was kept to a 12 h on/12 h off cycle. To compare experimental conditions with the ambient environment, another 100 duplicate pots were transferred to the nursery at the Australian Botanic Garden, Mount Annan (MtAn). These pots were kept outside and protected from rain by a clear plastic sheet. The pots at each location were watered using 5 L of water every 2-3 days. Ambient temperature was recorded at each site using a Thermochron iButton. Because we were examining emergence and not germination, we attempted to maximize the likelihood of germination by treating the soil with germination promoter in the form of a 1:100 smoke-water solution on the first and fifth days (Emery and Lacey 2010). Seedling emergence was recorded weekly from day 20, with final emergence recorded after 90 days. Non-germinated seeds were carefully exhumed from the soils, and seedlings were grown for a further 90 days. All seedlings were then carefully exhumed from the soil and stored between Kimwipe paper (Kimberly-Clark Corporation, NSW) at room temperature for six months. Dried seedlings were then measured for length, and dry biomass was determined for both shoots and roots.

In SPSS v22 (IBM Statistics, 2013), univariate general linear models (GLMs) were initially run with the emergence data split by site location (i.e. USyd and MtAn) to analyze data by population and soil. A linear mixed model (LMM) was run using the complete emergence data as the response variable to assess potential location (i.e. temperature) differences. Site location was included as a fixed factor with population and soil site used as random covariance parameters. To determine whether any edaphic variables could explain the variation in plant biomass, we used multivariate analyses in PRIMER (Clarke and Gorley 2006) & PERMANOVA+ (Anderson *et al.* 2008). The soil factors were normalized and a resemblance matrix was calculated using Euclidean distance. Plant biomass data were square-

root transformed and resemblances among samples were calculated using the S15 Gower measure. Patterns in the edaphic data were displayed using a Principal Components Analysis (PCA) and the biomass data were displayed and checked for similarity using a non-metric multi-dimensional scaling plot (MDS). A multivariate distance-based linear model (dbLM) was performed using the edaphic variables to explain the resemblances in the biomass data. The dbLM uses permutations to assess the relationship between a multivariate data matrix and one or more predictor variables.

Climate-and-soil models – these models were generated exactly as the *Climate-only models*, but with the following addition. Spatial layers for soil pH and soil sodicity (Na) were derived from the Harmonized World Soil Database v1.2 (FAO/IIASA/ISRIC/ISSCAS/JRC, 2012). These data were resampled in ArcGIS using the 'nearest' function from 30 arc-second grids to 2.5 arc-minute grids to match the bioclimatic data, and, therefore, satisfy the requirement in *MaxEnt* for input layers to have matching resolutions.

Comparisons between the climate-only and climate-and-soil models were made by calculating the differences in the probability of suitable habitat (P(suitability)). Spatial outputs were organized into five categories of P(suitability), ranging from 0% to 100%, and each category was compared amongst models. All area calculations were performed in ArcGIS.

Results

The total area from the minimum convex polygon of *Actinotus helianthi* occurrences encompassed 454,580km^2 (Fig. 2). The AUC for the climate-only model was 0.981 ± 0.011, indicating that the model performed better than random. Mean rainfall of the driest month and mean temperature of the driest quarter were the two variables which contributed the most

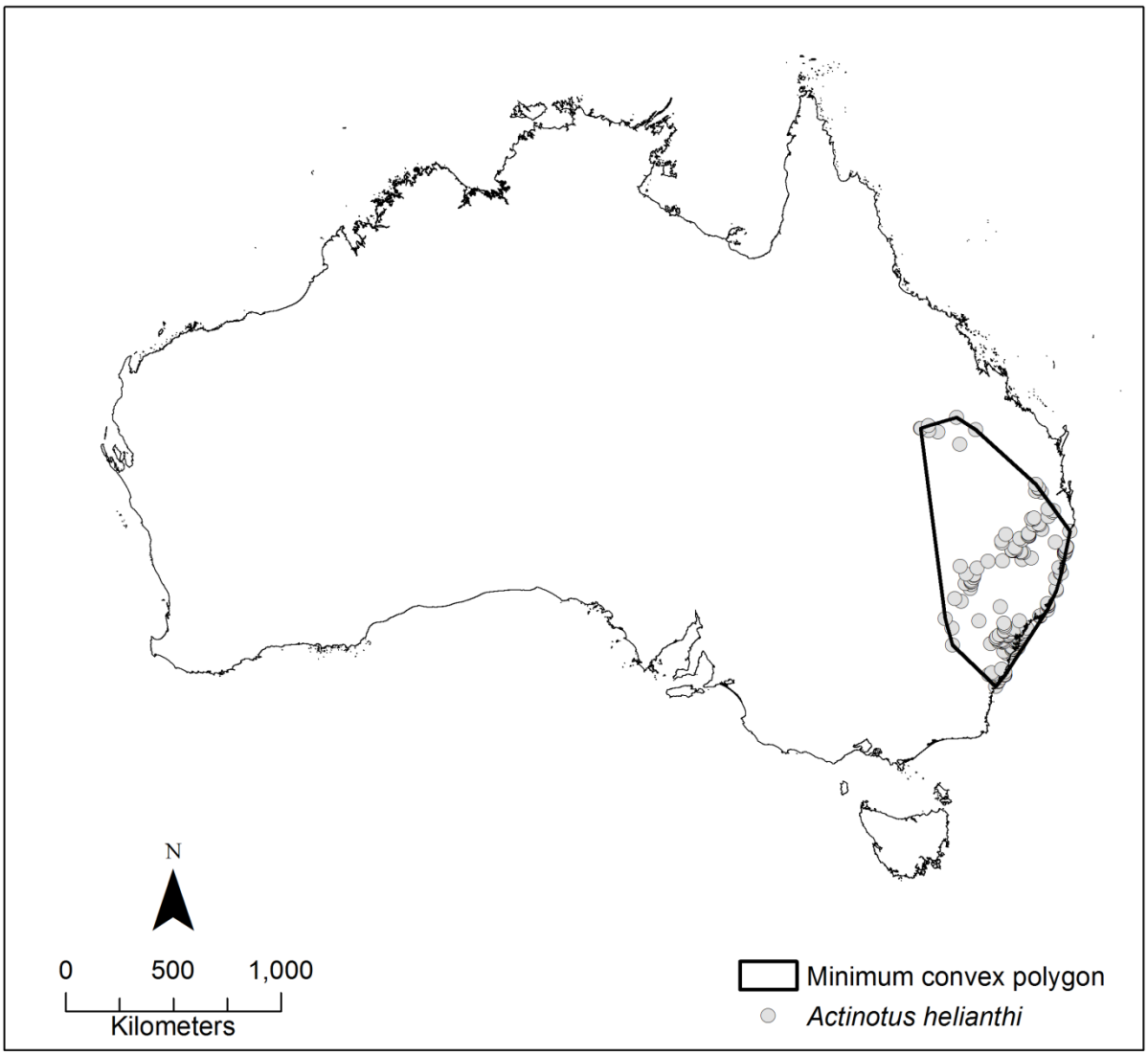


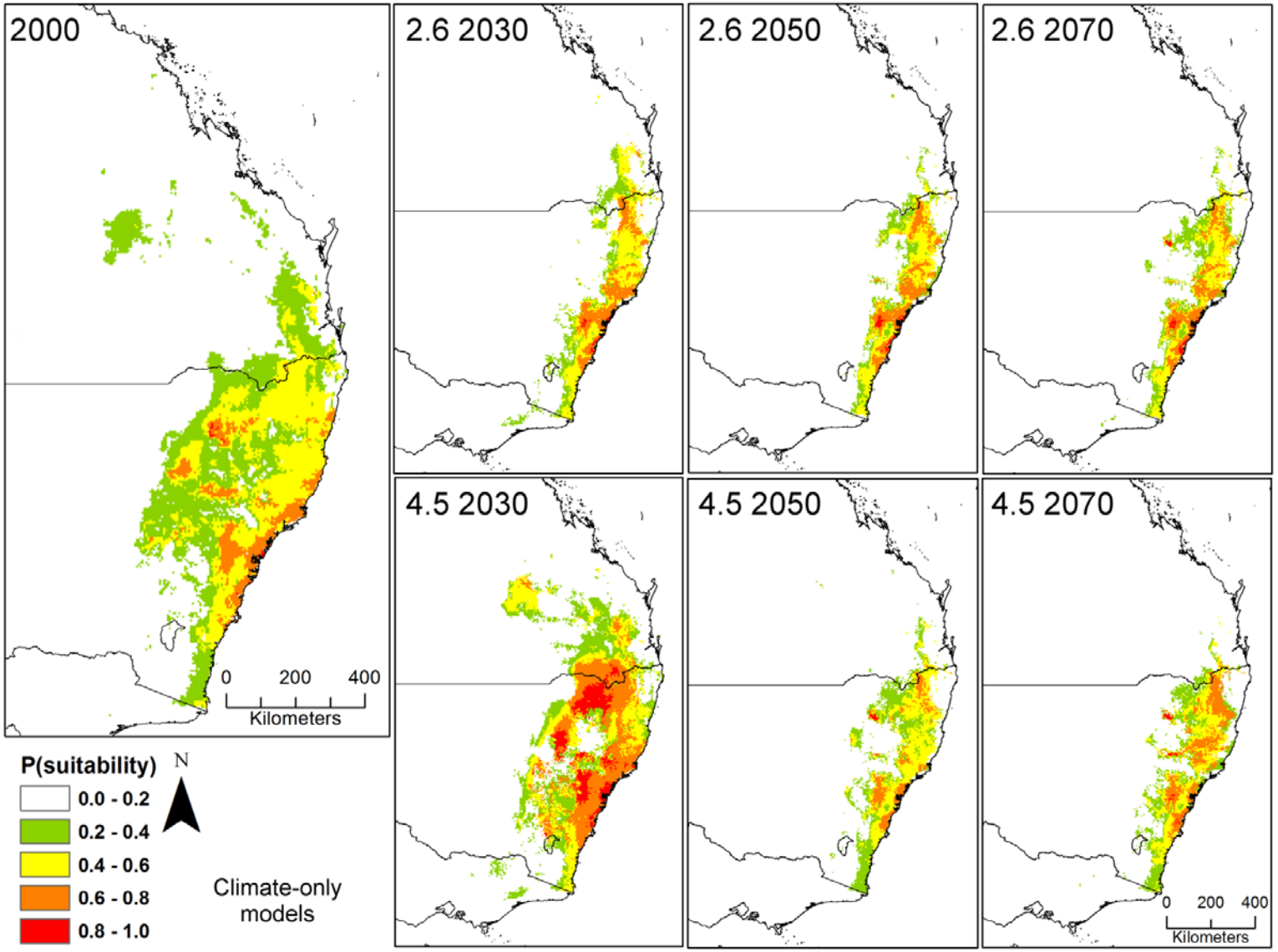
Fig. 2. The minimum convex polygon enclosing all mapped occurrences of *Actinotus helianthi* encompasses an area of 454,580 km^2.

to the overall model (Table 2). Predicted presence was highest when mean rainfall of the driest month was > 80mm and mean temperature of the driest quarter was < 15°C. The spatial output produced through ArcGIS indicated an overall trend of suitable habitats retracting south-south-east to the east coast of Australia (Fig. 3). While the four climate scenarios differed in output, the total area of P(suitability) declined in all scenarios between 44.5% (RCP4.5) and 67% (RCP8.5) by 2070. All four scenarios depicted an increase between 271% and 625% in the highest suitable habitats (80-100%) by 2070 (Fig. 4A). This equated to between 1% (RCP2.6) and 4% (RCP8.5) of the total predicted suitable area (20-100%) by 2070. All other projections, except 60-80% P(suitability) for RCP2.6 and RCP4.5, showed a decline in area by 2070 (Fig. 4A)

Mean ambient temperature at USyd and MtAn sites for the growing period were 20.4 ± 4.6 °C and 16.6 ± 8.8 °C, respectively. At MtAn, seedling emergence varied between 8 ± 2% (HN s40) to 47 ± 11% (TUN s40; Fig. 5). Seed from C6 and TUN had greater emergence on non-local soil than their local soil. Seed from three populations (excluding C6) showed greater emergence when grown in potting mix than their local soil. At USyd, seedling emergence ranged from 4 ± 3% (TUN s40) to 71 ± 13% (C6 sC; Fig. 5). Seeds from C6, HN and TUN experienced greater emergence on at least one non-local soil than on their local soil. Percent emergence also increased in all four populations when seeds were sown in the potting mix compared to the local soils. A significant interaction between population and soil site was identified at both MtAn and USyd sites ($P < 0.001$ and $P = 0.044$, respectively). When combined in the linear mixed model, the two sites were significantly different ($P < 0.001$). The covariate factors of population and soil were not found to be significant ($P = 0.375$ and $P = 0.362$, respectively).

Table 2. MaxEnt output of individual contributions of the included variables from the climate-only and climate and soil models.

Variable	**Climate-only**		**Climate and soil**	
	Percent contribution	*Permutation importance*	*Percent contribution*	*Permutation importance*
Mean rainfall of the driest month	51.9	0.4	46.6	3.6
Mean temperature of driest quarter	24	11.5	27.2	9.4
Mean annual rainfall	15.3	77.7	15.1	65.3
Mean temperature of warmest quarter	5.8	5	4.3	7.4
Rainfall seasonality	1.5	0.7	2.6	0.7
Mean temperature of coldest quarter	1	3.4	1.1	11.1
Soil pH	-	-	1.7	1.4
Soil sodicity (Na)	-	-	1.3	1.3
Isothermality	0.5	1.4	0.1	0


2000
2.6 2030
2.6 2050
2.6 2070
4.5 2030
4.5 2050
4.5 2070
0 200 400
Kilometers
P(suitability)
0.0 - 0.2
0.2 - 0.4
0.4 - 0.6
0.6 - 0.8
0.8 - 1.0
N
Climate-only models
0 200 400
Kilometers

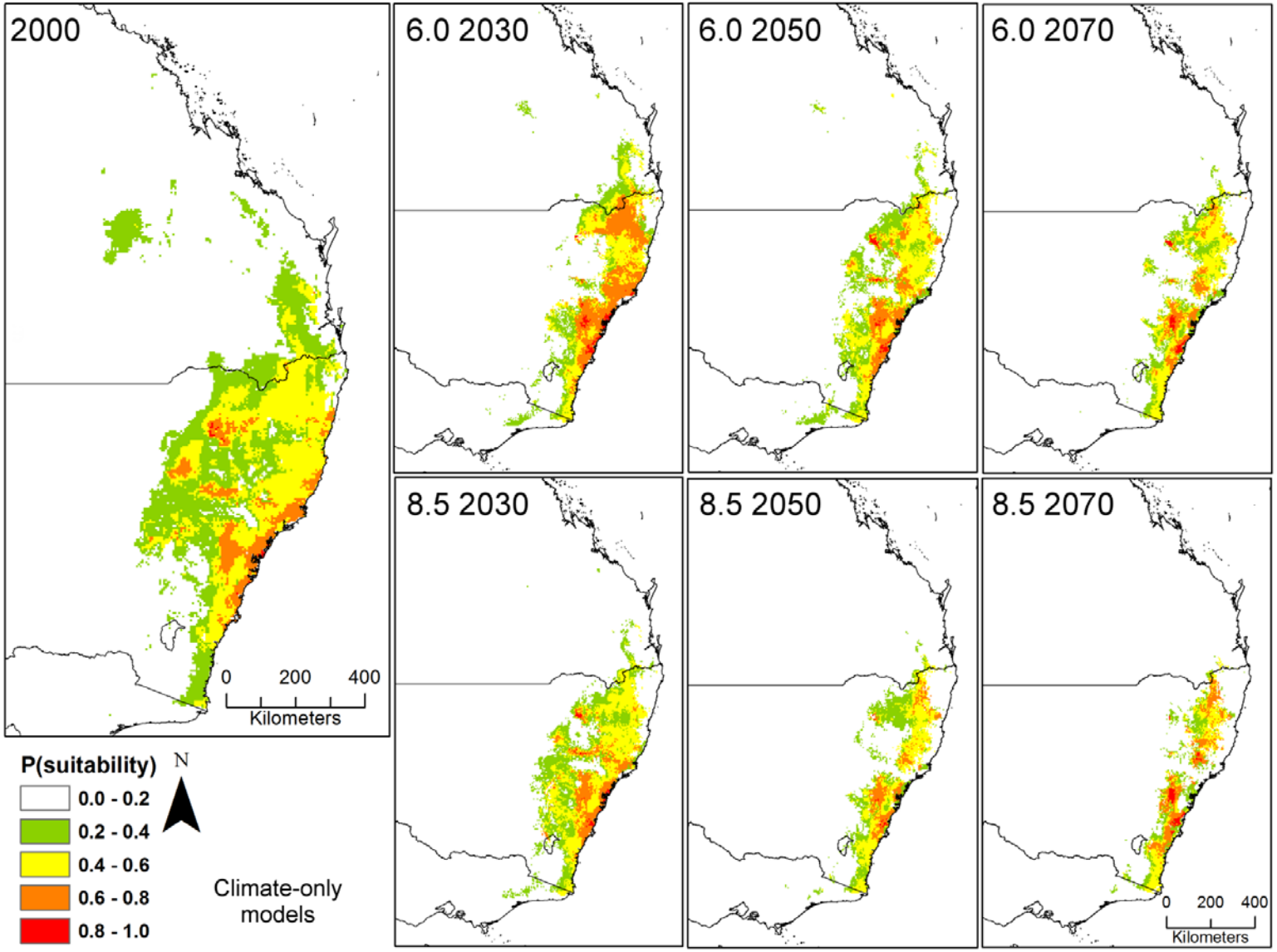


Fig. 3. Predicted change in distribution of *Actinotus helianthi* under the four AR5 climate scenarios (2.6, 4.5, 6.0 and 8.5). Different habitat suitability categories are derived from the probability of suitability (P(suitability)) from the climate only model.

