

Traits as units for prediction in ecological responses to climate change

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Abstract

Understanding and predicting ecological responses to climate change is crucial if we are to manage for the detrimental consequences that might ensue in its wake. This thesis looks to develop some new ideas and tools for ecological prediction under climate change, focusing on species traits rather than species themselves as units of prediction. Chapter One begins by reviewing the basis for a trait-based approach to prediction, presenting evidence from natural and experimental systems that responses to climate change cluster by traits. Chapter Two undertakes a proof-of-concept modelling study, using a well-known dataset of contemporary phenological changes under warming to test for trait-based links in the strength and direction of species responses. Chapter Three addresses the critical issue of transferability. One of the strongest justifications for the use of a trait-based approach is that inferences may extend more generally beyond the focal species and system. To test this, it develops trait-based models for long-term datasets of first-arrival dates for migratory birds in two neighbouring US states and assesses cross-applicability between them.

Having investigated the functionality and transferability of the trait-based approach, I then explore the bounds of its utility. Chapter Four uses a detailed record of community-wide changes in species abundance under ten years of experimental climate change to assess whether changes in abundance cluster by traits. It also re-evaluates the prevailing hypothesis guiding researchers' interpretations of ecological changes in this system. Chapter Five designs and implements an experiment building on the results of Chapter Four. This experiment tests the role of climatic effects on nitrogen-fixation in driving system dynamics via controlled removal of this trait from experimental communities. Overall, this thesis sheds new light on the role of traits in ecological responses to climate change, highlighting opportunities and limitations for using traits to organise our thinking over prediction and adaptation.

Declaration

All the work presented in this thesis is my own, with the following acknowledgements:

My supervisor, Blake Suttle, provided editorial input on all of my chapters.

The response data analysed in Chapter Two and Chapter Three were documented by others, who are cited appropriately throughout the thesis. The trait data I collated for these chapters were also documented by others across various sources. These are, again, appropriately cited.

David Orme kindly helped me develop the offset model used to compare trait-based predictions between regions in Chapter Three.

The data analysed in Chapter Four was collected by Blake Suttle and Meredith Thomsen.

In Chapter Five, September data for all years were collected by Blake Suttle. In 2013, all samples were collected by Blake Suttle and Meredith Thomsen.

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Chapter One

General Introduction

1.1 Species responses to climate change

There is a clear biological signal of recent climate change on Earth's species. Impacts have been recorded on every continent, in every ocean, and in most major taxonomic groups (Parmesan 2006). Changes in climate are driving changes in population dynamics (e.g. Croxall *et al.* 2002; Saino *et al.* 2009; Ozgul *et al.* 2010), composition of ecological communities (e.g. Brown *et al.* 1997; Pounds *et al.* 1999; Sagarin *et al.* 1999), functioning of ecosystems (e.g. Kurz *et al.* 2008; Schuur *et al.* 2008) and global distributions of species (e.g. Parmesan and Yohe 2003; Root *et al.* 2003; Chen *et al.* 2011). The Intergovernmental Panel on Climate Change (IPCC) predicts greater changes in climate in the next century than in the last (IPCC 2007), leading us to expect biological impacts to emerge in greater magnitude and across a wider range of taxa than are currently known.

Climate change results in temporal and spatial shifts in the occurrence of specific conditions. Warmer temperatures, for example, are occurring at higher latitudes and elevations, and at earlier times in the year across much of the planet. In response, species adjust their geographical distributions to track suitable conditions through space, and adjust their phenology to track suitable conditions through time. Species distributional changes are commonly manifest in shifts in latitudinal and elevational range that track species-specific physiological thresholds of temperature and precipitation (Woodward 1987). Species distributions are influenced by many factors beyond climate, and so correlations with temperature changes are not always apparent (Walther *et al.* 2002). Species phenological changes are commonly manifest in shifts in the timing of life history events, such as key aspects of annual breeding, migration, or growth cycles. Most of the evidence for phenological shifts related to climate change comes from advances in the timing of spring events, such as first appearance, first flowering, first arrival, or budburst, with nearly all spring phenophases correlating with temperatures in the months preceding their occurrence (Walther *et al.* 2002). There is also some evidence for changes in the timing of autumn phenological events, although these tend to be less pronounced and more heterogeneous in direction (Walther *et al.* 2002). Some of the early and most noteworthy evidence for

distributional and phenological shifts attributed to contemporary climate change is provided in Table 1.1.

Adjusting to changing climatic conditions is crucial if individuals are to maintain fitness and populations are to persist under a changing climate. Where rates of environmental change exceed the ability of a species to disperse to more favourable areas, modify its behaviour to adapt *in situ*, or evolve to suit emerging conditions, fitness declines in individuals, abundance declines in populations, and alterations of community structure will result (Rubolini *et al.* 2010). In many cases, the critical impact on fitness will come not directly from climatic change itself, but indirectly, from climate-driven changes in resources, competitors and predators. There are well-documented cases of populations that have shifted their own timing or location in line with interacting species, with the result of maintaining fitness in the face of changing climate (e.g. Charmantier *et al.* 2008; Bauer *et al.* 2010; Matthysen *et al.* 2011). Because physiological tolerances and optima differ from species to species with individual physiology, however, there is little reason to expect interacting species to respond generally in the same manner to a shared change in climate. This invites the opportunity for interspecific interactions to change under climate change, and for these changing interactions to exert selective pressure on species alongside the selective pressures of changing climate itself.

Examples of such “mismatches” come from a variety of taxa, including birds (Ahola *et al.* 2007; Both *et al.* 2009; Saino *et al.* 2009), plants (Visser and Holleman 2001), mammals (Post *et al.* 2008), and plankton (Edwards and Richardson 2004; Winder and Schindler 2004) (Table 1.2). These “indirect effects” of climate change are pervasive and potentially important, and they complicate efforts at ecological prediction.

1.2 Predicting species responses to climate change

One of the greatest challenges in modern ecology is to furnish information on how species will respond to future climate change, both temporally and spatially. If we can anticipate when and where species are likely to occur in the future, we can better-understand species-specific extinction probabilities (e.g. Thomas *et al.* 2004) and future community structure (e.g. Gilman *et al.* 2010). Such *a priori* knowledge will guide management solutions to the challenges posed by climate change and allow managers some measure of control over future consequences.