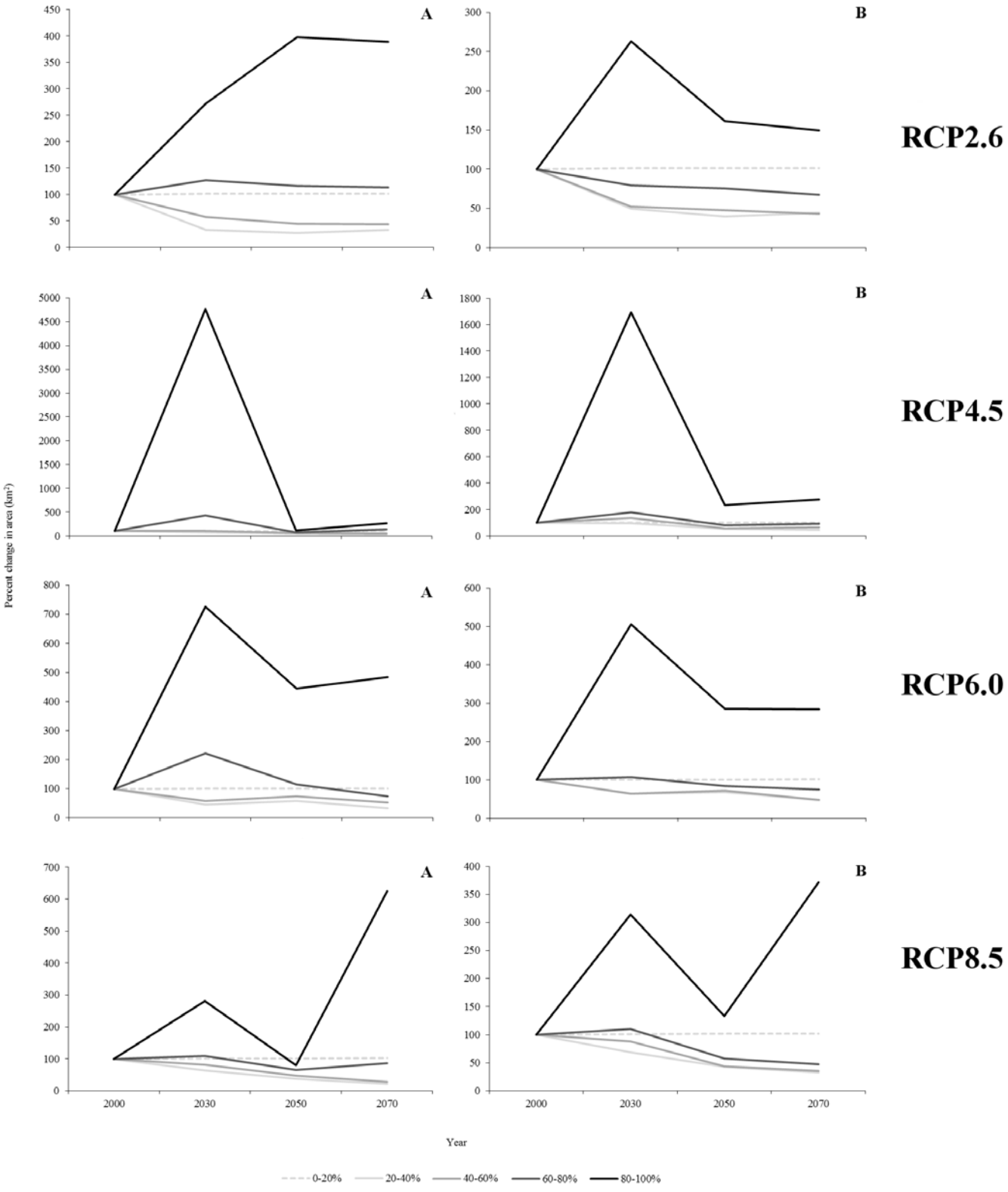


Fig. 4. Percent change in area of *Actinotus helianthi* from the current distribution under the four AR5 climate scenarios for the climate only (A) and climate and soil (B) models at different categories of habitat suitability (P(suitability)).

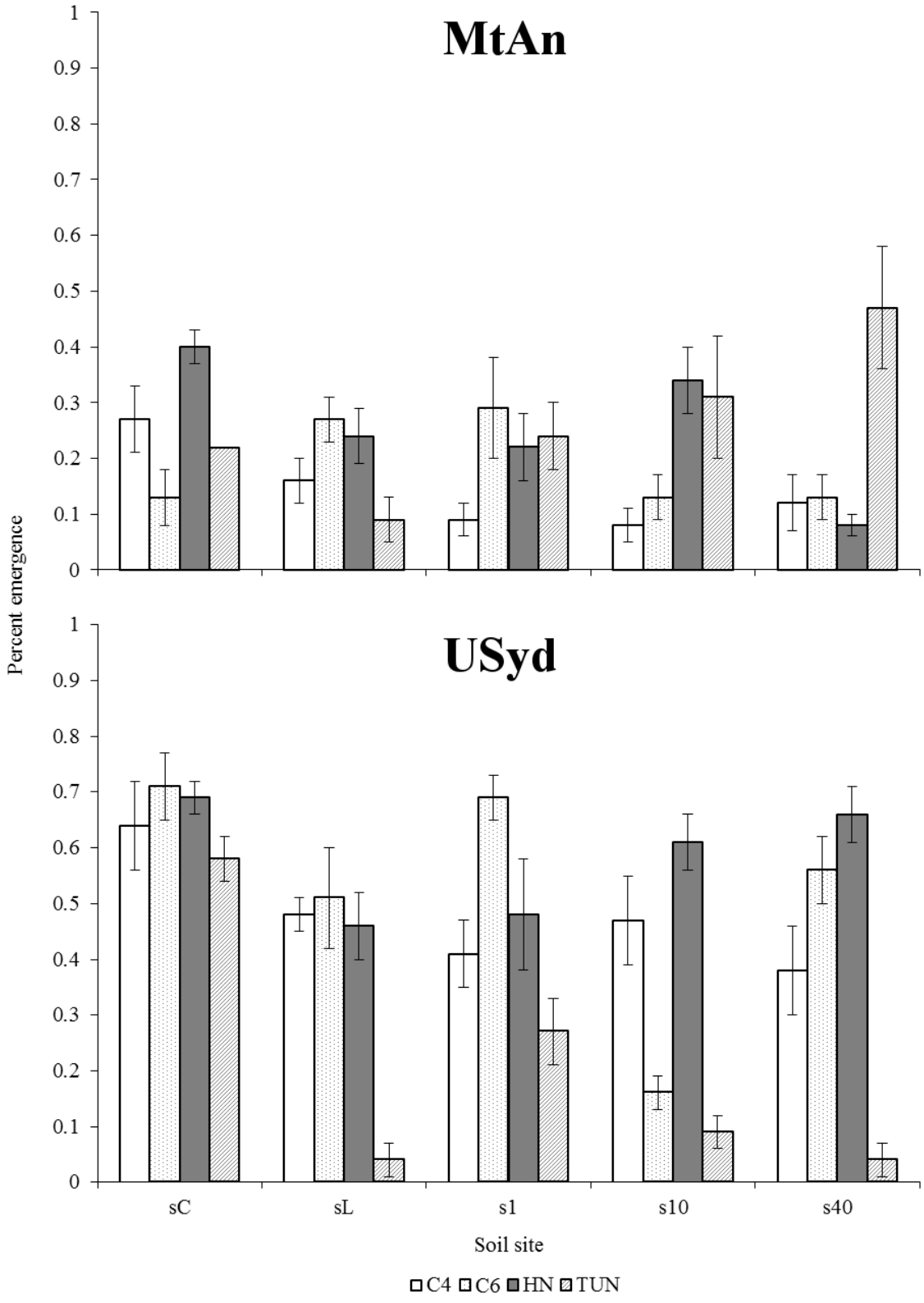


Fig. 5. Final percent seedling emergence at MtAn and USyd at day 100 across soil sites for each of the four local populations. Common potting mix (sC), local soil (sL), soil 1 km away (s1), soil 10 km away (s10), and soil 40 km away (s40).

There was an indication from the MDS plot of a longitudinal gradient of sampling from coastal clusters (left) to inland clusters (right). However, sites associated from three of the four populations area spread across the MDS plot (Stress = 0.08; Fig. 6). Pronounced variation in edaphic conditions across C6 and TUN sites was observed in the PCA plot (Fig. 7). The HN sites followed a gradient in edaphic conditions, while the separation of the C4 1 km was associated with site soil pH being non-acidic (7.1). When analyzed independently, five edaphic factors (salinity, Mg, percent Hydrogen, Mn and electrical conductivity) were significantly associated with the biomass resemblance data at $P < 0.05$, and a further seven factors (pH, Na, NO_3, PO_4, SO_4, Zn and organic carbon) at $P < 0.01$. The multivariate dbLM produced a model which included pH, salinity, Na and PO_4 to best explain the patterns in the biomass data ($R^2 = 15.1$, $P < 0.001$).

When soil pH and soil sodicity layers were added to the climate layers, the AUC of the new *MaxEnt* model was 0.981 ± 0.011, matching that of the climate-only model. Mean rainfall of the driest month and mean temperature of the driest quarter were the two variables which contributed the most (46.6% and 27.2%, respectively) to the overall model (Table 2). Soil pH and soil sodicity contributed 1.7% and 1.3%, respectively. Probability of presence was highest when soil sodicity was between 8% and 17%, and soil pH was negatively correlated with predicted presence. The climate and soil models illustrated suitable habitats to again retract south-south-east to the coast (Fig. 8). Total area of P(suitability) declined between 42.1% (RCP4.5) and 63% (RCP8.5) by 2070. All four scenarios predicted an increase between 149% and 371% in the highest suitable areas (80-100%) by 2070, equating to between 1.3% (RCP2.6) and 4.1% (RCP8.5) of the total predicted suitable area (Fig. 4B). All other combinations of P(suitability) and climate scenario were predicted to decline in area by 2070.

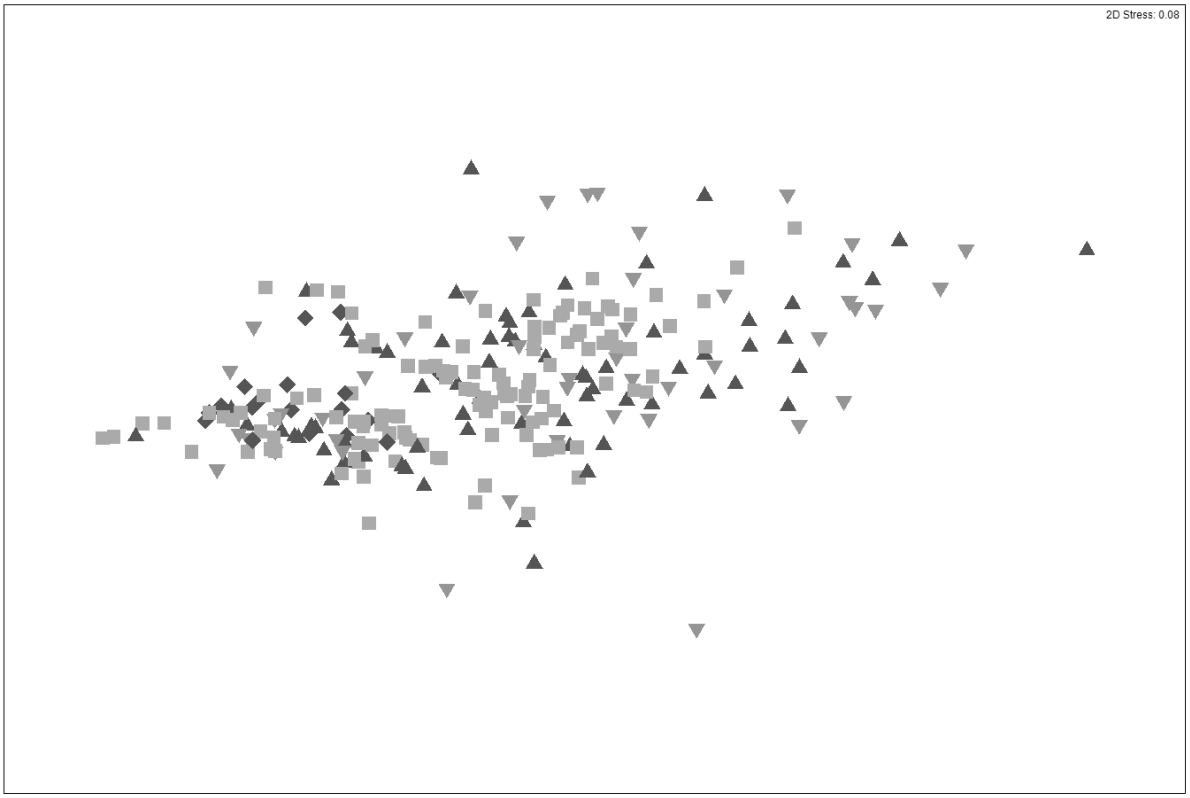


Fig. 6. Non-metric Multi-Dimensional Scaling (MDS) plot of the plant biomass data. C4 (▲); C6 (▼); HN (■); TUN (♦).

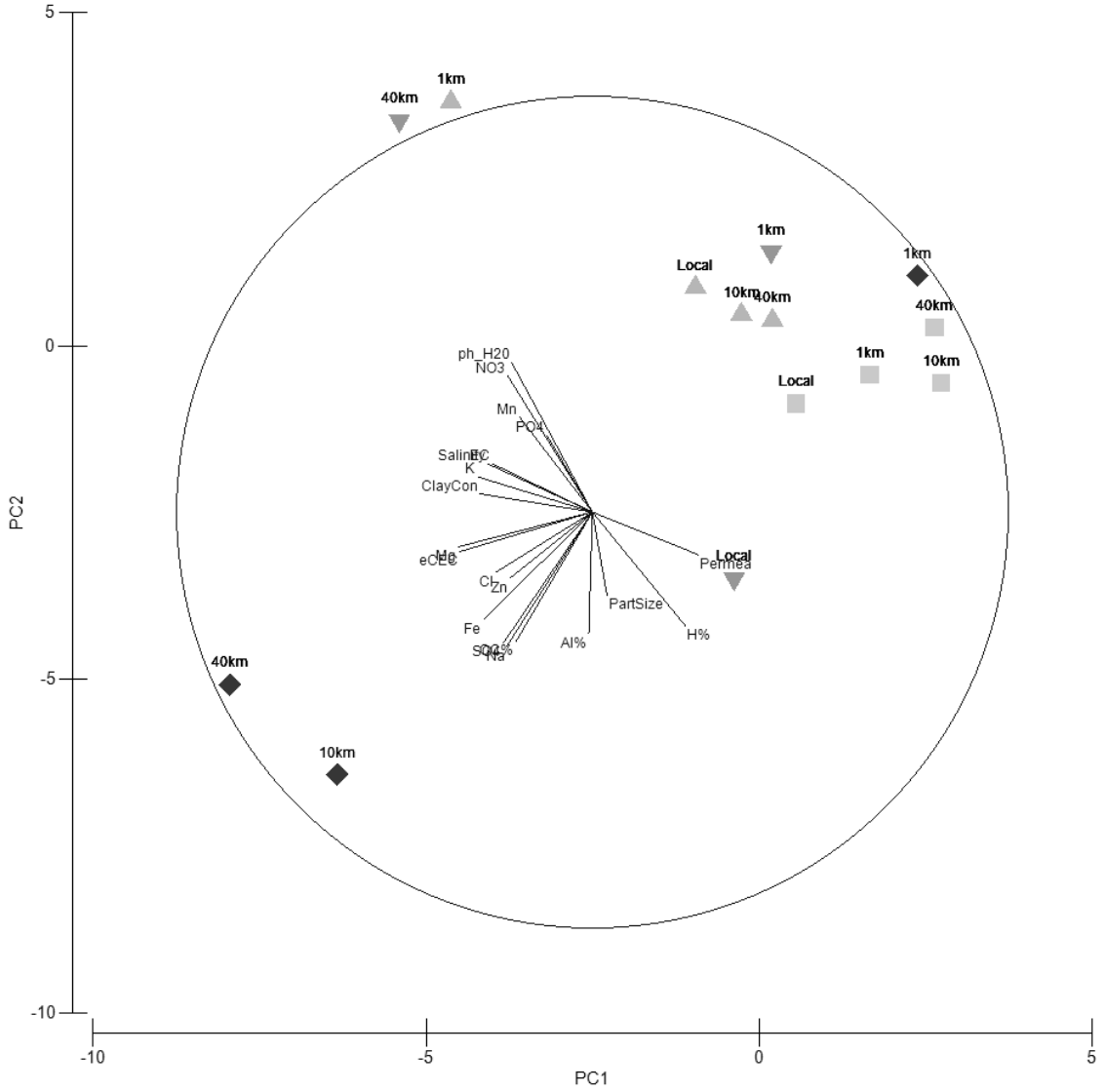
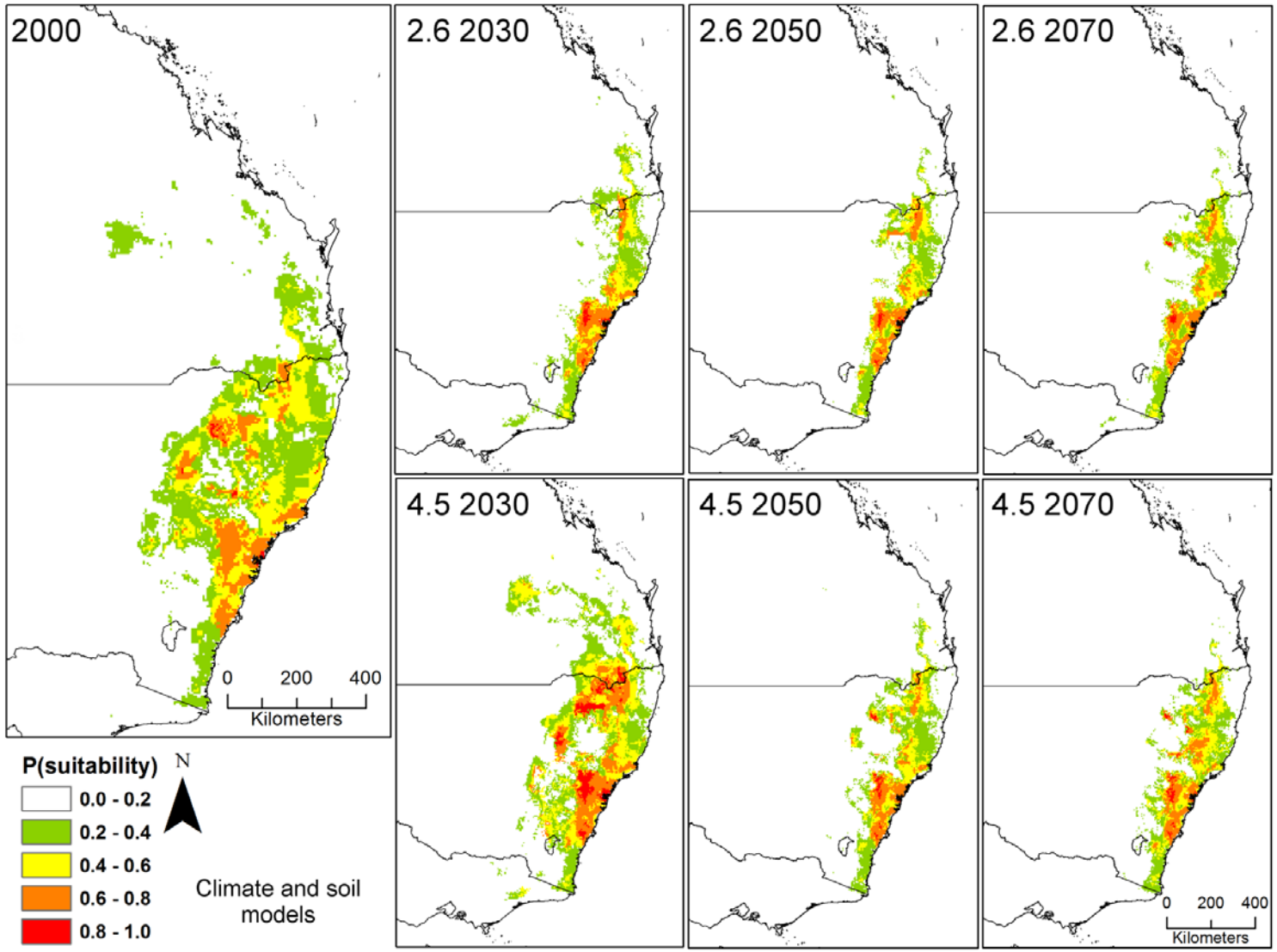


Fig. 7. Principal Components Analysis (PCA) displaying the patterns in the edaphic data. Some sites are missing from the data due to no emerged seedlings. C4 (▲); C6 (▼); HN (■); TUN (♦).


2000
2.6 2030
2.6 2050
2.6 2070
4.5 2030
4.5 2050
4.5 2070
0 200 400
Kilometers
P(suitability)
0.0 - 0.2
0.2 - 0.4
0.4 - 0.6
0.6 - 0.8
0.8 - 1.0
N
Climate and soil models
0 200 400
Kilometers

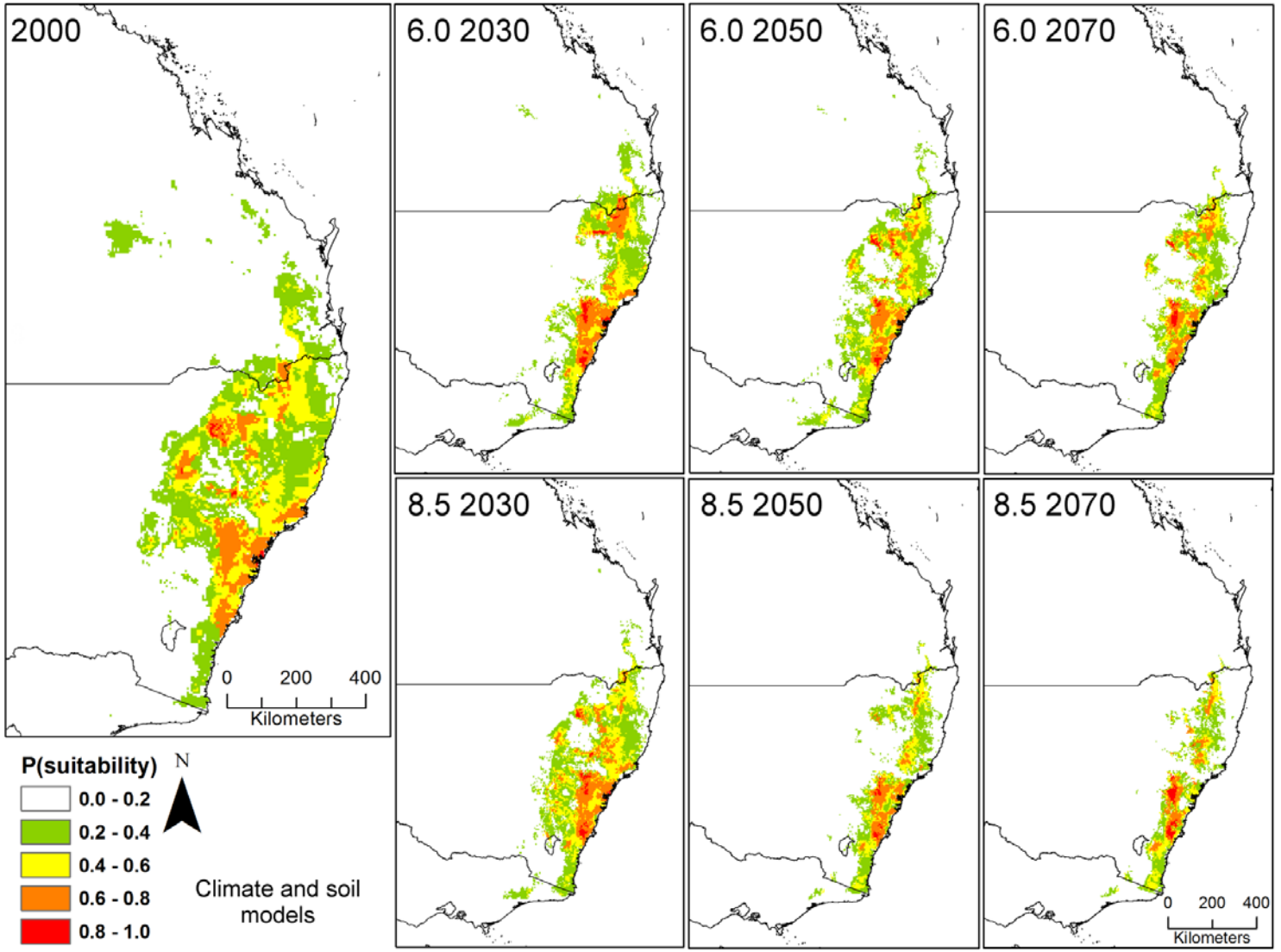


Fig. 8. Predicted change in distribution of *Actinotus helianthi* under the four AR5 climate scenarios (2.6, 4.5, 6.0 and 8.5). Different habitat suitability categories are derived from the probability of suitability (P(suitability)) from the climate and soil model

Comparisons between the climate-only and the climate and soil models identified the former to over-predict P(suitability). The climate-and-soil model predicted greater total suitable area to be retained by 2070 for all four scenarios (Fig. 9). Areas which were predicted to have the highest P(suitability) (80-100%) for the RCP2.6, RCP6.0 and RCP8.5 scenarios were over-predicted in the climate-only model by 240%, 199% and 254%, respectively (Fig. 10). Conversely, areas with the highest suitability in the RCP4.5 were under-predicted in the climate-only model by 4%. Areas projected to have a 60-80% P(suitability) were also over-predicted in the climate-only model in the RCP2.6, RCP4.5 and RCP8.5 scenarios by 45%, 50% and 39%, respectively (Fig. 10). The same areas in the RCP6.0 scenario were under-predicted by 0.2%. Least-suitable areas (0-20%) were consistently and slightly over-predicted across the four climate-only models (0.29 ± 0.07%). Mid-ranged suitable habitats (20-40%) were consistently under-predicted by the climate-only models (9.7 ± 4%).

Discussion

To progress our ability to understand the potential ecological impact of changing environments, it is crucial to identify the factors which might limit successful colonisation by species. In our study, including soil factors improved the projections of the climate-only model in three of the four climate scenarios. This is consistent with several previous studies that have added soil characteristics in distribution modelling (Coudun *et al.* 2006; De Frenne *et al.* 2014; Dubuis *et al.* 2013; Titeux *et al.* 2009). In a novel outcome, we validated experimentally, the need to include soil factors in the final model by growing seed in soil from sites which were predicted to have suitable climate in the future. The inclusion of the ecologically-appropriate edaphic variables was also robust as there was no change in the AUC between models.

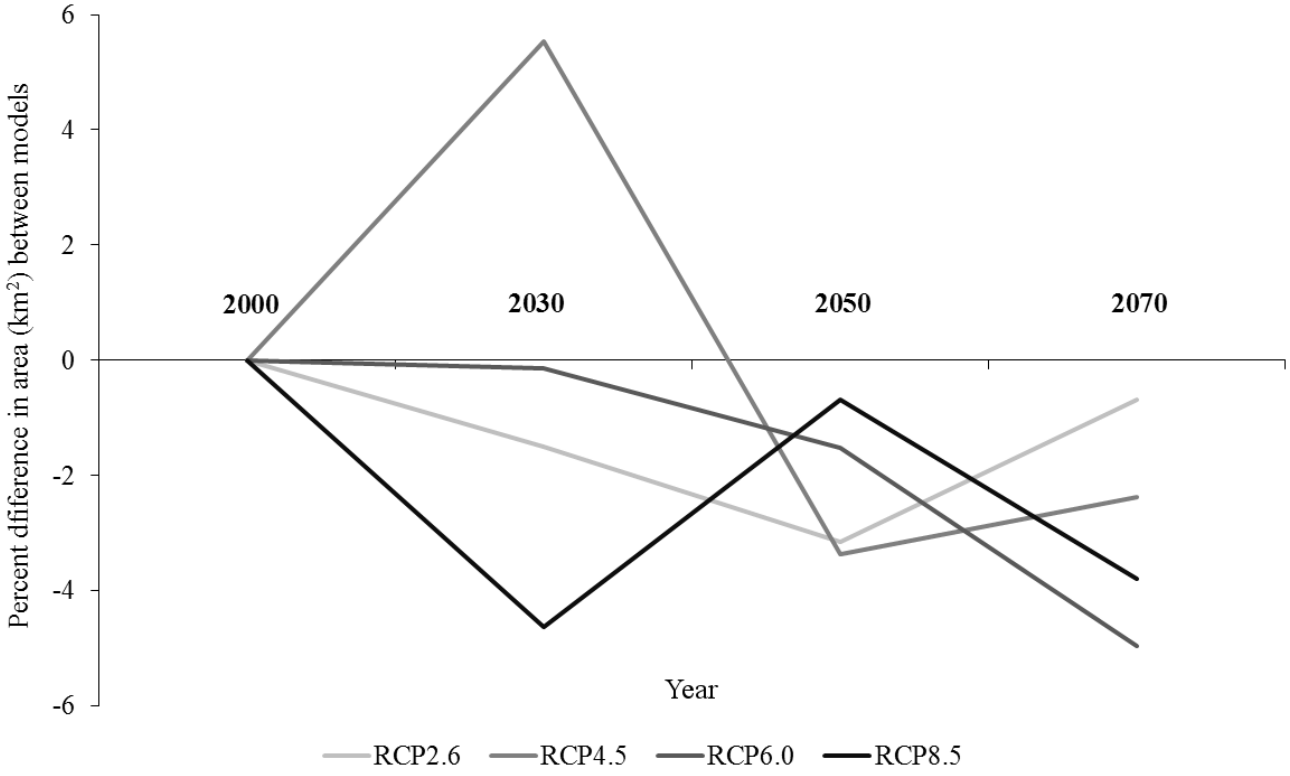


Fig. 9. Difference between the two models for the change in percent of total area of P(suitability) (20-100%) between 2000 and 2070 for the four AR5 scenarios. A positive value represents the climate-only model predicting a larger area to be retained at a time step than the climate-and-soil model. A negative value represents the climate-and-soil model predicting a larger area to be retained at a time step than the climate-only model.

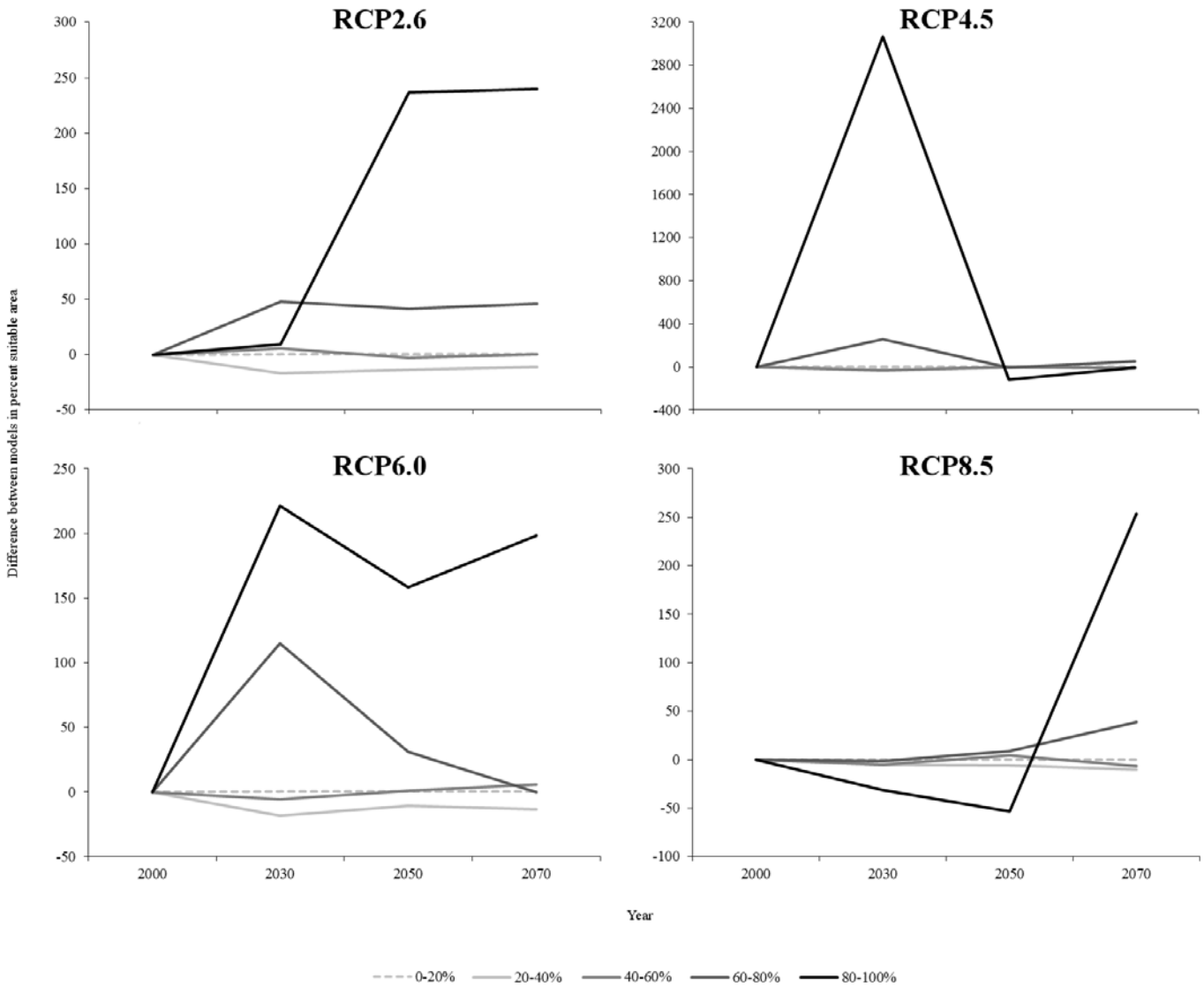


Fig. 10. Percent differences in the areas of suitable habitat between the climate only and climate and soil model projections from the current distribution. A positive value indicates an over-prediction of area from the climate only model, and a negative value indicates an under-prediction.