Table 1.1: A selection of studies documenting distributional and phenological shifts attributable to contemporary climate change.

Study	Distribution or phenology?	Taxa	Number of species	Location	Year	Main findings
Beebee 1995	Phenology	Amphibians	6	Hampshire and Sussex, UK	1971-1995	- 2/3 studied anurans advanced their first spawning date. - 3/3 urodeles advanced their date of first adult appearance.
Crick <i>et al.</i> 1997	Phenology	Birds	65	UK	1971-1995	- 20/65 birds advanced their first laying date by an average of 8.8 days.
Bradley <i>et al.</i> 1999	Phenology	Plants and Birds	55	Southern Wisconsin, USA	1963-1998	- 35% of species showed a significant advancement in the timing of spring events in the 1980s and 1990s, including first flowering date and first arrival date.
Parmesan <i>et al.</i> 1999	Distribution	Butterflies	35	Europe	1915-1997	- 63% of non-migratory European butterflies showed a significant northwards shift in their entire latitudinal range. - 3% showed a significant southwards shift.
Thomas & Lennon 1999	Distribution	Birds	59	UK	1968-1991	- 59 southerly species shifted their range 18.9 km northwards, independent of overall population expansions and retractions.
Luckman & Kavanagh 2000	Distribution	Plants	-	Canadian Rockies	1900-2000	- Latitudinal upwards movement of treelines in line with 1.5°C warming over the course of the last century.
Roy & Sparks 2000	Phenology	Butterflies	35	UK	1976-1998	- 22/35 species significantly advanced their date of first appearance.
Fitter & Fitter 2002	Phenology	Plants	385	UK	1954-2000	- 4.5 day average advancement in first flowering date in the final decade of the study compared with the average over the previous four decades.

Continued...

Study	Distribution or phenology?	Taxa	Number of species	Location	Year	Main findings
Parmesan & Yohe 2003	Distribution and Phenology	Multiple	1700	Global	Various	- Global cross-species meta-analysis documented average range shifts towards the poles of 6.1km per decade, and average advancement of spring events of 2.3 days per decade.
Hickling <i>et al.</i> 2005	Distribution	Odonata	24	UK	1960-1995	- 23/24 species extended their northern latitudinal range limit by an average of 88km.
Menzel <i>et al.</i> 2006	Phenology	Multiple	542 plants 19 animals	Europe	1971-2000	- 30% of all species showed a significant advancement in leafing, flowering and fruiting records. - 3% showed a significant delay.
Swanson & Palmer 2009	Phenology	Birds	44	Minnesota and South Dakota, USA	South Dakota: 1971-2006 Minnesota: 1964-2005	- 18/44 species showed a significant advancement in first arrival date in South Dakota. - 16/44 species showed a significant advancement in first arrival date in Minnesota.

Table 1.2: A selection of studies demonstrating mistimed interactions between species as a result of differential responses to climate change.

Study	Location	Year	Main findings
Visser & Holleman 2001	Netherlands	1988-2000	- Increased incidence of asynchrony between oak bud burst and winter moth larval hatch, with moths unable to keep pace with advancement in bud burst. - Thought to be driven by increases in spring temperatures with no concomitant decrease in the incidence of winter freezing.
Edwards & Richardson 2004	North Sea	1958-2002	- Variation in the phenological responses of plankton to ocean warming throughout the community and seasonal cycle, resulting in several trophic mismatches in the community food web.
Winder & Schindler 2004	Lake Washington, USA	1962-2002	- Variation in the extent of change in bloom phenology of Phytoplankton and Zooplankton in response to recent warming, resulting in trophic mismatches in the community food web and consequent declines in Daphnia populations.
Ahola <i>et al.</i> 2007	Finland	1953-2005	- Interspecific laying date interval for pied flycatchers and great tits decreased in warmer years of the study, resulting in a greater incidence of failed nest takeovers by pied flycatchers.
Post <i>et al.</i> 2008	Arctic	2000-2007	- Reduced spatial variation in plant phenology under warming, lowering forage availability to caribou and resulting in declines in offspring production.
Both <i>et al.</i> 2009	Netherlands	1985-2005	- No advancement of reproductive phenology in a Dutch population of Sparrowhawks, resulting in increasing temporal mismatches with food sources.
Saino <i>et al.</i> 2009	Europe	1947-2007	- Failure of the brood parasitic common cuckoo (<i>Cuculus canorus</i>) to advance its arrival onto breeding grounds in line with short distance migrant hosts across 20 sites, resulting in mismatches between cuckoo arrival and nest availability.

There is enormous research effort into ecological prediction under climate change. This applies not just to conservation, but to agricultural and fisheries production, and disease management as well. Most of this work involves extrapolation from relationships between climate and species that we can measure today. Such relationships may describe where species exist on the landscape in relation to climatic conditions, how they time crucial aspects of their life history in relation to these conditions, or how abundances have changed in relation to climatic conditions through some time series of observations. In each case, the observable relationship forms the basis of expectation for the future. Attempts are then made to account for sources of uncertainty in these expectations such as thresholds, biotic interactions, lags, and other non-linearities that will be important as conditions change but may not be evident from current relationships (e.g. Iverson and Prasad 1998; Bakkenes *et al.* 2002; Jetz *et al.* 2007). Another prominent approach to prediction is more explicitly rooted in process. Here, mechanistic relationships between climate change and species are modelled according to measured physiological tolerances and other processes linking the two (e.g. Sitch *et al.* 2008; Coma *et al.* 2009; Morin *et al.* 2009).

Each approach is challenged by numerous sources of uncertainty. Researchers have made some solid progress towards contending with these uncertainties however. Pearson and Dawson (2003) devised a hierarchical framework by which the uncertainties that come to bear upon prediction making can be partitioned by spatial scale, for example. At the broadest spatial scales of prediction (e.g. whole species ranges), species physiological tolerances alone may be successfully predictive of future dynamics. For management of ecological systems under climate change however, more local scales are often the most relevant. Predictions of these local-scale changes are likely subject to the greatest number and magnitude of uncertainties, and thus scaling predictions down towards such local scales requires consideration of additional influences, such as habitat cover, land use and biotic interactions.