Soil interacted with climate to impact seedling emergence, and as anticipated, the soil environment also influenced seedling growth. There was no obvious advantage of growing on local soil from the patterns in seedling emergence across sites, indicating a capacity of *Actinotus helianthi* plants to successfully colonize non-local soils. In a similar study with the Holarctic grass, *Millium effusum,* the percentage emergence in seedlings and time to emergence were significantly lower and slower, respectively, in local compared with non-local soils (De Frenne *et al.* 2014). The effect of location also had a strong influence on seedling emergence of *A. helianthi*, which was higher across all soils at USyd, excluding TUN sites. Seeds were sown in soils during mid-winter, and night temperatures at MtAn were as low as 2.5 °C (compared to the minimum 17 °C at USyd). This could have slowed the emergence of *A. helianthi* seedlings at MtAn since faster embryo development and hypocotyl elongation occurs at warmer temperatures (Emery *et al.* 2011; Forcella *et al.* 2000; Walck *et al.* 2011). Therefore, while temperature could have affected performance and seedling emergence, the extent of its effect on *A. helianthi* will be population-dependent. Temperature effects on emergence were reported in the study by De Frenne *et al.* (2014), who highlighted the need to not only determine colonization in non-local soils, but also from the impact of the local climate from origin populations.

Four edaphic variables were identified as best explaining the variability in plant growth. Soil pH is perhaps the most important and easily quantifiable variable, and has been documented to be correlated with other edaphic variables. For example, Goldberg (1982) noted pH to be significantly correlated with calcium and magnesium in soils along the Pacific slopes of Sierra Madre. The author noted that a boundary between deciduous and evergreen species was explained by soil pH – a direct response to soil fertility due to its correlation with calcium (Goldberg 1982). Soil pH has also been reported to have a close relationship with both seedling biomass and height in *M. effusum* and *Vincetoxicum incetoxicum* spp. (De

Frenne *et al.* 2014; Magidow *et al.* 2013). The importance of soil acidity is expected as it indirectly affects nutrient availability for plants. Low pH can reduce the available P or N, through the creation of ionic complexes that cannot be used by plants. High pH can prevent the release of ions which also causes a negative impact on plant growth (Gobat *et al.* 2004).

Like most terrestrial plant species, *A. helianthi* growth was negatively correlated with soil sodicity and salinity. Many of Australia's plants are adversely affected by these two properties. Australian sodic soils are defined as having an exchangeable sodium percentage (ESP) of ≥ 6 (Isbell 2002). Sodicity is associated with low water availability and reduced of microbial activity (Rengasamy 2002). Furthermore, these soils are often structurally degraded, exhibiting poor soil porosity, and N deficiency (Naidu and Rengasamy 1993; Rengasamy 2002). Salinity did not contribute to the climate and soil models, and was therefore removed for the final model. Since *A. helianthi* does not occur on these soils, we could expect the *MaxEnt* model might not be capable of detecting the fine-scale variation identified in our statistical analyses. Whether higher-resolution models (e.g. 1 km × 1 km grids) can pick up this level of variation requires further comparative investigation.

Phosphate (PO_4) was positively correlated with plant growth. Thuiller (2013) notes that some elements, including phosphorus, are not always identified as important edaphic factors due to the fact that level vary throughout the growing season. A previous study by von Richter and Offord (2006) tested the effects of slow release fertilizer on *A. helianthi*. The authors reported greater flower, bud and stem production when plants were treated with fertilizer. Furthermore, application rate of fertilizer was positively correlated with plant height, as well as flower, bud and stem production (von Richter and Offord 2006). It has also been demonstrated that soil phosphate increases in the short-term following fire (DeBano 1991; Schafer and Mack 2010). Much of this increase can be attributed to the ash layer which

is then leached into the top soil following rainfall (DeBano 1991). Since *A. helianthi* germination is known to increase significantly across populations following fire (Emery and Lacey 2010), it could be expected that subsequent growth is positively correlated with phosphate. That 'time since fire' might be substituted for PO_4 in a predictive model would require further investigation as these data were not available for this study.

Most of the differences between the climate-only and climate and soil models were in areas that were predicted to be most suitable (i.e. 60-100% P(suitability)). Climate-only models have a tendency to over-predict suitability due to the inherent assumption that each presence record is treated the same (Araújo and Peterson 2012). Furthermore, the broad geographic extent of climatic factors means that they have a poor capacity to restrict the extent of suitability at local (i.e. population) scales. However, including more limiting environmental factors, such as soil, provide a tighter constraint to model projections. In a study of 115 plant species, pH and N were reported as improving the predictive power of topo-climate models (Dubuis *et al.* 2013). When combined with climate, pH was the second most important variable, despite its influence varying across species and their ecological preferences (Dubuis *et al.* 2013). Including the edaphic dimension in climate models has permitted an interactive effect which has improved model projections for *Acer campestre* and *Quercus pubescens* in France (Bertrand *et al.* 2012; Coudun *et al.* 2006). In the present study, despite contributing only 3% to the overall model, the soil layers constrained three of the four modelled climate scenarios enough to reduce the areas with 80-100% P(suitability) by an average of 231%. In light of our experimental results, we are confident that the precision of the climate and soil models is greater than that of the climate-only models.

Given the limitations of using climate alone in distribution models, there are also limitations associated with the choice of global climate model (GCM). The choice of GCM is

likely to affect the scope and complexity of any interpretation of the data, and the best model(s) may potentially vary between regions. Uncertainty arises from the inherent differences in emissions scenarios and regions (Irving *et al.* 2012; Kang *et al.* 2002), and the IPCC have stated that global climates are likely to increase in variability, which may then cause uncertainty among projections (Stocker *et al.* 2013). We used the Mk3.6 model as it is based on Australia's natural climate and rainfall patterns that are associated with the El Niño Southern Oscillation (ENSO) (Jeffrey *et al.* 2013). For Australia, the model predicts an increase in drought-like conditions for south-west Western Australia and south-east Australia during 1981 to 2005 that reflected the observed climate in these areas over the time period (Syktus *et al.* 2011). While there was a second CSIRO model, ACCESS1.3, that is also relevant to Australia, the required spatial data was not available for this study. This model is defined as an 'aspirational model' and differenced from Mk3.6 as it takes advantage of new atmospheric physics (Bi *et al.* 2013). Importantly, however, the purpose of our study was to highlight the importance of including soil factors in a distribution model, and not to make comparisons among GCMs.

In summary, these results illustrate the capacity to build on the initial outputs of bioclimatic models by incorporating experimental evidence to better represent the ecological preferences of species. Despite the inherent assumptions of bioclimatic models that have been outlined in several studies (Hampe 2004; Heikkinen *et al.* 2006), these models are still widely used and remain an important means to explore a species relationship with climate. Models which consider climate to be the large-scale driver of a species distribution often accept other factors such as soil to be influential at a local scale. Contrary to this notion, we have demonstrated that the incorporation of soil factors acts as a limitation to the predicted suitability of habitats for a widely-distributed species. In the context of future management, the improved precision enables more efficient determination of suitable areas for

translocations, relocations and conservation areas. While we have demonstrated the importance of the edaphic environment for a unique species, it could be expected these factors might have an even greater impact on species which have a more restricted distribution. We also accept that soil factors are unlikely to remain stable in the future, particularly in Australia given the prevalence of fire and changes in land use (e.g. agriculture, grazing and mining). Therefore, developing dynamic soil predictions would be an important next step to further improve model precision.

Acknowledgements

We thank Miguel Bedoya-Pérez for helping with the collection of soil samples. We thank Amanda Rollason at the Australian Botanic Garden, Mount Annan, for monitoring and watering plants at Mount Annan.

Chapter VI

HERBARIA AS RESOURCES TO EXTRACT PLANT TRAIT DATA TO INFORM PREDICTIONS OF CHANGED DISTRIBUTIONS FROM BIOCLIMATIC MODELS

Abstract

Climate is a strong selective force in the geographic distribution of species, yet the utility of correlating climate factors with species presence records to predict current or future occurrence has been questioned. Herbarium records are commonly used in bioclimatic models as points of occurrence, thus allowing for presence across geographic space to be interpolated using similarity in climate. These models can then be used to extrapolate to predict future presences outside of the original geographic space. Herbarium specimens present an excellent opportunity to explore the relationship between plant traits with both climate and non-climate factors. In this study, we provide an overview of how specimen data have been previously used in bioclimatic modelling and other historical studies. We then propose a use of herbarium specimens by collecting trait data and test their relationship with local climate. Such an approach is embedded within ecological biogeography where patterns in trait diversity are linked with patterns of species occurrence across space. We identified five, eight and three climate envelopes for *A. forsythii*, *A. minor* and *A. suffocatus*, respectively. The Analyses of similarity showed these climate envelopes to not be significantly associated with the plant trait data from *A. forsythii*, *A. minor* and *A. suffocatus* ($P = 0.559$, $P = 0.84$ and $P = 0.247$, respectively). We have illustrated that the capacity to detect patterns between plant traits and climate may be dependent on the spatial distance where specimens have been collected from. By exploring the relationship between plant traits

and their associated local climate, we can then make informed decisions regarding the capacity for changes to a species distribution.

Introduction

Bioclimatic modelling (BM) has been used to predict the impacts of temperature and rainfall parameters on species occurrence (Elith and Graham 2009; Franklin 2009). These models use a correlative analysis to make a predictive assessment of the impact of future environmental scenarios on the distribution of a species (Sommer *et al.* 2010). Coupled with improvements to GIS mapping and the availability of environmental data, BMs can also be applied to other fields, such as evolutionary biology, examining such phenomena as hybrid zones (Kozak *et al.* 2008) or the spread of disease (Kearney *et al.* 2009). The adaptability of BMs to a wide range of different research fields implies that an understanding of the required data and modelling type for the intended application is important. In particular, BMs rely heavily on the quantity and accuracy of the input data to avoid issues associated with biased sampling and statistical uncertainty surrounding means derived from a low sample size (Hernandez *et al.* 2006; Stockwell and Peterson 2002; Wisz *et al.* 2008).

The source of species occurrence data can be broadly divided into two methods; observational ecological surveys and collections of herbarium or museum specimens. Both approaches now typically include accurate GPS spatial locations but past records may have been only map references or general localities with wide margins of spatial uncertainty. Ecological surveys of permanent plots and long-term studies provide an even richer understanding of occurrence, as the persistence of a species at the locality is known directly (Lindenmayer *et al.* 2012). Due to time constraints, logistical reasons or financial limitations, occurrence records may be sparse, incomplete or only encompass a small proportion of a species distribution (Willis *et al.* 2003). This leads to a poor understanding of a species actual

distribution, often referred to as a ‘Wallacean shortfall’ (Mokany and Ferrier 2011). Future information may be substantially improved by widening the sources of records. For example, public databases of species records such as the Atlas of Living Australia (Atlas of Living Australia, 2014) draw from the traditional herbarium or museum records but are also being supplemented by citizen science observations that are then verified by experts before being ingested into the online resources.

Beyond the issues of the quantity or quality of the data, a basic assumption of BMs is that all occurrence records are equivalent in terms of the information they contribute on the environments experienced by the species. This overlooks the extra certainty of a fit to environment provided by a large population compared to a few scattered individuals that may not persist. Collections from the edge of a range compared to central or throughout the range may provide different information for predictive modelling. Further, BMs assume that a species is at equilibrium with climate, thereby occurring in all climatically suitable areas (Hutchinson 1957). This approach ignores the potential for species to not occupy some climatically suitable areas due to variations in traits, such as dispersal, or reproductive traits which may, in part, define the species adaptive potential (Araújo and Pearson 2005). With uncertainty surrounding whether BMs should be performed, it is difficult for researchers to build on previous methods to subsequently improve BMs so they may compliment empirical evidence.

With the arrival of online databases, such as TRY: Plant Trait Database (Kattge *et al.* 2011) that provide data for a large number of plant traits, we propose the following question: to what extent can trait data extracted from herbarium species be used to reduce the uncertainties in BMs? A plant collected for depositing in a herbarium consists of a physical sample, and that material will of course include important traits such as leaves and usually

also inflorescences, flowers or even seeds. The presence of flowers, or fruit indicate the phenological state of the individual at the time of collection, and such observations contribute to documenting the potential impact of climate change in altering phenology of that species (Chambers and Keatley 2010; Chuine 2010; Primack *et al.* 2004).

Typically, plant samples accompanied by information recorded about the habitat are often geo-referenced, permitting the data to be used in spatial modelling. Older records, however, may be missing locality data or have only broad locality details that may, nonetheless, still be valuable for modelling. For example, herbarium records for some species span several hundred years, providing a long-term dataset with which to study changes in plant traits over time. In addition, records are also often collected throughout a species geographic range allowing for some inferences on spatial or population trait differentiation to be made.

Herbarium specimens have previously been used to collect data to determine the impacts of global warming on phenology (Miller-Rushing *et al.* 2006), invasive plant spread (Fuentes *et al.* 2013), changes to flowering time (Bolmgren and Lönnberg 2005; Hart *et al.* 2014; Primack *et al.* 2004), species range shifts (Applequist *et al.* 2007; Elith and Leathwick 2007), changes to plant traits (Dalrymple *et al.* 2015; Molnár *et al.* 2012; Parkhurst 1978) and harvesting effects on plant size (McGraw 2001). Perhaps the greatest value of using herbaria as data sources is when the data are examined over a temporal scale to illustrate a measureable impact on plant phenology. For example, Dalrymple *et al.* (2015) report three asexual plant species have shown significant change up to 561% in leaf area, and another species had declined in height by 75% per 100 years. Using herbaria data, Molnár *et al.* (2012) note pollination mode and life span of orchids to have greatest associated relationship with an advancement of flowering time. However, it is important to note that variations in

trait values recorded from herbaria data cannot be extrapolated as being either adaptive or plastic (Dalrymple *et al.* 2015), rather these data provide a useful means to explore relationships between plant traits and climate.

There are several benefits to using herbarium specimens. First is the capacity to independently verify records. When herbarium specimens have been taxonomically verified through expert identification, collections of endemic species will be highly reliable (Ter Steege *et al.* 2000). Secondly, improving model precision by manipulating or otherwise removing records that are inaccurate due to inconsistencies in cataloguing or coarse-resolution sampling (Austin 1998). Finally, the potential of attaining large sample sizes means that uncertainty surrounding the performance of BM outputs is also minimized. While this is also true for ecological survey data, herbarium records have the major benefit of often being already available for use, as well as being collected over a temporal scale. Regardless of the data source, however, ecological niches of species are often highly complex and large, large sample numbers, either through herbaria or surveying, may be required in order to encompass the range of environments that the species can survive (Hutchinson 1957).

Despite potentially involving large numbers of individuals, models which use biological data from herbarium collections can also suffer from sample bias (Delisle *et al.* 2003; Loiselle *et al.* 2007). For example, locations of records are often clumped in areas with easy accessibility (e.g. roads and fire-trails). Furthermore, some models are unable to delineate sample bias from model outputs as clumped distributions are often associated with better data fitting (i.e. higher AUC values) (Luoto *et al.* 2005; Segurado and Araújo 2004). Additionally, most collections are targeted for individuals that are flowering or fruiting to incorporate reproductive characteristics. There is uncertainty surrounding using specimens for flowering responses to climate without any field observations of populations (Miller-

Rushing *et al.* 2006). Although the collected specimen may have flowers, there is often no indication whether any other individuals in the population were also flowering. Additionally, both observational and herbaria data are also presence-only records, and very few species will have absence records available from surveys. While presence-only models can compensate the lack of absence data by generating pseudo-absences, the selection and manipulation of absences needs careful consideration so they may help to minimise the effect of sampling bias in presence records (Radosavljevic and Anderson 2014; Warton and Aarts 2013). Therefore, issues regarding the collection and sampling of both presence and absence records can be related to how the model deals with such data.

However, uncertainty remains regarding if extracting trait data from herbarium specimens can provide a resource for assessing the uncertainty between climate and phenology, and, therefore, potential changes to modelled distributions. Here, we propose a method of extracting and testing phenological trait data from herbarium specimens to inform researchers as to whether climate can predict the patterns in plant traits. To illustrate this, our aim was to sample plant trait data extracted from herbarium specimens of three *Actinotus* species to then determine whether patterns in plant traits were explained by macroclimatic factors. Long-term average climate variables are publically accessible and are common practise for bioclimatic modelling (Hijmans *et al.* 2005). If our approach could be used to detect whether patterns in species traits are associated with their local climate, it could greatly improve our ability to determine whether macroclimatic or topoclimatic factors drive a species distribution (Slavich *et al.* 2014). If traits are not associated with climate, then we argue that BMs may predict inaccurate future shifts in distributions. We have previously documented climate envelopes to be poorly associated with *A. helianthi* plant trait values recorded in the field (Chapter 2). As *A. helianthi* is widespread and occurs over a latitudinal range of 14 degrees it is perhaps in hindsight not unexpected that there is little pattern of

plant traits in relation to local climate. However, this finding may not extend to species that are more restricted in their distribution. To test this idea we selected three other *Actinotus* species that are more restricted in their spatially distribution and that have partially sympatric distributions and thus experience a similar range of climates. By testing three species with restricted distributions, our aim was to determine whether species with restricted spatial distributions have plant trait values that are related to the local climate. It was predicted that life-history might affect a species interaction with climate. Therefore, each species was selected as having a different life-history, as well as occurring in different bioregions.

Materials and methods

Plant specimens from the National Herbarium of New South Wales and the John Ray Herbarium (The University of Sydney) were used in this study. A total of 24 *Actinotus forsythii* Maiden & Betche, 60 *A. minor* Sm. and 20 *A. suffocatus* Hook.f. specimens were sampled (Fig. 1). *A. forsythii* is a fire-ephemeral herb up to 50 cm in height, recorded from the Sydney Basin and South Eastern Highlands bioregions (IBRA, 2007) in New South Wales (NSW) as well as east Gippsland (Victoria). The species comprises a single tap root and inflorescences protrude from a central rosette of compound leaves at ground level (Benson and McDougall 1993). Flowers have small pink petals which give the capitulum a characteristic pink hue (Fig. 2A). *A. minor* is a perennial herb comprising wiry branches up to a height of 50 cm, and is endemic to the Sydney Basin, Brigalow Belt South and South Eastern Highlands bioregions (Benson and McDougall 1993). The species can persist in the landscape as seed in the soil seedbank or by re-sprouting following fire. *A. minor* has small clusters of apetalous flowers approximately 12 mm in diameter (Robinson 2003) (Fig 2B). Umbels contain 6-12 central hermaphroditic flowers surrounded by up to 50 peripheral staminate flowers (Willmott 2000). Both *A. forsythii* and *A. minor* occur on nutrient-poor, Hawkesbury-derived sandstone soils. Both species have flowers arranged into densely packed

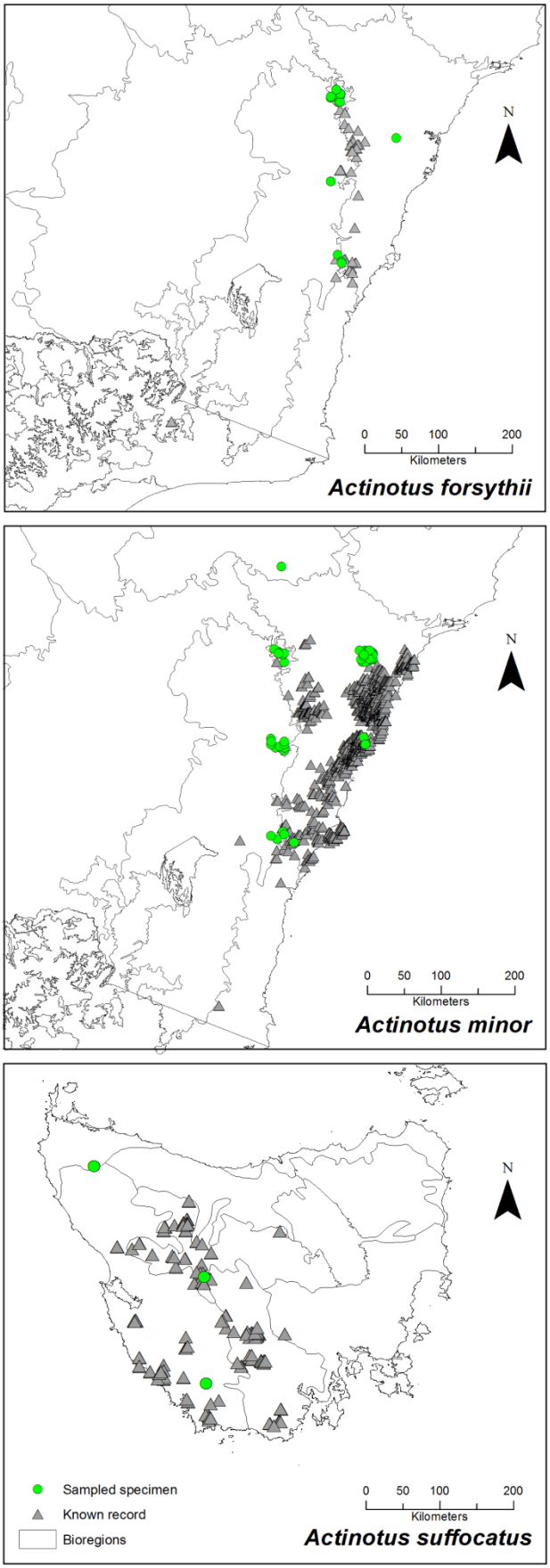


Fig. 1. Locations of sampled herbarium specimens compared to their known distributions. Known records were downloaded from the Atlas of Living Australia (www.ala.org.au).

Fig. 2. A capitulum of *Actinotus forsythii* (A), *Actinotus minor* (B), and *Actinotus suffocatus* (C). Photos by Nathan Emery.

umbels, with central female flowers surrounded by male-only (staminate) flowers. Both have umbels which are pseudanthia. *A. suffocatus* is a rosette-forming herb to 5 cm tall, found in the sub-alpine regions of Tasmania (TAS). The umbel is 1 cm wide and contains 5-10 flowers, and subtended by green involucral bracts. The species has long, rhizome-bearing roots and plants form dense mats along the ground (Fig 2C).

Current baseline climatic data comprising 19 variables were downloaded from the WorldClim online database (www.worldclim.org) at the highest resolution available (30 arc-second; $1km^2$ grids). These data are the most current long-term (1950 to 2000) climate averages currently available. Although some specimens were originally sampled pre-1950, we made the assumption that the WorldClim climate averages were representative of the historical local climates. The climate data were clipped to Australia, and climate values were extracted for each species spatial presence record using ArcGIS v10.1 (ESRI, 2012). In SPSS v21 (IBM, 2012), we ran univariate correlations using Pearson's coefficient to check for collinearity between the climatic variables for the three datasets. Due to low sample sizes, we used a conservative value of $\geq \pm 0.85$ to determine collinear variables (Dormann *et al.* 2013; Elith *et al.* 2010). A total of four, six and three climate variables were retained for *A. forsythii*, *A. minor* and *A. suffocatus*, respectively (Table 1; Appendix 4).

Six plant traits were measured on each specimen (Table 1). Average leaf length was highly correlated with longest leaf length, and the former was not included in the analysis. Specimens that were missing data from more than one trait were removed from the database, leaving a total of 19 *A. forsythii*, 53 *A. minor* and 20 *A. suffocatus* specimens for analysis.

For each species, the trait and climate datasets were imported into PRIMER v6.1.16 (Clarke and Gorley 2006) & PERMANOVA+ v1.0.6 (Anderson *et al.* 2008). Trait data were

Table 1. Plant trait and climate variables used in this study. Trait variables in bold were used included in analyses for all three species. Plant trait measurements are in mm. Capitulum width was measured as the distance from one bract tip to the opposite, including the umbel.

Plant traits	**Climate variables**
Umbel no. on specimen	Annual mean temp. *+^
Umbel width	Mean diurnal range
Capitulum width	Isothermality *
Average leaf length	Temp. seasonality
Length of longest leaf	Max. temp. warmest month
Width of longest leaf	Min. temp. coldest month +
	Temp. annual range
	Mean temp. wettest quarter
	Mean temp. driest quarter
	Mean temp. warmest quarter *
	Mean temp. coldest quarter
	Annual rainfall *+^
	Rainfall of wettest month ^
	Rainfall of driest month *
	Rainfall seasonality
	Rainfall wettest quarter
	Rainfall driest quarter
	Rainfall warmest quarter *+
	Rainfall coldest quarter

* denotes climate variable was used for *Actinotus minor*.
+ denotes climate variable was used for *Actinotus forsythii*.
^ denotes climate variable was used for *Actinotus suffocatus*.

normalised before resemblances were calculated using the Euclidean Distance measure. Resemblances were also calculated for normalised climate data using Gower's dissimilarity measure (Gower 1971). Climate resemblances were then run through an agglomerated Hierarchical Cluster Analysis (HCA). Samples were grouped into 'climate envelope clusters' if the resemblances had at least an 85% similar climate. The trait datasets and their respective climate envelopes were then projected and visualised in a Multi-Dimensional Scaling (MDS) analysis. One-way Analyses of Similarity (ANOSIM) tests were performed for each of the datasets using the climate envelopes as predictors of plant traits. We then ran Distance-based Linear Models (dbLM) to determine which climate factors, if any, were significantly correlated with the plant trait resemblances.

Results

The average number of umbels per specimens was 39.58 ± 8.22, 8.11 ± 1.81, and 0.9 ± 0.1 for *A. forsythii*, *A. minor* and *A. suffocatus*, respectively (Table 2). Similarly, the other four trait values were representative of plant size, with *A. forsythii* having larger umbels, capitulums and leaves than *A. minor* and *A. suffocatus* (Table 2). From the HCAs, five, eight and three climate clusters for *A. forsythii*, *A. minor* and *A. suffocatus*, were identified respectively. The climate envelopes showed poor differentiation across two-dimensional space in the MDS plots (Stress = 0.09, 0.15 and 0.08 for *A. forsythii*, *A. minor* and *A. suffocatus*, respectively; Fig. 3). Results from the ANOSIMs identified the respective climate envelopes to not be significantly associated with the trait data for *A. forsythii*, *A. minor* and *A. suffocatus* ($P = 0.559$, $P = 0.84$ and $P = 0.247$, respectively). Consequently, no climate variables were significantly correlated with either *A. forsythii* or *A. suffocatus*. A significant model was identified with *A. minor*, which comprised mean temperature of the warmest quarter and isothermality ($P = 0.007$). The correlation of this model was weak ($R^2 = 0.106$).

Table 2. Mean and standard errors for the five plant traits used for analysis. The oldest and most recent collection years from the sampled specimens are given.

	A. forsythii (n = 19)	***A. minor*** (n = 53)	***A. suffocatus*** (n = 20)
Range of collection dates (years)	1906 - 2010	1900 - 2010	1960 - 2005
Umbel no. on specimen	39.58 ± 8.22	8.11 ± 1.81	0.9 ± 0.1
Umbel width (mm)	15.16 ± 1.01	10.73 ± 0.45	3.05 ± 0.33
Capitulum width (mm)	8.37 ± 0.53	4.94 ± 0.17	1.6 ± 0.27
Length of longest leaf (mm)	48.16 ± 4.32	21.98 ± 0.98	5.95 ± 0.21
Width of longest leaf (mm)	18.79 ± 3.02	12.79 ± 0.67	1.9 ± 0.12

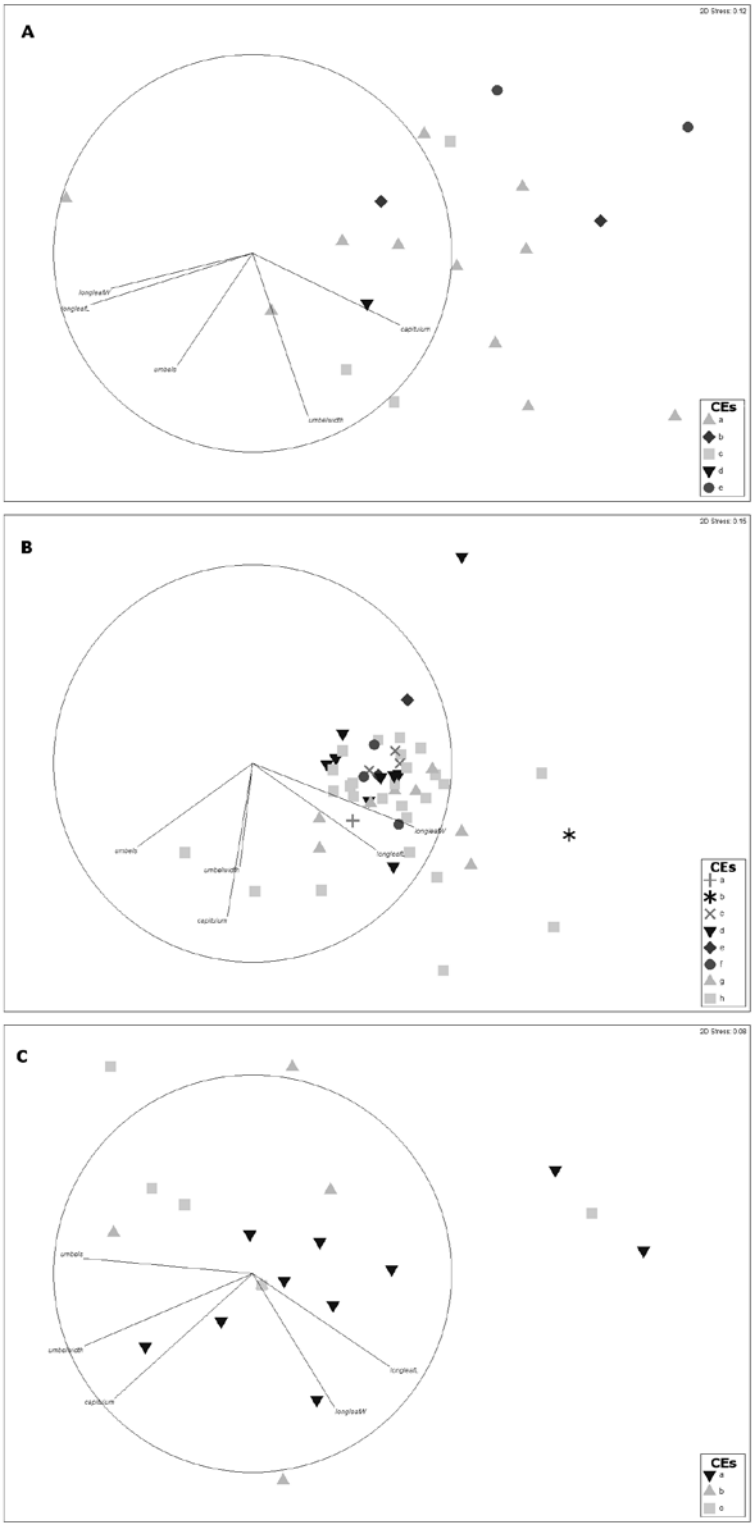


Fig 3. Multidimensional Scaling (MDS) plots of *Actinotus forsythii* (A), *Actinotus minor* (B), and *Actinotus suffocatus* (C). The trait resemblance data are visualised in space by climate envelopes. Traits were number of umbels on specimen (*umbels*), umbel width (*umbelwidth*), capitulum width (*capitulum*), length of longest leaf (*longleafL*), width of longest leaf (*longleafW*). Symbols represent the different climate envelopes for each species. CEs: climate envelopes.

Discussion

We have illustrated that plant traits can be successfully extracted from herbarium specimens for use in climate studies. Furthermore, we were able to collect data from specimens which represented the known distributions of each of the three *Actinotus* species. When analysed, we demonstrated that climate had little predictive power on the plant traits recorded. Since all three species have geographically-restricted distributions, which encompass between two and five bioregions, it is possible that climate is too broad to be influential at such a fine scale. *Actinotus helianthi*, which is known to occur across a wider geographic extent (14 bioregions) than the three *Actinotus* species tested here, also showed little relationship between plant traits and climate envelopes (Chapter 2).

While *A. helianthi* traits were recorded in the field, for annuals or fire-ephemerals, such as *A. forsythii*, herbaria records may be more abundant and easily accessible than field observations, providing greater power and precision to phenological studies of such species. Specimens may also extend well into the past and contain phenological data from numerous records across the species known distribution. However, we do not discount the importance of field records. Both observational and vouchered data are subject to uncertainties. For example, if examining the effects of climate on flowering times, peak flowering is only approximated by a herbarium specimen, and could be missed by up to several weeks (Miller-Rushing *et al.* 2006). For this reason, the number of umbels counted on each specimen is unlikely to be indicative of the total number or reproductive effort of the whole plant. Furthermore, it may be unreasonable to suggest from one or two collected specimens from an area that they are indicative of peak flowering within the population. Without any descriptive observational data on the local population and habitat, it is then difficult to extrapolate any generalisations about intra- and inter-population variation in plant traits. Further

investigations are required to determine the relative importance of these uncertainties, and how they may subsequently influence BMs.

When interpreting our results, it is important to note that we assumed that the climate baseline averages between 1950 and 2000 would be representative of the local climate of each herbarium specimen, regardless of the year it was collected in. We made this assumption so our results would be relevant for distribution modelling, as these data are commonly used in BMs (Hijmans *et al.* 2005). While we could have also used the appropriate annual climate data for each recorded specimen, it would then be difficult to standardise these data as spatial layers for modelling using the same herbarium records. Additionally, for this study we examined the variation in plant traits across a spatial extent to create climate envelopes rather than the relationship between traits and climate over time. Some species have records that are poorly represented over time. For example, the 20 *A. suffocatus* records had been collected in either 1960 or 2005. However, to limit the uncertainty of our assumption, future studies could examine and compare bioclimatic averages as well as annual climate variables where temporal historical records are available.

It has long been a criticism of BMs that they do not, or are unable to, incorporate factors which vary at finer geographic scales (Luoto and Heikkinen 2008; Pearson and Dawson 2003). Several factors have been shown to be more influential at a local scale when compared with climate, which can influence distributions at a macro-scale. These may also have contributed to the variation exhibited between the traits recorded in our study. For example, when land-cover was added to climate variables in a hierarchical model approach, the predictive power was found to increase in two plant species in Britain, *Rhynchospora alba* and *Salix herbacea* (Pearson *et al.* 2004). Specifically, their model was better able to determine areas with suitable climate but unsuitable land-cover at the finest resolution of 1

km, but not at 10 km. The authors state that factors, such as land cover, should be modelled at fine scales in order to represent the variation across the landscape. When performing BMs at course resolutions, it is possible for a saturated prediction of suitability due to 'suitable' patches of land cover existing in cells that contain presence records (Pearson *et al.* 2004). However, it is suggested that models which use multiple topological variables, such as land cover, geology and elevation, can outperform models which use land cover only (Illán *et al.* 2010; Slavich *et al.* 2014). This is likely to be more important for species that are present in mountain areas, such as the three species in this study.