Community ecologists are focusing on better understanding the mechanisms of local-scale species responses to climate change (e.g. Barton and Schmitz 2009). In turn, this understanding is being built into predictive models (e.g. Gilman *et al.* 2010). In addition to accounting for uncertainties within existing methods for prediction however, we need to be seeking new sources of predictability as well. Indeed, the more divergent approaches we can use to inform upon understanding and anticipating such complex local-scale ecological changes, the greater our likelihood of success will be in minimising the negative consequences of climate change and maximising positive opportunities. The work of this

thesis then, is presented not with a mind to replacing existing techniques for prediction, but towards complementing those techniques for an “every tool in the box” approach toward contending with climate change (Dawson *et al.* 2011). Development of alternative, complementary techniques that might refine, rescale and unite current approaches to prediction is imperative if these predictions are to be most relevant and useful in both mitigation and adaptation senses.

1.3 Biological traits as units for prediction

The use of biological traits as predictors of species responses to climate change may offer the kind of new approach we are looking for. A trait is a well-defined, measurable property of an organism that is usually characterised at the level of the individual and can be used comparatively across species (McGill *et al.* 2006). A functional trait is one that has a strong influence on organism performance and includes characteristics like egg or seed size, and basal metabolic rate (McGill *et al.* 2006). When environmental conditions change, certain traits might make species more or less sensitive to the change. Indeed, when we look across the many studies where clear directional changes in distribution or phenology have been reported in response to recent climate change (e.g. Table 1.1), variation in the extent and direction of response is obvious. This strongly suggests a role for Biology in predicting these responses.

The use of a trait-based approach to prediction carries many potential advantages. Primarily, because traits can be mapped to phylogenetic trees (Pau *et al.* 2011), trait-based rule-sets established for well-studied species may be applied to other species for which we have information on phylogeny and biology, but not necessarily on responses to climate change. Supporting this, recent studies have shown that phylogenetic relationships are important predictors of species responses to climate change. Rubolini *et al.* (2007) demonstrated that changes in arrival date of European migratory bird species at their breeding ground in relation to climate change was linked to phylogeny. Willis *et al.* (2008) showed that the phenological sensitivity of East-American plants to changes in spring temperature over the past 150 years was also phylogenetically conserved. Davis *et al.* (2010) demonstrated a similar trend for UK plants, although only when certain clades were excluded, suggesting that community context may be important to some extent (Pau *et al.* 2011).

Trait-based rule-sets for how species can be expected to respond to climate change may also be transferable between sites. There are no two identical ecosystems. The physical and biological context in which species experience climate change will vary from place to place and time to time. Locally varying factors are an important source of uncertainty in predicting how species will respond to future climate change. Stepping back from species toward a more probabilistic view of expected changes based on traits may help to diminish the influence of local variability in context, allowing individual species to behave idiosyncratically while responses are more consistent at the level of shared traits. This is a further virtue of a trait-based approach: the probabilistic nature of expectations is made explicit. This forces caution into our interpretation of predicted changes that perhaps should be adopted for virtually all forms of prediction, given the uncertainties involved. Such caution is sometimes discounted with quantitative outputs from species-focused modelling.

Finally, trait-based approaches can make use of the large body of natural history and ecological response data already available for a wide number of taxa, and thus will be comparatively quick to implement (e.g. from PLANTATT (Hill *et al.* 2004); Animal Diversity Web (Myers *et al.* 2013); Birds of North America Online (Poole 2005); *etc.*). Overall the potential transferability of trait predictions between species, sites and scales and the immediacy of knowledge gains they offer may make them useful tools for refining and uniting current approaches to prediction.

Certain traits might be expected to be more useful than others in predicting the responses of species to climate change. Firstly, traits that vary more between species than within them will be of greater value (McGill *et al.* 2006). Furthermore, one might expect that functional traits would be particularly important, given their impact on fitness, although it is possible that some traits not classed as “functional” may act as good proxies. Body mass for example, is a good proxy for certain life history traits (Purvis *et al.* 2000). There are seven groups of traits that might affect how species change their distribution and/or phenology under climate change, and thus be useful predictors:

1. **Life History** e.g. gestation period; perennation strategy; clutch size. The speed of a species’ life history might be an important determinant of its distributional and phenological responsiveness to climate change. Species with slow life histories typically have low fecundity and high adult survival, and adult survival is normally the life history trait which is under the strongest selective pressure (Sandvik and Erikstad 2008). Consequently, such species might invest less in any specific breeding

attempt at the cost of future ones (Sandvik and Erikstad 2008). Since distributional and phenological shifts are methods of tracking appropriate conditions through space and time, so maintaining immediate fecundity under climate change, species with slower life histories might show smaller shifts.

2. **Ecological Generalism** e.g. diet breadth; habitat breadth. The breadth of a species ecological niche might be expected to impact its distributional and phenological shifts in response to climate change, however the direction of this effect is unclear (Buckley and Kingsolver 2012). The responses of species displaying high ecological generalism will be less constrained by needing to spatially and temporally track specific habitat types, pollinators or food sources. This might mean that ecological generalists are able to persist better at new spatial or temporal locales than ecological specialists. Alternatively, if ecological specialists are constrained by tracking the specific phenology or range of certain species with which they interact, they may show stronger shifts in distribution or phenology than do generalists, so as to keep pace with their interactors.
3. **Genetic diversity** e.g. total range size; population proximity to a species range limit. Genetically depauperate populations may show lower adaptive capability. They might thus be expected to show weaker distributional and phenological shifts if such responses are related to evolutionary adaptation. Some genetically depauperate populations however (such as those proximal to a range edge) may be under strong selective pressure (Arnaud-Haond *et al.* 2006) and thus may show stronger responses to the changing climate.
4. **Seasonal readiness:** e.g. overwintering stage; flowering time; migration distance. Species more readily able to respond to increasing temperatures (e.g. by flowering during months that have seen the greatest temperature increase or overwintering as a mobile larval stage that can then disperse more rapidly at Spring warm up) might show stronger distributional and phenological shifts in response to a longer-term change in climate.
5. **Preadaptation:** e.g. species adapted to warmer climes. Species that have some level of preadaptation to warmer temperatures might be expected to show greater distributional and phenological responses to a change in climate as a result of this preadaptation.