We cannot also discount the potential of soil to be influencing plant traits at the local level. Indeed, climate alone has been documented to poorly predict species distributions when direct soil factors are added to the original climate model (Dubuis *et al.* 2013). Indeed, we have previously demonstrated that *A. helianthi* plant traits were better predicted by soil type and bioregions than climate (Chapter 2). Climate was also reported to over-predict the extent of future suitable habitats of *A. helianthi* when soil factors were added to the model (Chapter 5). At a rudimentary level, plants are impacted by soil pH as it controls the release of important ions (Gobat *et al.* 2004). Dubuis *et al.* (2013) reported soil pH, and to lesser extent Nitrogen, significantly improved model accuracy over BMs when tested across 115 plant species. Model improvement was not ubiquitous across species, rather soil factors were important for plants with low surface leaf area or high leaf dry matter content (Dubuis *et al.* 2013). These traits are common to species which uptake nutrients slowly and are often found on acidic and nutrient-poor soils (Pellissier *et al.* 2010). Furthermore, the capacity of a plant to change its traits to better suit different soil environments is likely to be species specific (Hancock *et al.* 2013). Since we have determined that climate factors are not correlated with *A. forsythii* and *A. suffocatus* traits (and only very slightly with *A. minor* traits), the next step would be to test other 'environmental envelopes', such as soil, topology, or bioregions.

Herbarium specimens represent a large source of data which can then be used to improve bioclimatic modelling by indicating whether finer scale climatic data are needed, or even whether climate data influence those particular plant traits in a predictable way. The capacity for species to adapt traits to environmental factors will play an important role in species persistence and, therefore, the distribution across the geographic landscape. For the three *Actinotus* species used in our case study, we have demonstrated that climate may be too broad to be associated with traits for plant species which have restricted distributions. This raises the question of whether more fine-scale environmental factors, such as soil, might be better suited to examining plant trait variation. While our approach may be novel, we have outlined a foundation and encourage further testing on a wider array of species with varying life-histories, as well as screening for relationships between traits and non-climate factors across multiple species.

Acknowledgements

We thank Lisa Popenhagen for collecting trait data from the herbarium specimens.

Chapter VII

GENERAL DISCUSSION

Main findings

Species Distribution Models (SDMs) are an integral part of ecological management in predicting the impact of future environmental conditions on species or communities. Starting with known records of occurrence, is it possible to ask what drives the extent of the geographical distribution and is that closely related to known differences in environmental factors? Presumably, at larger spatial scales, climate plays some role in setting the context for whether the conditions support the continued persistence of a species. However, if a species exists as multiple populations, each with its own locally different environmental conditions, what can we expect in any mapping of occurrence to present or future climate envelopes?

The obvious utility of SDM approaches means that it is also necessary to examine the model assumptions. As with all models, there is a balance between the level of generality of broad models that may lack predictive power (Evans *et al.* 2013) and highly specialised models that require copious amounts of data to parameterise them, and yet risk overfitting complex data (Lonergan 2014). We also need to be aware of instances where the quality of the information in terms of the spatial resolution of either the occurrence records or the climatic data may impact on the predictions. Therefore, it is vital to test the extent that some of the basic assumptions might have on model predictions in order to improve model outputs. To this end, I identified several areas where progress could be made, and appropriate experimental integration with SDMs can be used to address these. Using *Actinotus helianthi* Labill. as a model system, I examined several factors relevant to ecological preferences of

species, including whether: (1) phenological traits could be predicted by climate factors, (2) early performance traits, such as germination and survival, were genetically fixed factors, (3) insect visitor abundance and diversity were site-specific, and what effect this might have on reproductive success, (4) edaphic characteristics limited the capacity of the species to colonise new, climatically-suitable habitat, and (5) patterns in plant trait data extracted from herbaria specimens could be related to macroclimatic factors.

Species are defined by their populations, which are defined by the individuals and their traits. Although the major assumption of most SDMs is that populations will respond to climate in the same way, it is apparent that populations are not equivalent in their plant traits. There was little evidence to support a strong match between climate and the phenotypes of plants at a given location. It is expected that populations experience different climates throughout a species distribution. In this case, if populations exist across a wide temperature range, then individuals must be able to survive at different temperatures. However, the capacity of individuals to thrive in different temperatures will vary across populations, and not all temperatures within the species range will be equally suitable for all individuals. For example, plants at the margins of their distribution range may have a more narrow range of suitable temperatures for survival then individuals towards the centre of the distribution (Bridle *et al.* 2010; Eckhart *et al.* 2011). Without the capacity to match climate with individual traits, the main premise of SDMs is less compelling.

Species with wide geographic distributions experience a wide range of climates, and thus provide an appropriate system to explore the relationships among environmental variables, traits and ecological interactions. This study used a model system to test several assumptions surrounding SDMs, and then build on macroclimatic models by incorporating limiting environmental data. Firstly, phenology, which is expected to be closely linked to

climate as flowering and other events can be highly seasonal in many species (Chambers and Keatley 2010), was shown to have a poor relationship with local climate for *A. helianthi*. Furthermore, I obtained similar results when using traits extracted from herbarium specimens of *A. forsythii*, *A. minor* and *A. suffocatus*. Secondly, recruitment is also expected to be strongly influenced by the environment in plants (Ooi *et al.* 2009; Ooi *et al.* 2012; Walck *et al.* 2011). If so, then early stages of the life cycle should be closely matched to their local environment. The early performance traits of *A. helianthi* significantly varied at multiple scales, including plant, population and bioregion (according to the Interim Biogeographic Regionalisation of Australia). Thirdly, if phenotypes are well matched to the environment, then species interactions may also be matched to local conditions, but these may be affected by changing climate. Seed set varied among populations and local climate was found to be partially influential. While insect abundance and diversity differed among sites, there was an indication that higher seed set, and, therefore, reproductive output, was positively associated with the presence of flies. Low reproductive output suggests that there is a poor match between individuals and their environment, signaling the possibility of poor persistence in the future. That fact that *A. helianthi* is partially reliant on interactions with insects for its reproductive output suggests the species may be unlikely to expand its range beyond that of its visitors. Finally, even if a species can disperse into new environments, individuals must already possess the traits to successfully colonise these areas. The success of plant colonising an area is largely influenced by the physical and chemical properties of the soil. The edaphic environment is one of the most commonly recognised environmental variables that can limit a species distribution (Thuiller 2013). *A. helianthi* seedlings emerged and grew at significantly different rates across local and nonlocal soils, and showed no adaptive advantage on local soil. Furthermore, soil pH, sodicity, salinity and phosphorus were associated with *A. helianthi* seedling emergence and growth. Distribution models which included some of these important environmental variables predicted a more restricted extent

of suitable habitat in the future. In particular, areas with high suitability were found to be over-predicted when the environmental variables were added to the model. Taken together, the results from this thesis provide strong evidence that throughout the geographic distribution, populations are not necessarily at equilibrium with climate, nor are they likely to respond in a similar fashion to changes in environmental conditions. These population differences suggest that purely correlative climate SDMs do not encompass the dynamic processes that represent the relationship between a species and the environment.

Implications for Species Distribution Models

Understanding the factors that limit a species distribution is fundamental to ecological management. Specific niche is likely to be extremely complex in nature, and, therefore, extensive experimentation is required to test the validity of the assumptions inherent in SDMs (Hutchinson 1957). Thus, studies investigating potential changes to plant and animal distributions using climate alone have less predictive certainty. It is evident from this thesis that topological factors are better suited to defining species distribution than macroclimatic factors. Macroclimatic factors are broad-scale climate factors which have been derived from weather stations around the world (Hijmans *et al.* 2005; Slavich *et al.* 2014). However, macroclimatic variables are too broad to successfully interpolate other topological factors, such as elevation, slope, aspect and light availability (i.e. canopy cover), which can influence climate and operate at a finer spatial resolution (Austin and Van Niel 2011b; Harris *et al.* 2014; Slavich *et al.* 2014). The question of what spatial resolution is most appropriate has been raised throughout this thesis, and warrants further attention. Here, I discuss why spatial resolution in SDMs is important. Coupled with this, I then argue that if climate is included in SDMs, then an appropriate assessment of what macroclimatic data must be made to best represent the study area. For this reason, I outline the rationale for the choice of macroclimatic data used in the analyses of *A. helianthi* populations. Similar decisions were

made for each experiment, where appropriate, so that the most accurate and current climate data were used.

Recent studies have suggested that climate is not the most influential factor at a regional (i.e. $\geq 50km^2$ spatial grid size) or finer resolution scale (i.e. ≤ 10 km^2 spatial grid size) (Pearson and Dawson 2003). Rather, topological environmental factors are more important as these directly influence the microclimate through changes in the landscape, such as slope, aspect or altitude (Slavich *et al*. 2014). If these topological factors are shown to be important predictors resulting in different interpretations of the species response to future climate, then climate-only models need to be verified with these factors. For example, the extent of predicted optimal habitats for *Eucalyptus fastigata* was almost four-fold more than that of the climate-only model when several landscape variables (such as radiation, topography and lithology) were included in a generalized additive model (GAM) (Austin and Van Niel 2011a). Topography, in particular, was highly significant and demonstrated the species preference for gullies. This led to strong differences between models at the distribution margins, mainly due to the landscape variables buffering the species against changing climate (Austin and Van Niel 2011a). Similar outcomes have been recorded in mobile (animal) species. For two species of kangaroo, *Macropus rufus* and *M. fuliginosus*, non-climate factors were highly influential on the model fit and performance at a 50 km resolution but not at finer resolutions (Harris *et al.* 2014). This is likely due to the movement patterns of the species being better modelled at the landscape scale rather than a local scale (Harris *et al.* 2014). These studies demonstrate that models which do not include topological factors can often produce interpretations which are unnecessarily pessimistic. I also demonstrated this to be the case for *A. helianthi* when soil factors were added to the climate-only model (Chapter 5).

The importance of scale for non-climate factors has been shown in bird distributions across Europe. At resolutions of 10 km and 20 km, 90% and 88% of models had an increase in cross-validation performance when land-cover was added to the BMs (Luoto *et al.* 2007). Climate was postulated to be the major factor of bird distributions at coarser scales, as the addition of nonclimate factors made no improvement to model performance at 40 km and 80 km resolutions (Thuiller *et al.* 2004a). These results are likely to be consistent across most widely-distributed or highly mobile species, as land-cover and even other topological factors are likely to exhibit some degree of heterogeneity within a grid cell which covers a spatial extent of $\geq$ 40 km. Therefore, when modelling species over a regional extent, such as *A. helianthi*, it is important that the appropriate resolution is used so that environmental heterogeneity is successfully represented in the model.

The geographic extent of a model, as well as what resolution to use will be partly dependent on the availability and quality of data. For presence records, *ad hoc* herbarium collections and ecological observations are supplemented by public record databases (i.e. citizen science). However, environmental data are also needed when distributional data are recorded. Although more environmental data are becoming available online for use in models, they are often not at a scale that could be recorded when a species is recorded or collected for herbaria. This means that for many fine resolution models, we are using interpolated data, which is not ideal. Spatial climate data may also suffer similar issues at fine resolutions (Hijmans *et al.* 2005).

In this thesis, I obtained baseline (current) climate data at a global extent from the WorldClim online database (www.worldclim.org). These data are derived from global climate stations and represent the climate averages between 1950 and 2000 (Hijmans *et al.* 2005). WorldClim data are commonly used in SDMs in the published literature, and the most

recent data were employed in this thesis. However, there is uncertainty surrounding whether climate baselines such as the 1950-2000 or 1960-1990 periods should be used when species data are being collected from the 21st century. To examine this, Roubicek *et al*. (2010) tested the 30- and 50-year baselines against custom decadal climate time steps up to 2005 and found model performance to be consistently higher across decadal baselines for five Australian crop species. Furthermore, these results were consistent across both tropical and temporal climates in Australia (Roubicek *et al.* 2010). Climate baselines are of pivotal important in SDMs as the International governmental Panel on Climate Change (IPCC) AR5 has indicated that global climates are expected to increase in variability (Stocker *et al.* 2013). By contrast, both the IPCC and World Meteorological Organisation (WMO) recommend using a 30-year climate baseline for future projections (i.e. 1960-1990). For this reason, it is appropriate to question whether we are under-valuing the impact of climate on species distributions, and, therefore, over-estimating future distributions. If the variance across current global climates can be used in SDMs, then climate at finer scales may be found to impart a comparable impact to nonclimate factors.

The choice of climate model will affect the outcomes of SDMs and the best model is likely to vary for each region. As the development of climate models is itself an area of specialty, most biologists choose from available models that have been verified for the study region. For my thesis, I used the CSIRO's Mark 3.6 climate model for future climatic conditions. The Mk3.6 model is the most current climate projection model, which includes improved ocean, sea-ice and soil-canopy data generated from the Mk3.5 model. The Mk3.6 model includes a new aerosol treatment and radiation pattern in the projections of the four Representative Concentration Pathway (RCP) scenarios (Jeffrey *et al.* 2013). There are dozens of submitted models based on the RCP scenarios of the IPCC AR5; however, the CSIRO's Mk3.6 model is most relevant for Australia as it focuses on Australia's natural

climate and rainfall variability associated with El Ni*ño Southern Oscillation* (ENSO) (Jeffrey *et al.* 2013). There is also a second CSIRO model built from Australian climate and weather modelling, ACCESS1.3, which is defined as an 'aspirational model' and takes advantage of new atmospheric physics (Bi *et al.* 2013). Although the appropriate spatial data was not available when it was required for Chapter 5, the progress of these and other models will help to improve future model predictions.

Species distributions are determined by their niche, which is defined by the interactions of numerous environmental conditions (Higgins *et al.* 2012; Hutchinson 1957). Now is the opportunity to develop methods to build on climate-only SDMs to include other important niche factors. Recent studies have started to include environmental factors in models by initially confirming their importance using ecological knowledge from experimental testing (Dubuis *et al.* 2013; Eckhart *et al.* 2011; Harris *et al.* 2014). While I have demonstrated the importance of species traits and interactions in defining the variation that exists among populations, I have also shown how the inclusion limiting environmental factors (soil) changes the original climate-only SDM projection. This has also been demonstrated in other recent studies of plant distributions (Coudun *et al.* 2006; De Frenne *et al.* 2014; Dubuis *et al.* 2013).

While these studies are timely and pertinent, there is still further work needed. One of the greatest knowledge gaps is often precise biological information of a species. Higgins *et al*. (2012) remind us that we are ultimately modelling the responses of individuals to future conditions. Only when the variation among individuals is minimal, or can be expressed by a generalist or surrogate factor, such as growth rate, should we then focus on the population responses (Higgins *et al.* 2012). Demographic factors link the Hutchinsonian niche with species range dynamics by combining the persistence of a population, measured by its growth

rate (i.e. abundance), with the relationship between predicted dispersal and environmental variability (Schurr *et al.* 2012). Understanding a population's growth rate requires an extensive knowledge of the phenology, reproductive traits and environmental conditions that determine the overall birth and death rates of individuals over time (Schurr *et al.* 2012). However, the success of this approach was demonstrated by Eckhart *et al*. (2011) who manipulated their SDMs based on the demographic data they had previously recorded. Specifically, the authors compared a control SDM with a filtered model using only presence records from populations which had a positive growth rate, and a model that weighted records based on their quartiles of the growth rate (Eckhart *et al.* 2011). Both the filtered and weighed models predicted a greater decline in suitable habitat due to precipitation and topology being more important factors than for the control model (Eckhart *et al.* 2011). This approach can be referred to as a stochastic spatio-temporal model (Marion *et al.* 2012) that better matches the dynamic nature of a species biology. It is through these approaches that we should be targeting efforts for future data collection and experimentation.

Future directions

This thesis creates exciting avenues for future avenues of exploration. In particular, I have demonstrated several methodologies that can be used to provide experimental evidence of the influence of climate and nonclimate factors on species distributions (Chapters 2–6). These types of approaches are necessary steps towards enhancing the predictions of SDMs. Future work requires an integration of biological population data that furthers our understanding of what limits a species niche. Here, I discuss some important areas which should be investigated further to help develop dynamic SDMs. These are logical next steps following the experiments which have been undertaken in this thesis.

Experimental data

It is important for data to be collected or recorded across multiple populations that are spread throughout the species known geographic distribution. However, this is not always possible due to financial or logistic restrictions. Therefore, we should be open to the usefulness of citizen science databases to help collect species presences, such as the Atlas of Living Australia (www.ala.org.au, 2014). Furthermore, other databases which store species data, such as the plant trait database TRY (Kattge *et al.* 2011), can be used to extract information for SDMs. The types of data needed are those that can help determine the variation that exists among individuals and can then be extrapolated to potential population effects. These build on the experiments undertaken in this thesis.

Firstly, experiments which examine the thermal tolerance of seed dormancy and germination would help to determine the upper limit for a species to survive under increasing future temperature. These data would be crucial for species whose distribution occurs over a topologically-variable extent, such as mountain regions. To understand the relationship between climate and topology, Ooi *et al.* (2012) examine how an increase in atmospheric temperature would impact soil temperatures, and, in turn, how that would affect seed dormancy trait in *Acacia suaveolens* and *Dillwynia floribunda*. The authors note that soil temperatures increased by 1.5 times that of atmospheric temperatures. While there was no clear effect on seed dormancy and viability, there was a positive relationship between dormancy release and heat wave conditions (Ooi *et al.* 2012). This study exemplifies the value of performing experiments to elucidate the mechanistic relationship(s) between the environment and species traits by indirectly examining the effects of local climate. Furthermore, the response of both species exhibited a positive correlation between heat treatment and the temperature of the maternal environment, which indicates that the populations may be locally adapted (Ooi *et al.* 2012) through an epigenetic mechanism. A

reduction in dormancy could have detrimental effects on seed bank longevity, particularly if conditions deteriorate after germination and before successful reproduction by the germinants.

Species in alpine environments represent extreme cases where the capacity for range shifts is limited under changing climate. These species often require a burst of cold temperatures to stimulate their seeds to germinate. For these species, it is important to determine whether shorter winters might have detrimental effects on species recruitment. In the alpine herb *Aciphyllya glacialis,* however, shorter periods of cold stratification were not detrimental to germination, indicating that a shorter winter season is unlikely to affect the succession rate of this species (Hoyle *et al.* 2014). However, this is not always the case, as *Aegopodium podagraria* required a longer period of cold stratification before germination occurred (Vandelook *et al.* 2009). While changes in temperature are expected to cause changes in recruitment rates so too will rainfall, as well as the duration of climatic conditions which are suitable for dormancy to be released. Germination as well as early growth and survival are the stages most sensitive to change, and play an important role in maintaining population growth rates. Identifying the climatic tolerance for a species will illuminate any restriction on the spatial extent of its distribution shifts in the future.

Secondly, to enhance future distribution predictions, several models have been enhanced by the inclusion of plant traits. Increasingly, there are also on-line databases that aim to provide extensive global trait data, such as the plant trait database TRY (Kattge *et al.* 2011). Violle *et al*. (2014) argue that the notion of plant functional traits could be used as a means to move beyond a species by species approach, and work towards a 'common currency' among species. The authors state that such an approach will better present a species by species comparison, leading to an integrated framework for assessing the environmental

effects on biodiversity. A number of studies have illustrated these points. For example, Albert *et al.* (2010b) illustrate that a single plant trait can exhibit significant variation among individuals within a population. Reich *et al.* (2014) report several needle traits in gymnosperms to show significant intraspecific variation with latitude. Duoma *et al*. (2012) used four functional plant traits, including stem-specific density and three nutrient traits, in a community habitat distribution model to determine how they affected model performance in predicting different vegetation types. Similarly, Pollock *et al*. (2012) quantified seed mass, surface leaf area (SLA) and plant height for 20 species of *Eucalyptus* across environmental gradients. By using an approach that combined plant traits with multiple environmental variables, the authors determined that taller plants exhibited a positive relationship with solar radiation and rainfall gradients. Taller plants with low SLA were predicted to perform better in areas which also had fine-textured soil (Pollock *et al.* 2012). The information derived from these trait-based distribution studies permit the modeler to better evaluate the capacity of a species to colonise novel habitats than using physical data alone.

Thirdly, a common interest of some models is not where the species are found, rather where they might be. These studies often examine the translocation of a species using SDM outputs as guides to subsequently evaluate and optimise experiments to determine the tolerance of species to new environments. Since SDMs identify other habitats that are climatically similar to those where the species is currently known to exist (Araújo and Peterson 2012), this presents the possibility for the species to be translocated to predicted areas. Hancock and Hughes (2014) recently demonstrated that there was no advantage in using individual local provenance for restoration projects. Specifically, the authors found no significant difference between individuals from different populations of *Eucalyptus tereticornis* and *Themeda triandra* in terms of non-reproductive growth and survival. However, modelled suitable habitats with the intention for translocating a species are made as

areas which are only abiotically suitable. Therefore, the predictions do not take into account any biotic restrictions or interactions (Araújo and Peterson 2012). These impacts must be investigated to determine the risks of translocating to 'predicted suitable' locations, particularly if the species is rare or endangered. Reciprocal transplantation of seed between coastal and inland populations of *A. helianthi* in order to examine reproductive success was beyond the scope of the study time-frame, but highlights the need for longer-term field validation of plant biology to enhance the accuracy of model predictions.

Finally, genetic characterisation of individual plants and populations would further complement the results in this thesis. Due to time and financial limitations, I was unable to examine the genetic diversity of populations. Higgins *et al*. (2012) state that in order to understand the origin of a species distribution, there must be an understanding of how its niche occurred and evolved. Again, this can be examined at an individual level. The interaction between an individual and its environment depends on the species traits and the evolution of those traits determines the evolution of its niche (Higgins *et al.* 2012). Whether the macroclimate, in terms of temperature and rainfall, drives evolutionary preferences of species provides an insight into the potential rate of diversification through subsequent niche evolution (Schnitzler *et al.* 2012). Therefore, it is important that the genetic basis for species traits is determined, and that studies to use cross-discipline techniques to produce estimates of species fitness (Dostálek *et al.* 2010). For example, a genetic study on the waratah (*Telopea speciosissima*) identified three population groups (Rossetto *et al.* 2011). One population was genetically isolated by the edaphic environment whereas the remaining two groups shared some genetic similarity from intermediate populations along an altitudinal gradient. These results indicate that population genetic studies have the capacity to provide evidence for population persistence as well as the capacity for reproductive outcrossing. Despite the importance of combining demographic data with genetic diversity, there is an obvious dearth

of studies which examine both (Oostermeijer *et al.* 2003). Ideally, these data would be collected across multiple environments where a species is present, as well as environments that have not been colonised by the species, but are predicted to be suitable in the future.

We expect that populations will not respond similarly to environmental change, and population abundance may not necessarily be a proxy for genetic diversity (Dostálek *et al.* 2010; Oostermeijer *et al.* 1994). For example, if populations have formed from a recent common ancestor, then they are unlikely to show poor gene flow (Leimu and Mutikainen 2005). Similarly, self-compatible species are unlikely to exhibit a strong relationship between population size and genetic diversity (Leimu *et al.* 2006). There are also several genetic techniques that can facilitate significant progress when employed alongside ecological experiments. The availability of molecular protocols and techniques to identify single-gene markers (or single nucleotide polymorphisms, SNPs) for specific traits means that genetic diversity within a species can be easily mapped. This is especially true for electrophoretic methods for examining the genetic diversity in allozyme loci (Hamrick and Godt 1996). Techniques, such as sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), can be used to quickly and effectively identify differences in seed proteins, thereby providing an indication of the level of diversity (Ferguson and Grabe 1986). The development of next generation sequencing (NGS) has also provided a cheaper, faster and comprehensive method of analysing large numbers of DNA sequences and producing genetic maps (Bräutigam and Gowik 2010). This augments SDS-PAGE to enable to rapid genome screening and estimation of genetic diversity in individual populations. Furthermore, quantitative trait loci (QTL) can be identified to determine which loci contribute to particular phenotypic traits (Nordborg and Weigel 2008). For example, Huang *et al.* (2010) identified QTLs which were responsible for several early fitness components at the seed stage, including a single QTL that explained almost 14% of the variation in the ability of seeds to

germinate. For these reasons, it is strongly encouraged that genetic data should be generated in tandem with ecological studies. Specifically, demographic data from both field and genetic experiments will permit more informative demographic simulations and SDMs.

Model manipulation

To better evaluate model predictions, more refined modifications and improvements should be made to popular SDM programs, such as *MaxEnt*. While the benefits and superiority of *MaxEnt* have been detailed in the thesis, the capacity to further improve the mechanical process of the model warrants discussion.

MaxEnt is a machine-learning program and like most computer models its performance is only as good as the input data used (Phillips *et al.* 2006). Furthermore, *MaxEnt* is a presence-only model, which means it suffers from several critical issues. A meta-analysis by Yackulic *et al.* (2013) illustrated that almost 90% of published studies which used *MaxEnt* were likely to suffer from sampling bias due to misconceptions or by ignoring the controls for background absence sampling. Perhaps more alarming was that over 50% of the sampled papers incorrectly addressed *MaxEnt*'s output as probability of occurrence instead of probably of occupancy (Elith *et al.* 2011; Yackulic *et al.* 2013). Therefore, as implemented in this thesis, for presence-only models such as *MaxEnt*, it is recommended that studies examine and apply appropriate model refinement and optimisation for the available species occurrence dataset. Ideally, protocols tested across multiple species to examine effects of sample size and spatial autocorrelation would improve the level of correlation among environmental variables.

Since most presence record databases are built from samples that were collected without any stratified design, some discrimination of the data is appropriate. Austin and

Heyligers (1989) used a set of transects directed along multiple environmental gradients (gradsects) in attempt to encompass greater variation in the vegetation in New South Wales. The authors report that the gradsects succeeded in capturing the variability of the study region when compared across different rock-derived landscapes. However, the capacity to perform such surveying is often very limited, and the financial costs of such practises can be easily underestimated (Austin and Heyligers 1989). Bias can also be minimised during the model tweaking in *MaxEnt* by using sample bias grids or controlling the selection of background absence points. Syfert *et al*. (2013) used a sample bias grid to prevent *MaxEnt* from selecting background points from grid cells which did not contain at least one presence record. When implemented, mean 'average under the ROC curve' (AUC) value significantly improved for both herbarium and ecologically-sampled data when sampling bias was neutralised (Syfert *et al.* 2013).

Further improvements can also be made when mechanistic data or models are incorporated (Elith *et al.* 2010). However, if these are not available, then attention should be given to spatial extent of background samples in these instances. Radosavljevic and Anderson (2014) tested several geographically structured methods to partition occurrence records into four distinct regions or spatial subsets (bins) using a *k*-fold cross-validation technique. Occurrences were then divided randomly so that each subset contained an equal number of records. However, since this approach modifies niche estimates, it is important to then mask out environmental data for *MaxEnt* to perform its background sampling. This avoids any environmental bias, thereby mimicking the likelihood that a species currently inhabits less than its fundamental niche (i.e. realized niche). These new advances are encouraging signs towards further optimising and enhancing SDMs. Furthermore, new tools are now becoming available that can assemble spatial and bioclimatic dataset and develop software that enhances the integration of these data into modelling programs. A good example of this is the

ecosystem Modelling and Scaling Infrastructure Facility (eMAST; www.emast.org.au). In light of these recent developments, it would be beneficial to return to the *MaxEnt* model used in Chapter 5 and to implement some of these advances to further improve the model's projections.

Data quality

As discussed above, irrespective of the modifications and improvements made to modelling programs, the final output will be only as good as the data used. Ecological surveys are presence-absence data and are, therefore, the most useful in SDMs. However, for the majority of species, records are available as presence-only records. Specimens stored in herbaria or museums represent a physical confirmation of a presence record, and can be independently identified or corrected to account for changes in taxonomy (Delisle *et al.* 2003). Sampling biases inherent with most herbarium records are well-documented in the literature (Delisle *et al.* 2003; Elith and Leathwick 2007; Loiselle *et al.* 2007). However, I have demonstrated that plant traits extracted from herbarium specimens can be used in multivariate climate models (Chapter 6).

Building on this principle, I strongly encourage collectors to record environmental factors when sampling species in the landscape. As an example, a simple estimation of species abundance and population extent would enable opportunities to model the relationship between distribution and population size (Brown 1984; Van Der Wal *et al.* 2009; Young *et al.* 2012). Duff *et al*. (2012) illustrated that the influence of abundance on the distribution of the species would depend on whether there were different environmental factors driving presence and abundance. For example, while climate was the main factor affecting the distribution of *Xanthorrhoea australis*, soil and vegetation were the main determinants of abundance (Duff *et al.* 2012). Other data which would prove informative

include a list of dominant species in the area, a qualitative (or, where possible, a quantitative) description of the soil, and an indication of any phenological event (i.e. peak flowering). Furthermore, systematically recording search intensity and recording absences from locations would be beneficial towards directing models where pseudo-absences should be chosen, since the selection method may constrain the output (Chefaoui and Lobo 2008). This may become more important in the future if absences are more prevalent in the future, if a species cannot cope with climate change.

Given the importance of using sound science to inform decision and policy making, it is necessary that we make full use of the global herbaria resource. Now is the perfect time to update the protocols for recording environmental data when collecting a sample species, as historical records have moved their data to online databases, such as Australia's Virtual Herbarium (AVH), hosted by the Atlas of Living Australia (www.ala.org.au) and Climate Watch (www.climatewatch.org.au). During my fieldwork, I recorded estimates of species abundance, percentage of mature plants in the population, and population extent. I included these factors in some of these data analyses. These methods will reduce model uncertainty. Ultimately, achieving this will require the cooperation and collaboration of different scientific disciplines in order to maximise resources and optimise efforts for synergistic and meaningful outcomes. If we consider SDMs simply as tools that assist in our understanding of the factors that limit the species niche, then the focus should be on the acquisition of credible and comprehensive biological data and the best way to collect it.

Conclusion

Distribution models are now common throughout the scientific literature. However, there is still a need for further development and improvements. My thesis provides a means to experimentally test the assumptions inherent in most SDMs by analysing and determining the

environmental and biotic factors that limit the geographic distribution. Furthermore, I have shown how environmental topological factors can be added to a bioclimatic model. The importance of climate will depend on the geographic scale used by the modeler. While bioclimatic models should not be discounted, it is important for the modeler to outline their questions and use quality data that provide the most informative predictive summary. For *A. helianthi*, traits associated with the seed and seedling stage are highly variable and potentially exhibit a genetically fixed type. Coupled with growth rates varying among local and non-local soils, populations may struggle to disperse into novel environments. If populations are unable to adapt to the changing window of future climates then it could be predicted that populations may become locally extinct.

SDMs are powerful tools that help to fill gaps in the species data through the inclusion of assumptions; however, they cannot be the sole focus underpinning decisions for conservation and management. Rather, models and their assumptions should be challenged with the inclusion of species and environmental data. The main conclusion of these arguments is that for all species, developments should be made towards incorporating the mechanistic factors which define the niche of a species. When we examine a species 'niche', we are in fact defining the spatial extent of suitability for reproductive success based on ecological concepts and factors associated with individuals and populations. Therefore, it makes sense that our predictions are made following the investigation of appropriate ecological research so that subsequent modelling techniques have reduced predictive uncertainty and are more likely to be validated in the field.

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Appendix 1 – List of field locations of *Actinotus helianthi* populations which were sampled.

Number	Pop^{n} code	Location	Latitude	Longitude	Abundance
1	AHAR01	Anvil Rock Lookout; Anvil Rock Rd; Blue Mountains NP; from hat hill Rd; Blackheath	-33.60	150.34	1000
2	AHBW01	On a granite outcrop in Broadwater State Forest; at the back of 201 Glenlyon Rd; Stanthorpe	-28.63	151.91	1500
3	AHC01	Pilliga Nature Reserve; 40km N of Coonabarabran on Newell Hwy; on the side of the road	-30.95	149.42	3000
4	AHC02	Pilliga Nature Reserve; 37km N of Coonabarabran on Newell Hwy; on the southern ridge	-30.98	149.42	2000
5	AHC03	Coonabarabran lawn cemetary; northern side; adjacent to Columbian wall	-31.25	149.29	1000
6	AHC04	Along the Newell Hwy c. 70km N of Coonabarabran; Piliga Nature Reserve	-30.68	149.55	500
7	AHG01	On a granite outcrop E of track to Underground Creek; c. 100m from intersection with Dr Robert's Waterhole	-28.83	151.97	30
8	AHGR01	Georges River National Park; on south side of Georges River immediately adjacent to northbound lanes on ridge	-33.98	151.03	160
9	AHHN01	Myall Lakes NP; along Mungo Track entrance from Mungo Brush Rd; ca. 300m from golf course	-32.66	152.18	1500
10	AHLP01	2.3km along track to Lockley's Pylon; Blue Mountains NP	-33.64	150.37	1000
11	AHLP02	East side of Lockley's Pylon on the edge of the track at fork to Blue Gum Forest; Blue Mountains NP	-33.63	150.36	200
12	AHMD01	Manly Dam; SE side on edge of track at intersection between the circuit track and Dam wall track	-33.78	151.25	2000
13	AHMD02	Manly Dam; along bike trail from entrance at Cootamundra Drive	-33.77	151.25	3000
14	AHMD03	Manly Dam; along Alambie Rd; between Alambie Rd and fire trail from Martin Luther Ln	-33.76	151.25	3000
15	AHMD04	Manly Dam; ca. 500m along track going N from the western side; 2km from Wakehurst Pkwy and Warringah Rd	-33.77	151.23	157
16	AHMW01	Mount White; on eastern side of gravel road to Greenmans Valley Caravan Park; off Morgans Rd	-33.46	151.17	500
17	AHPB01	Palm Beach; McKay Reserve on corner of Cynthea Rd and Ebor Rd	-33.61	151.33	1000
18	AHSH01	Sydney Harbour NP; Grotto Point Reserve at the end of Culter Rd; along Manly Spit walk towards Manly	-33.81	151.26	2000
19	AHSR01	Along the northern base of Spyglass Rock; Salvator Rosa section of Carnarvon National Park	-24.82	147.20	463
20	AHTUN01	On sub-divided land block left of Chapmans Rd; from Lakes Way; Tuncurry	-32.16	152.49	4000
21	AHUL01	ca. 5km S of Ulladulla from 80/60km speed sign to Princess Street; on embankment on E side of road	-35.39	150.45	300
22	AHW01	Wyrrabalong NP; Pelican Beach Rd; on the right 100m from intersection with Central Coast Hwy; Magneta	-33.29	151.55	1000
23	AHW02	Wyrrabalong NP; Central Coast track; ca. 300m from Crackneck Lookout	-33.40	151.48	10000
24	AHWOL01	Wollemi NP; Putty Rd; 200m S of Morilla Rd	-33.51	150.82	280
25	AHWOL02	Wollemi NP; Putty Rd facing NE at High Wollemi 2389/122 entrance; 73km N of Wilburforce on the left	-33.04	150.68	200
26	AHYEN01	Yengo NP; 80km N of Wilburforce; along Putty Rd; on E side of road on ridge	-33.12	150.70	3000
27	AHWAT01	Along the Princes Highway; c. 2km S of Waterfall on E side of road	-34.15	150.67	120
28	AHTUN02	Along Parr Road; adjacent to cemetary and substation; Tuncurry	-32.16	152.49	1000
29	AHBS01	Baradine State Forest	-30.91	149.09	100
30	AHC05	c. 15km N of Coonabarabran on Newell Highway; E side of road; Pilliga State Forest	-31.07	149.39	3000

Number	Pop^{n} code	Location	Latitude	Longitude	Abundance
31	AHRNP01	c. 500m along Bungoona Track from the Park Research Centre; Royal National Park	-34.06	151.06	50
32	AHRNP02	Along Sir Bertram Stephens Drive; c. 1km S of Garie Beach Road turnoff; along roadside in several patches; Royal National Park	-34.17	151.04	50
33	AHA01	At the end of the road to overflow campsite at Arakoon Gaol; Arakoon	-30.88	153.07	500
34	AHMIN01	Along the headland side of Grevillea Avenue; Minnie Water	-29.78	153.30	500
35	AHMEN01	On E side of road to Mendooran; c. 37km N of Mendooran; Mendooran Road	-31.53	149.26	5000
36	AHPAT01	c. 200m S of the turnoff to Pearl Beach; Patonga Drive; Brisbane Waters National Park; E side of road	-33.54	151.30	500
37	AHTH01	Ku-Ring-gai Chase National Park; Terrey Hills; c. 200m along fire trail entrance on N side of Booralie Road; 200m from Kallaroo Rd travelling W	-33.68	151.21	200
38	AHMD05	Manly Dam; SE side on edge of track immediately adjacent to the N side of the dam wall	-33.77	151.25	1000
39	AHC06	c. 2km N of Coonabarabran on Newell Highway; E side of road	-31.23	149.32	150
40	AHMEN02	On W side of road to Mendooran; c. 45km N of Mendooran; Mendooran Road	-31.51	149.27	136

Appendix 2 – Pearson's correlations for the climate data. Significant correlations (i.e. ≥ ± 0.85) are in bold.