6. **Dispersal ability (for range responses):** e.g. length of dispersal period. Species with a greater ability to disperse might show stronger distributional responses under the changing climate.
7. **Proxies** e.g. body mass. Body mass has been suggested to be a useful proxy for other suites of life history traits, with animals of greater mass tending to have slower life histories (Purvis *et al.* 2000). Such proxies might thus be valuable predictors of distributional and phenological responses to climate change. This final point is worth discussing in more detail. Mechanistic insights are a key pursuit in climate change ecology, and whilst preferable, they are not necessary for this approach to be useful. A statistical association between strength of response and some shared characteristic among species is still beneficial in this context, even if it cannot be made sense of mechanistically.

1.4 Evidence for the value of traits as predictors

Evidence is accruing to suggest that traits might indeed be useful predictors of the ecological responses of species to the changing environment. Traits have already emerged as predictors of a variety of biological phenomena, most notably extinction risk (Owens and Bennett 2000; Cardillo 2003; Cardillo *et al.* 2005; Cooper *et al.* 2008; Fritz *et al.* 2009) and invasiveness (Grotkopp *et al.* 2002; Blackburn *et al.* 2009; van Kleunen *et al.* 2010), but also responsiveness to ocean acidification (Halloran *et al.* 2008), responsiveness to habitat loss (Bommarco *et al.* 2010), and tendency to become dominant following other extinctions (Doak and Marvier 2003). There are now an increasing number of field and experimental studies looking at traits as predictors of species responses to climate change. These demonstrate that responses of a variety of species to the changing climate break down according to traits or certain suites of traits.

Buckley and Kingsolver (2012) performed an ISI Web of Knowledge and Google Scholar search (including all combinations of the three search terms: climate change, phylogeny or trait(s), and phenology or range shifts) in order to identify studies that have examined traits or phylogeny as predictors of cross-species ($n > 5$) distributional and phenological responses to climate change, up to the end of 2011. Where a particular dataset was analysed several times, they cited the study that had performed the most thorough investigation of trait-based predictors. They identified 20 studies where traits have been used as predictors of species

distributional or phenological shifts in relation to climate change. 17 of these demonstrated a significant relationship between trait(s) and response, whilst 3 found no significant relationships in their analyses (Hill *et al.* 2002; Gienapp *et al.* 2007; Forero-Medina 2011). Performing the same Web of Knowledge search from 2012 to October 2013, and excluding papers considering a timeframe of ≤ 5 years (1 study), I identified 8 more studies that relate traits to species distributional or phenological responses. One study (Zhu *et al.* 2012) showed no significant relationship between traits and response. The remaining 7 studies were combined with the 17 significant studies from Buckley and Kingsolver's search, and are summarised in Table 1.3.

These 24 studies provide evidence that traits are significant predictors of the phenological and distributional responses of a broad variety of both terrestrial and aquatic species to climate change, displaying moderate explanatory power. Traits from all the groups listed in section 1.3 are represented as being important predictors in one or more of these studies. Whilst generally there is consensus across studies with the hypotheses surrounding these proposed trait groups, certain results within some studies show direct contradictions (e.g. Jonzen *et al.* 2006; Diamond *et al.* 2011; Molnar *et al.* 2012) suggesting some context dependence to trait-predictions. Furthermore there are considerable methodological inconsistencies between the different papers. One of the most promising ways to test for trait-based correlates of response across species is to analyse the effects of several traits using a phylogenetically controlled comparative analysis with model selection on linear or linear mixed effects models, following the methods of Burnham and Anderson (2002). This approach allows multiple, independent predictors to be identified whilst controlling for phylogenetic non-independence. The latter is crucial in analyses that include evolved traits, since closely related species will display greater trait similarities than expected by chance, violating statistical assumptions of data independence. The approach also avoids the caveats of stepwise regression modeling, which include parameter estimation bias, model selection algorithm inconsistencies and an inappropriate focus on a single "best" model (Whittingham *et al.* 2006). Despite this, only 6 of the studies in Table 1.3 used this method and clearly stated that a phylogenetic signal was tested and controlled for (specifically: Vegvari *et al.* 2010; Rubolini *et al.* 2010; Angert *et al.* 2011; Diamond *et al.* 2011; Molnar *et al.* 2012; Reif and Flousek 2012). This brings into question the reliability of some of the other trait studies. Finally, whilst some studies (e.g. Wolkovich *et al.* 2013) compare predictions across different

Table 1.3: The 24 studies identified by the literature searches of Buckley and Kingsolver (2012) and myself, documenting significant relationships between species traits and their distributional and phenological responses to climate change. These responses (column 2) refer to polewards shifts in latitude, upwards shifts in elevation and advances in spring phenophases. P denotes a phenological response and R a distributional response. R^2 values (column 7) are given, where available, for the minimal model in stepwise approaches and the R^2 of the range of candidate models from model selection analyses. If this range is not available, the R^2 of the average model is given. R^2 values marked with “*” represent that the value is for the single regressor with the greatest explanatory power. R^2 values marked with “-” represent those studies where the R^2 value was not reported. R^2 values marked with an NA are for non-parametric studies where R^2 is not an appropriate metric. The trait categories listed in section 1.3 are attributed to the results of each study (column 8), where appropriate. These are denoted LH, EG, SR, GD, D and P for Life History, Ecological Generalism, Seasonal Readiness, Genetic Diversity, Dispersal Ability and Preadaptation respectively. AIC denotes Akaike Information Criterion. The studies highlighted in blue are those that implement a phylogenetically controlled comparative analysis with model selection on linear or linear mixed effects models.

Study	Response attributable to climate change	Taxa	<i>n</i>	Location	Time period	R^2	Specific traits linked to greater responsiveness	Method
Altermatt 2010	P: Flight period shift	Butterflies and moths	556	Europe	1964-2008	0.08	- Overwintering as eggs - Larvae feed on woody plants - Larger range (↑GD)	Multiple regression with stepwise simplification
Buskirk <i>et al.</i> 2012	P: First arrival date at breeding ground	Birds	27	Eastern USA	1961-2006	-	- Shorter migratory distance (↑SR)	Mixed effects models
Butler 2003	P: First arrival date at breeding ground	Birds	103	Eastern USA	1903-1993	NA	- Shorter migratory distance (↑SR)	Non-parametric
Diamond <i>et al.</i> 2011	P: Date of first appearance	Butterflies	44	UK	1976-2010	0.43-0.52	- Fewer host plants (↓EG) - Adult overwintering stage - Earlier baseline date of first appearance (↑SR) - Less widespread, less dense species (↓GD)	Model selection on multiple regression models using AIC
Fitter & Fitter 2002	P: First Flowering Date	Plants	385	UK	1954-2000	0.19*	- Spring flowering (↑SR) - Annual life cycle (↑LH) - Insect pollination - Closer centre of distribution(↑SR)	Chi-square, ANOVA, Spearman's rank

Continued...

Study	Response attributable to climate change	Taxa	<i>n</i>	Location	Time period	R ²	Specific traits linked to greater responsiveness	Method
Hassall <i>et al.</i> 2007	P: Date of first adult emergence	Odonata	25	UK	1960-2004	0.31*	- Spring emergence (↑SR)	Multiple regression, no simplification or model selection
Hurlbert & Liang 2012	P: Mean arrival date at breeding ground	Birds	18	USA	2000-2010	0.63	- Slower migration - Shorter migratory distance (↑SR) - Broader climatic niche (↑EG)	Model selection on multiple regression models using AIC
Jonzen <i>et al.</i> 2006	P: Arrival date at breeding ground	Birds	34	Scandinavia	1980-2004	-	- Longer migratory distance (↓SR)	95% Confidence Intervals around regression Beta-values
Molnar <i>et al.</i> 2012	P: Mean flowering date	Plants	39	Hungary	1837-2011	0.34-0.54	- Autogamous pollination strategy (↑EG) - Earlier flowerers (SR↑) - Long lived (↓LH) - Mediterranean species (↑P)	Model selection on multiple regression models using AIC
Rubolini <i>et al.</i> 2007	P: First and mean arrival date at breeding ground	Birds	184	Europe	1960-2006	0.18	- Shorter migratory distance (↑SR)	Mixed effects model with stepwise deletion
Rubolini <i>et al.</i> 2010	P: First spring singing date	Birds	56	Northern Germany	1977-2006	0.11*	- Shorter migratory distance (↑SR)	Model selection on multiple regression models using AIC
Stefanescu <i>et al.</i> 2003	P: Mean adult flight date	Butterflies	17	Northwest Mediterranean	1988-2002	-	- Grass host plants	ANOVA with Tukey-Kramer post-hoc tests
Thackeray <i>et al.</i> 2010	P: Multiple spring events	Multiple	726	UK	1976-2005	0.06*	- Primary producers and primary consumers advanced more rapidly	Mixed effects models

Continued...

Study	Response attributable to climate change	Taxa	<i>n</i>	Location	Time period	<i>R</i> ²	Specific traits linked to greater responsiveness	Method
							than secondary consumers (↑EG)	
Vegvari <i>et al.</i> 2010	P: First arrival date	Birds	117	Hungary	1969-2007	-	- More complex diets (↑EG) - Shorter migratory distance (↑SR) - More broods per year - No prebreeding moult (↑SR) - Summer-time post-breeding moult (↑SR)	Model selection on multiple regression models using AIC
Wolkovich <i>et al.</i> 2013	P: Mean annual flowering time	Plants	198 - 477	Multiple sites across UK and North America	1851-2010, but variable between sites	-	- Non-native (↑EG)	Mixed effects models (cross site) and linear regression models (within site)
Angert <i>et al.</i> 2011	R: Northern latitudinal range margin	Birds	254	North America	1775-2004	0.07-0.12	- Broader diets (Passeriformes) (↑EG)	Model selection on multiple regression models using AIC
	R: Northern latitudinal range margin	Odonata	24	UK	1960-1995	0.10-0.24	- Exophytic egg habitat (↑EG)	
	R: Upper elevational margin	Plants	133	Switzerland	1885-2004	0.01-0.18	- Longer dispersal period (↑D)	
	R: Upper elevational margin	Mammals	28	Western North America	1914-2008	0.10-0.31	- Lower historical range limit (↓P)	
Betzholtz <i>et al.</i> 2013	R: Northern latitudinal range margin	Butterflies and moths	282	Sweden	1973-2010	-	- Diet and habitat generalism (↑EG) - Forest-based species - Specialist species with Nitrogen-based larval diets - Species active during warmer periods of the year (↑SR)	Model selection on multiple regression models using AIC

Continued...

Study	Response attributable to climate change	Taxa	<i>n</i>	Location	Time period	<i>R</i> ²	Specific traits linked to greater responsiveness	Method
Dulvy <i>et al.</i> 2008	R: Mean latitudinal range	Fish	28	North Sea	1980-2004	-	- Abundant, widespread thermal specialists shifting northwards to a greater extent than other groups	Separate robust regression analyses for each species, with average departures from mean slope calculated for trait-categorised species assemblages
Felde <i>et al.</i> 2012	R: Optimum and maximum elevation	Plants	106	Norway	1922-1932, 2008	-	- Higher preference for prolonged snow cover (↑P)	Linear regression for each trait
Grewe <i>et al.</i> 2013	R: Northern latitudinal range margin	Odonata	112	Europe	1988-2000	0.32	- Lentic species at Southern range boundary (↑D)	Multiple regression, no removal of non-significant terms
Lenoir <i>et al.</i> 2008	R: Optimum elevation	Plants	171	Western Europe	1905-2005	-	- Mountainous habitats (↑P) - Herbaceous life form (↑LH)	Multiple regression
Perry <i>et al.</i> 2005	R: Mean latitudinal range	Fish	90	North Sea	1977-2001	0.24*	- Fast life cycle (↑LH) - Small body size (↑LH)	t-test on difference between mean values for each trait for shifting and non-shifting species
Poeyry <i>et al.</i> 2009	R: Northern latitudinal range margin	Butterflies	48	Finland	1992-2004	-	- Mobile species (↑D) - Woody plant host for larvae	Hierarchical partitioning analysis
Reif and Flousek 2012	R: Upper elevational margin	Birds	51	Central Europe	1996-2008	0.02-0.12	- Use of open habitats	Model selection on multiple regression models using AIC

sites, none of them formally examine the transferability of trait-based predictions between different geographies. This was one of the key values of the trait approach proposed earlier. It is clear that further research is required to establish reliable trait models for more groups of species, and to ascertain whether trait rule-sets can be applied between different groups of the same taxa, and between such groups at different locales.