	Bio1	*Bio5*	*Bio6*	*Bio8*	*Bio9*	*Bio10*	*Bio11*	*Bio12*	*Bio13*	*Bio14*	*Bio16*	*Bio17*	*Bio18*	*elevation*
Bio1														
Bio5	0.4277													
Bio6	0.6385	-0.4033												
Bio8	0.6223	0.8225	-0.0798											
Bio9	0.8247	-0.0707	**0.8850**	0.0964										
Bio10	0.8284	**0.8572**	0.1161	**0.8663**	0.4187									
Bio11	**0.8802**	-0.0477	**0.9206**	0.2451	**0.9540**	0.4673								
Bio12	-0.0315	**-0.8613**	0.7162	-0.6086	0.4210	-0.5452	0.4206							
Bio13	0.0205	-0.7594	0.6868	-0.5495	0.4291	-0.4597	0.4298	**0.9610**						
Bio14	-0.1399	-0.7890	0.5860	-0.6170	0.2845	-0.5412	0.2643	0.8290	0.7242					
Bio16	-0.0888	-0.8460	0.6265	-0.6060	0.3435	-0.5775	0.3463	**0.9716**	**0.9677**	0.7024				
Bio17	-0.0862	-0.8192	0.6550	-0.6060	0.3539	-0.5321	0.3374	**0.9134**	0.8171	**0.9636**	0.8042			
Bio18	-0.2501	-0.8486	0.4584	-0.6608	0.1703	-0.6743	0.1677	**0.9074**	**0.8958**	0.6304	**0.9669**	0.7185		
elevation	-0.7785	-0.0532	-0.7775	-0.2896	-0.8089	-0.4823	**-0.8526**	-0.2955	-0.2833	-0.2766	-0.1762	-0.3070	0.0042	

Pearson's correlations for the climate data for the larger populations only. Significant correlations (i.e. ≥ ± 0.85) are in bold.

	Bio1	*Bio5*	*Bio6*	*Bio8*	*Bio9*	*Bio10*	*Bio11*	*Bio12*	*Bio13*	*Bio14*	*Bio16*	*Bio17*	*Bio18*	*elevation*
Bio1														
Bio5	0.2553													
Bio6	0.7206	-0.4764												
Bio8	0.4579	0.7285	-0.1194											
Bio9	0.8099	-0.2439	**0.9161**	-0.1257										
Bio10	0.7806	0.8014	0.1383	0.7641	0.3380									
Bio11	**0.9004**	-0.1878	**0.9467**	0.1203	**0.9413**	0.4321								
Bio12	0.1071	**-0.8964**	0.7587	-0.5677	0.5524	-0.5091	0.5159							
Bio13	0.1066	-0.8180	0.7117	-0.5656	0.5281	-0.4699	0.4924	**0.9513**						
Bio14	0.2681	-0.7403	0.8059	-0.4586	0.6615	-0.2963	0.6048	**0.9265**	0.8408					
Bio16	-0.0651	**-0.9387**	0.6270	-0.6171	0.3896	-0.6475	0.3621	**0.9736**	**0.9532**	0.8332				
Bio17	0.2195	-0.7736	0.7867	-0.4865	0.6304	-0.3482	0.5690	**0.9344**	0.8268	**0.9768**	0.8396			
Bio18	-0.3056	**-0.9471**	0.4075	-0.6717	0.1623	-0.7999	0.1192	**0.8853**	**0.8513**	0.7159	**0.9580**	0.7201		
elevation	**-0.9099**	-0.0590	-0.7994	-0.2921	-0.8388	-0.5958	-0.9027	-0.3256	-0.3359	-0.4360	-0.1729	-0.4022	0.0602	

Pearson's correlations for the plant trait data. Significant correlations (i.e. ≥ ± 0.85) are in bold. For a description of variables see Chapter 2, Table 1.

	height	*umbel*	*stem*	*leaf1*	*leaf2*	*leaf3*	*leaf4*	*diam*	*condist*	*leaf*	*umbelstem*	*abundance*	*maturity*
height													
umbel	0.3586												
stem	0.2937	**0.8649**											
leaf1	0.2531	0.0532	0.0281										
leaf2	0.2219	0.0403	0.0231	**0.8890**									
leaf3	0.2184	0.0252	-0.0056	**0.8549**	**0.8790**								
leaf4	0.2199	0.0286	0.0127	0.8373	**0.8626**	**0.8805**							
stem	0.3639	0.7411	0.6916	0.0574	0.0608	0.0299	0.0409						
condist	0.0200	0.1312	0.1221	-0.1180	-0.1087	-0.1041	-0.1138	0.1460					
leaf	0.2404	0.0387	0.0152	**0.9426**	**0.9568**	**0.9527**	**0.9435**	0.0497	-0.1171				
umbelstem	0.1956	0.2271	-0.0498	0.1479	0.1496	0.1518	0.1553	0.1454	0.0366	0.1593			
abundance	0.3087	0.0116	0.0348	0.1497	0.1563	0.1459	0.1543	0.1193	-0.2376	0.1597	-0.0028		
maturity	0.3718	0.0852	0.1109	-0.0224	-0.0428	-0.0335	-0.0499	0.0126	0.1055	-0.0393	0.0000	0.0815	

Appendix 3 – Pearson's correlations for the MaxEnt models in Chapter 5. Significant correlations (i.e. ≥ ± 0.75) are in bold.

	Bio1	*Bio2*	*Bio3*	*Bio4*	*Bio5*	*Bio6*	*Bio7*	*Bio8*	*Bio9*	*Bio10*	*Bio11*	*Bio12*	*Bio13*	*Bio14*	*Bio15*	*Bio16*	*Bio17*	*Bio18*	*Bio19*
Bio1																			
Bio2	0.005																		
Bio3	0.639	0.069																	
Bio4	-0.173	**0.944**	-0.232																
Bio5	0.486	**0.845**	0.232	**0.768**															
Bio6	0.643	**-0.750**	0.385	**-0.819**	-0.308														
Bio7	-0.101	**0.986**	-0.097	**0.981**	**0.806**	**-0.812**													
Bio8	**0.821**	0.477	0.510	0.337	**0.817**	0.180	0.390												
Bio9	**0.832**	-0.060	0.515	-0.180	0.374	0.600	-0.144	0.619											
Bio10	**0.846**	0.509	0.466	0.377	**0.873**	0.168	0.431	**0.951**	0.694										
Bio11	**0.891**	-0.434	0.614	-0.599	0.044	**0.904**	-0.536	0.510	**0.766**	0.516									
Bio12	0.140	**-0.869**	0.133	**-0.889**	-0.713	0.722	**-0.887**	-0.340	0.208	-0.343	0.524								
Bio13	0.141	-0.689	0.141	-0.725	-0.575	0.574	-0.710	-0.254	0.175	-0.258	0.445	**0.934**							
Bio14	-0.135	-0.741	-0.190	-0.612	-0.637	0.506	-0.706	-0.457	0.024	-0.438	0.186	0.676	0.504						
Bio15	0.216	0.500	0.350	0.319	0.414	-0.285	0.432	0.392	-0.061	0.343	0.001	-0.312	-0.058	**-0.750**					
Bio16	0.150	**-0.762**	0.196	**-0.822**	-0.651	0.633	**-0.794**	-0.283	0.135	-0.307	0.492	**0.943**	**0.957**	0.490	-0.015				
Bio17	-0.069	**-0.805**	-0.107	-0.711	-0.679	0.587	**-0.782**	-0.453	0.114	-0.492	0.286	**0.790**	0.626	**0.945**	-0.743	0.583			
Bio18	0.095	-0.651	0.217	-0.731	-0.606	0.510	-0.689	-0.249	0.067	-0.318	0.400	**0.827**	**0.872**	0.319	0.146	**0.919**	0.425		
Bio19	0.063	**-0.830**	0.000	**-0.769**	-0.650	0.683	**-0.824**	-0.373	0.258	-0.338	0.418	**0.862**	0.710	**0.891**	-0.680	0.672	**0.968**	0.495	

Appendix 4 – Pearson's correlations for the climate variables for *Actinotus forsythii* in Chapter 6. Significant correlations (i.e. ≥ ± 0.85) are in bold.

	Bio1	*Bio2*	*Bio3*	*Bio4*	*Bio5*	*Bio6*	*Bio7*	*Bio8*	*Bio9*	*Bio10*	*Bio11*	*Bio12*	*Bio13*	*Bio14*	*Bio15*	*Bio16*	*Bio17*	*Bio18*	*Bio19*
Bio1																			
Bio2	0.75																		
Bio3	**0.89**	0.702																	
Bio4	.354	**0.868**	.338																
Bio5	0.922	**0.944**	**0.82**	0.674															
Bio6	0.842	.278	0.729	-.203	0.572														
Bio7	0.64	**0.985**	0.58	**0.929**	**0.886**	.127													
Bio8	**0.966**	**0.89**	**0.853**	0.582	**0.988**	0.675	**0.814**												
Bio9	**0.859**	.321	0.706	-.116	0.605	**0.975**	.181	0.712											
Bio10	**0.978**	**0.867**	**0.874**	0.54	**0.979**	0.713	0.782	0.998	0.748										
Bio11	**0.979**	0.603	**0.872**	.157	**0.826**	**0.932**	0.473	**0.893**	**0.933**	**0.916**									
Bio12	-0.664	**-0.942**	-0.512	**-0.855**	**-0.888**	-.196	**-0.963**	-0.824	-.240	-0.788	-0.509								
Bio13	-.283	-0.725	**-.087**	-0.81	-.588	.179	-0.812	-0.488	.139	-.435	-.111	**0.89**							
Bio14	-0.828	**-0.932**	-0.665	-0.76	**-.957**	-.433	**-0.913**	**-0.936**	-0.496	**-0.914**	-0.705	**0.952**	0.737						
Bio15	**0.945**	**0.905**	**0.883**	0.623	**.980**	0.633	0.828	**0.989**	0.676	**0.988**	**0.865**	-0.803	-.446	**-0.917**					
Bio16	-.344	-0.77	-.178	-0.807	-.644	.117	-0.844	-0.542	.101	-0.491	-.179	**0.915**	**0.986**	0.76	-0.502				
Bio17	**-0.855**	**-0.93**	-0.69	-0.718	**-.971**	-0.48	**-0.903**	**-0.95**	-0.525	**-0.93**	-0.742	**0.951**	0.729	**0.994**	**-0.923**	0.763			
Bio18	-.384	-0.648	-.145	-0.589	-.603	-.045	-0.704	-0.523	-.029	-0.478	-.264	0.845	**0.928**	0.71	-.443	**0.947**	0.737		
Bio19	-0.78	**-0.951**	-0.624	-0.799	**-.947**	-.355	**-0.945**	**-0.908**	-.404	**-0.88**	-0.646	**0.984**	**0.804**	**0.99**	**-0.886**	0.833	**0.99**	0.78	

Pearson's correlations for the climate variables for *Actinotus minor* in Chapter 6. Significant correlations (i.e. ≥ ± 0.85) are in bold.

	Bio1	*Bio2*	*Bio3*	*Bio4*	*Bio5*	*Bio6*	*Bio7*	*Bio8*	*Bio9*	*Bio10*	*Bio11*	*Bio12*	*Bio13*	*Bio14*	*Bio15*	*Bio16*	*Bio17*	*Bio18*	*Bio19*
Bio1																			
Bio2	-.129																		
Bio3	.733	.28																	
Bio4	-.425	**.914**	-.084																
Bio5	.707	.595	.716	.328															
Bio6	**.897**	-.553	.494	-.765	.329														
Bio7	-.332	**.971**	.060	**.976**	.429	-.712													
Bio8	**.962**	.108	.735	-.174	**.860**	.760	-.087												
Bio9	**.959**	-.357	.670	-.636	.500	**.963**	-.550	.849											
Bio10	.347	.331	.446	.153	.485	.146	.222	.405	.266										
Bio11	**.983**	-.300	.674	-.582	.569	**.960**	-.495	**.899**	**.989**	.283									
Bio12	.081	**-.937**	-.187	**-.883**	-.629	.482	-.929	-.171	.327	-.212	.252								
Bio13	.421	**-.862**	.041	**-.888**	-.290	.731	**-.915**	.206	.602	-.047	.558	**.908**							
Bio14	-.689	-.583	-.814	-.276	**-.942**	-.322	-.393	-.806	-.515	-.518	-.563	.582	.295						
Bio15	**.935**	.065	.819	-.234	.768	.754	-.150	**.932**	**.862**	.473	**.887**	-.030	.324	-.783					
Bio16	.457	-.811	.196	**-.897**	-.255	.740	**-.898**	.223	.654	.023	.593	**.902**	**.964**	.188	.396				
Bio17	-.609	-.676	-.767	-.386	**-.952**	-.212	-.505	-.755	-.411	-.481	-.467	.691	.408	**.983**	-.714	.321			
Bio18	.442	-.672	.293	-.781	-.191	.666	-.779	.225	.622	.123	.556	.822	**.878**	.079	.460	**.964**	.218		
Bio19	-.258	**-.890**	-.545	-.694	-.830	.177	-.787	-.465	-.026	-.416	-.090	**.899**	.713	**.852**	-.413	.637	**.917**	.506	

Pearson's correlations for the climate variables for *Actinotus suffocatus* in Chapter 6. Significant correlations (i.e. ≥ ± 0.85) are in bold.

	Bio1	*Bio2*	*Bio3*	*Bio4*	*Bio5*	*Bio6*	*Bio7*	*Bio8*	*Bio9*	*Bio10*	*Bio11*	*Bio12*	*Bio13*	*Bio14*	*Bio15*	*Bio16*	*Bio17*	*Bio18*	*Bio19*
Bio1																			
Bio2	**-.853**																		
Bio3	**.928**	-.597																	
Bio4	**-.991**	**.914**	**-.871**																
Bio5	**.976**	-.718	**.987**	**-.939**															
Bio6	**.998**	**-.881**	**.906**	**-.997**	**.962**														
Bio7	**-.951**	**.973**	-.767	**.983**	**-.860**	**-.967**													
Bio8	**.999**	**-.878**	**.908**	**-.997**	**.963**	**1.000**	**-.965**												
Bio9	**.997**	-.813	**.952**	**-.979**	**.989**	**.992**	**-.926**	**.993**											
Bio10	**.997**	-.813	**.952**	**-.979**	**.989**	**.992**	**-.926**	**.993**	**1.000**										
Bio11	**1.000**	**-.852**	**.929**	**-.991**	**.976**	**.998**	**-.950**	**.999**	**.998**	**.998**									
Bio12	-.578	.066	-.840	.465	-.742	-.531	.296	-.535	-.634	-.634	-.579								
Bio13	-.033	-.494	-.402	-.099	-.250	.023	-.279	.018	-.104	-.104	-.034	.835							
Bio14	-.559	.043	-.827	.445	-.726	-.512	.274	-.516	-.617	-.617	-.560	**1.000**	.847						
Bio15	.681	-.198	**.905**	-.579	.824	.639	-.420	.643	.731	.731	.682	**-.991**	-.754	**-.988**					
Bio16	-.444	-.090	-.745	.322	-.628	-.393	.144	-.397	-.506	-.506	-.445	**.988**	**.910**	**.991**	**-.958**				
Bio17	-.578	.066	-.840	.465	-.742	-.531	.296	-.535	-.634	-.634	-.579	**1.000**	.835	**1.000**	**-.991**	**.988**			
Bio18	-.578	.066	-.840	.465	-.742	-.531	.296	-.535	-.634	-.634	-.579	**1.000**	.835	**1.000**	**-.991**	**.988**	0.425		
Bio19	-.182	-.359	-.534	.050	-.391	-.126	-.133	-.131	-.251	-.251	-.182	**.908**	**.989**	**.917**	**-.844**	**.962**	**.907**	**.907**	