1.5 Methodological considerations

In investigating this area further, we need to consider a number of obstacles to the implementation of any form of comparative investigation into traits as predictors of species responses to climate change. Firstly, the abundant published literature describing biological responses to recent climate change often provides only summary statistics across large groups of species, rather than species-by-species data that could be mined with an eye toward trait-based correlates of response (e.g. Parmesan and Yohe 2003; Root *et al.* 2003; Amano *et al.* 2010). More detailed records are surely abundant, but often closely held. For the records that are freely available, few are standardised and comprehensive. Others have limited scope, calculating trends for only a few species or a few years, rather than over more useful, decadal scales (e.g. Donnelly *et al.* 2006). Others still are inconsistent in or vague regarding the sites from which data are recorded (e.g. Swanson and Palmer 2009). Information on traits, too, is often inconsistent in breadth and detail. Between the limited availabilities of both species response and trait data, low n values restrict potential analyses and demand careful consideration in selection of data (e.g. what traits to use) and choice of analysis structure (e.g. whether to examine interactions between traits or just main effects). There are also a variety of caveats inherent to any analysis that uses a phylogenetic approach. Indeed, phylogenetic trees are biased with regards to the chosen method of reconstruction (Barracough and Nee 2001), molecular dating (Bennett and Owens 2002), the fundamental definition of species (Agapow *et al.* 2004), the choice of taxonomic scale (Harvey and Pagel 1991) and the reliance on correlative approaches rather than experimental ones (Purvis and Agapow 2002).

Finally, the way in which trait-based predictions are interpreted and linked back to species should be carefully considered. One of the main issues confronting any species-focused model is over-interpretation of outputs and consequent expectations that they can provide definitive spatiotemporal answers if the uncertainties involved can be understood. In reality, some uncertainties are simply intractable with respect to predictive modelling of ecological responses to climate change, and will be likely to persist regardless of efforts to eliminate

them (Carpenter 2002; Doak *et al.* 2008; Walther 2010). Open-ended efforts focussed on reducing uncertainty can therefore be at odds with the urgency with which action is required to prevent further damage (Carpenter 2002). Interpretation of trait models then, should not be focussed on using the output to say “this species will”, but rather should be framed in terms of “these kinds of species should”. Ultimately, humility, flexibility and frequent reassessment of projections for the future is crucial, as is a healthy awareness of what we cannot know, as much as what we can.

1.6 Thesis outline

This thesis attempts to develop some new ideas and tools for predicting ecological responses to climate change. Earlier chapters aim to provide assessment of the effectiveness of biological traits as predictors of species’ climate change responses across different taxa and geographically distinct populations. Chapter Two is a proof-of-concept modelling study that establishes the value of traits as predictors by using appropriate statistical methods to build trait-based rule-sets of plant phenological responses. Chapter Three provides a further test of traits as predictors of phenological responses, but in a different taxon: birds. It further addresses the critical issue of transferability, asking whether trait-based predictions can be transferred between regions by assessing if bird phenological responses in one area can be predicted from relationships between traits and responses in another area. Chapter Four moves towards a more conservation-relevant metric of species response to climate change: abundance. It examines whether traits are useful predictors of the abundance responses species show using a detailed record of community-wide changes in species abundance under ten years of experimental climate change. It also re-evaluates the hypothesis that has guided researchers’ interpretations of the ecological changes seen in this experimental system using new data and analyses. Chapter Five retains a focus on local responses. It designs and implements experimental research testing the role of climatic effects on a key trait that may be driving system dynamics via a controlled removal of this trait from experimental communities. It asks whether the indirect effects observed in this experimental community can be resolved to the level of a single major driver, and whether by targeting this driver the role of indirect relative to direct effects can be reduced in this system. Finally, Chapter Six reviews the principal findings of this thesis and their broader implications, and also postulates future directions for research in this area.

Chapter Two

Traits as predictors of phenological change in plants

2.1 Abstract

Predictions of species responses to climate change face challenges of scale, precision and efficiency. Biological traits may offer additional information on how species respond to climate change that could be used to refine and rescale current approaches. At this point, the utility of traits in this regard has received relatively little study. Here, I test the value of traits in explaining cross-species variation in the phenological changes observed in 351 British plant species from 1954 to 2000. Characteristics related to the broader distribution and the native origin of species significantly correlated to their extent and direction of phenological change, but with low overall explanatory power. My results indicate that species traits can effectively predict their responses to climate change, but will be of most practical relevance when used in conjunction with other predictive techniques.

2.2 Introduction

Changes in the timing of life history events (*i.e.* phenology) are a common response by species to climate change. Phenological shifts allow species to track suitable conditions and resources through time, just as distribution shifts allow tracking through space. The recent biological record is filled with examples of changes in the annual timing of life history events across diverse taxa. Most of this evidence relates to changes in the timing of spring events such as bud burst (Bradley *et al.* 1999), flowering (Fitter and Fitter 2002), egg laying (Crick *et al.* 1997), migration from wintering to breeding grounds (Swanson and Palmer 2009) and emergence from hibernation (Ozgul *et al.* 2010). Overwhelmingly, the direction of these changes accord with expectations for adaptive responses to climate change (Parmesan and Yohe 2003; Root *et al.* 2003; Menzel *et al.* 2006; Parmesan 2007): species springtime life history events are trending toward earlier Julian dates in line with temperature increases. These changes span taxa and continents, with major meta-analyses establishing the global extent of this phenomenon and implicating contemporary climate change as the primary driver (Parmesan and Yohe 2003; Root *et al.* 2003; Menzel *et al.* 2006; Rosenzweig *et al.* 2008; Chen *et al.* 2011). The preponderance and global extent of evidence of phenological responses to contemporary climate change led the Intergovernmental Panel on Climate Change (IPCC) to designate phenological advance and delay an important proxy for the overall biological signal of climate change (IPCC 2007).

Phenological responses to climate change have important implications for individual fitness, population persistence and community structure (e.g. Winder and Schindler 2004; Both *et al.* 2006; Willis *et al.* 2008). These implications extend beyond the species directly affected to the communities around them – co-occurring species with physiologies that allow them to escape detrimental effects of climate change may be affected all the same. Recent research demonstrates how indirect effects of phenological changes can spread through food chains, competitive relationships and plant-pollinator networks. Indeed, while many important effects will be direct (e.g. Ozgul *et al.* 2010), there is tremendous opportunity for important population-level effects from trophic mismatch (Winder and Schindler 2004; Both *et al.* 2009; Saino *et al.* 2009). Understanding and potentially predicting phenological changes under climate change is therefore an important goal, particularly given the more substantial climate change expected still to come (IPCC 2007). One of the greatest challenges in modern ecology is to furnish information on how species will respond to climate change so that natural resource managers and other conservation practitioners can make preparations that

will limit the negative consequences of it. Such *a priori* knowledge will be necessary to guide management solutions to the challenges posed by the changing climate and to allow managers some measure of control over future consequences. Predictive understanding of changes in species ranges and phenologies could help us assess species extinction probabilities (e.g. Thomas *et al.* 2004) and inform adaptation planning.

Prediction of climate change impacts is a major focus of scientific research. Ecologists have developed a number of approaches to predicting various forms of responses (e.g. species abundances, species ranges, species phenologies, ecosystem function, ecosystem composition or diversity) at scales ranging from individual populations (e.g. Wolf *et al.* 2010) to the whole biosphere (e.g. Cramer *et al.* 2001; Scholze *et al.* 2006). Species-level predictions probably represent the greatest focus as species are fundamental to how we conceptualise and categorise the natural world. Predictions of species-level responses to future climate change are often built around physiological tolerances, extrapolations from already documented trends, or correlations between climate change and observed species distributions, phenologies or abundances (Helmuth *et al.* 2005; Kearney and Porter 2009; Morin and Thuiller 2009, Buckley *et al.* 2010). These predictions are challenged by a number of uncertainties (Carpenter 2002; Doak *et al.* 2008; Walther 2010), including the potential for indirect effects from species interactions, as discussed above. While much of the field is devoted to identifying, investigating, and reducing these uncertainties, it is also important to seek out new pathways to prediction that may circumvent some of them.

A focus on species traits may offer one such pathway forward. A trait is a well-defined, measurable property of an organism that is usually defined at the level of the individual and can be used comparatively across species (McGill *et al.* 2006). When we look across those studies documenting clear, directional responses to recent climate change for large numbers of species, variation in the extent and direction of response is immediately evident (e.g. Parmesan and Yohe 2003; Menzel *et al.* 2006). The variation in phenological responses of species to climate change in existing records implies that certain characteristics make species more or less phenologically responsive to a given change in climate.

Traits may be a useful addition to our predictive toolkit for several reasons. Firstly, traits can be mapped to phylogenetic trees (Pau *et al.* 2011) and thus trait-based rule sets established for well-studied species may also be applicable to other species for which we have information on phylogeny and biology, but not necessarily on responses to climate. Secondly, trait-based predictions may be transferable between sites and scales. If traits are acting as functional

respondents to climate change, in that species responses can be predicted based on the traits they possess, then insights should carry over from region to region. Finally, trait-based approaches can make use of the large body of natural history and ecological response data already available for a wide number of taxa. Overall, trait-based predictions may be broadly relevant across both sites and species at a variety of scales.

There are currently 15 published studies demonstrating that traits can statistically explain cross-species variation in phenological responses to climate change (for full list see Table 1.3). There is extensive methodological variation across these studies, with one of the main weaknesses in several of them being the lack of appropriate testing and control for phylogenetic non-independence in models (e.g. Butler 2003; Altermatt 2010; Buskirk *et al.* 2012; Hurlbert and Liang 2012). While certain similarities between species can arise despite independent evolutionary origins, most occur as a result of shared evolutionary history. Closely related taxa therefore share various similarities due to common descent. If analyses on evolved traits do not take account of this, pseudoreplication is introduced as differences between major groups are counted multiple times (Purvis 2008). Consequently, it is essential that a phylogenetic signal is tested and controlled for in trait-based analyses if results are to be reliable. Another weakness is the use of a stepwise regression approach in simplifying models of multiple traits (e.g. Rubolini *et al.* 2007; Altermatt 2010). Stepwise regression is subject to parameter estimation bias and model selection algorithm inconsistencies, and has an inappropriate focus on a single “best” model (Whittingham *et al.* 2006). A more appropriate technique that avoids these stepwise caveats is a model selection approach, as laid out in Burnham and Anderson (2002). Only 4 of the 15 trait studies looking at phenological responses to climate change use this model selection approach and explicitly state that they test and control for phylogenetic non-independence in their models (Rubolini *et al.* 2010; Vegvari *et al.* 2010; Diamond *et al.* 2011; Molnar *et al.* 2012). Despite significant traits being identified in all these studies, the explanatory power of the models they produce is highly variable (R^2 values range from 0.11-0.54) indicating uncertainty around the practical relevance of trait based predictions. It is clear that further work with rigorous methods is required to test the utility of traits as predictors of phenological responses to climate change and to develop this approach for more general application.

Plants are well suited for this kind of analysis. Plant phenological responses to climate change are well documented and not complicated by short-term motility, as animal responses might be. Plants represent the energy-base of virtually all of Earth’s terrestrial ecosystems,

so changes in phenology will influence resource availability for co-occurring consumer species (Visser and Holleman 2001). This improved understanding of plant responses to the changing climate may help us anticipate vulnerabilities in the food chains and webs that form above them.

Here I present a reanalysis of the classic Fitter and Fitter (2002) dataset on changes in first flowering date (FFD) for 385 plants over 47 consecutive years at a site in Oxfordshire, England, to determine whether FFD changes can be understood in terms of species traits. This dataset offers several advantages to a trait-based analysis. Indeed, the UK flora is exceptionally well characterised, with detailed information available on morphological characteristics, life history, evolutionary relationships, and native origins. Further, the Fitter and Fitter dataset spans a large number of species, a long timeframe and has factored importantly into our understanding of the ecology of climate change, as one of the earliest and most comprehensive pieces of evidence that the biological world was changing along with the physical world. As such, it represents an excellent choice as a dataset to build a proof-of concept trait-analysis with.

I define traits in accordance with McGill *et al.* (2006) as “well-defined, measurable properties of organisms that are...defined at the level of the individual and [can be] used comparatively across species”. Ten traits representing several of the trait categories detailed in Section 1.3 were selected for analysis (Table 2.1). Five hypotheses were tested:

1. Small, annual, or herbaceous species have faster life histories and thus will show greater phenological responsiveness, advancing their first flowering date to a greater extent. This is due to the fact that species with faster life histories tend to be under stronger selective pressure to maximise the effectiveness of immediate breeding attempts as opposed to future ones, and thus might show stronger responses to climate change (Sandvik and Erikstad, 2008).
2. Species with broader habitat usage, those independent of biotic pollination mechanisms (e.g. insect pollination) and species that are non-native to the UK (and thus are adapted to a broader range of habitats and diets), display greater ecological generalism and thus will show greater phenological responsiveness, advancing their first flowering date to a greater extent. This is because more generalist species are not constrained by needing to track the phenology or range of

specific interactors, and thus are able to respond more readily to changes in climate and perhaps persist better at new temporal or spatial locales.

3. Populations proximal to range edges often show low genetic diversity but high divergence (e.g. Durka 1999; Bouzat and Johnson 2004; Krauss *et al.* 2004). This indicates that there may be strong selective pressure acting on these populations (Arnaud-Haond *et al.* 2006). If this is the case, we might expect stronger responses to climate change from species with small ranges and those whose range edge is proximal to the study locale, if responses are driven by evolutionary adaptation. Such traits might thus promote more extensive shifts in first flowering date. Alternatively, if low genetic diversity greatly limits adaptive capacity, we might expect weaker responses from species possessing these traits.
4. Species that flower in months where temperature changes have been greatest will be able to respond more readily to changes in climate (i.e. will have greater Seasonal Readiness), showing greater phenological responsiveness and advancing their first flowering date to a greater extent.
5. Species adapted to more southerly biomes will show some degree of preadaptation to warmer conditions and so will show greater phenological responsiveness, advancing their first flowering date to a greater extent.

Results will be compared in detail with these hypotheses and other comparative trait analyses.

Table 2.1: A summary of the traits analysed as predictors of plant phenological responsiveness to climate change in this chapter. Further detail is provided in Methods.

Trait	Trait Category*	Variable type	No. of factor levels	Factor level names	Factor level <i>n</i> value	Primary Data Source**
Height (m)	LH	Continuous	N/A	N/A	N/A	PLANTATT (Hill <i>et al.</i> 2004)
Native Status	EG	Categorical	2	Native Non-native	279 75	PLANTATT
Perennation Strategy	LH	Ordered categorical Annual<Biennial<Perennial	3	Annual Biennial Perennial	52 29 273	PLANTATT
Woodiness	LH	Categorical	2	Woody Non-woody	313 41	PLANTATT
Biome	P	Categorical	4	Northerly Temperate Southerly Broad	63 172 102 17	PLANTATT
Total Range in Britain (km ²)	GD	Continuous	N/A	N/A	N/A	PLANTATT
Flowering time***	SR	Categorical	2	Peak Sub-peak	102 249	Plants For a Future (http://www.pfaf.org) The Met Office (http://www.metoffice.gov.uk/climate/uk/stationdata)
Northern Range Limit in Britain?	GD	Categorical	2	Yes No	40 314	PLANTATT

Continued...
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Trait	Trait Category*	Variable type	No. of factor levels	Factor level names	Factor level <i>n</i> value	Primary Data Source**
Pollen Vector	EG	Categorical	4	Insect	267	Ecoflora (Fitter and Peat 1994)
				Wind	68	
				Selfed	13	
Habitat Breadth	EG	Continuous	N/A	N/A	N/A	PLANTATT

* LH = Life History; GD = Genetic Diversity; EG = Ecological Generalism; SR = Seasonal Readiness; P = Preadaptation

**Missing values were filled in from other sources in the table and from the wider literature, including: NatureGate (<http://www.luontoportti.com/suomi/en/>); ISSG (<http://www.issg.org/index.html>); ARKive (<http://www.arkive.org/>); BioNET (<http://keys.lucidcentral.org/keys/v3/eafrinet/plants.htm>)

*** Flowering time represents whether a species flowers first during months that had experienced the greatest temperature increase from 1954-2000 (peak months), or not (sub-peak months). More detail in Methods.

2.3 Methods

2.3.1 Raw plant response data

Fitter and Fitter (2002) provided freely available FFD data for 385 species of British plant from 1954-2000. FFD values are numbered from 1 to 365, representing each day of the year chronologically. The long and consistent time frame over which these data were recorded (each species had FFD records for at least half of the study years) ensured reliable trends for analysis. Furthermore, the data were collected by a single observer (R.S.R. Fitter) eliminating issues of observation bias. Most records came from Chinnor, Oxfordshire. Chinnor is a rural area that did not experience much land use change during R.S.R Fitter's recording period (Fitter and Fitter 2002). A small amount of data came from counties adjacent to Oxfordshire. I allowed this variation since these locations were proximal to the main sampling site and represented only a small number of the total data points.

2.3.2 Temperature trends in Chinnor

As discussed previously, changes in phenology are an important response of many species to the changing climate. Consistent with the original Fitter and Fitter paper, this study examines phenological trends over time. That being said, if assertions about climate change are to be made in this analysis, it is important to look at the strength of the climate signal at the study site. Collating maximum and minimum yearly temperature data from a Met Office weather station in Oxford (4509E, 2072N), I calculated an average of the two values for each year of Fitter and Fitter's study. I then ran a linear regression of this data against time to examine the strength of the average climate signal (Table 2.2, Figure 2.1). I repeated this for monthly temperature data in order to discern whether the strength of the climate signal varied by month (Table 2.2, Figure 2.2). Results showed a signal of temperature increase from 1954-2000, although some months displayed stronger increases than others: January, February, March, July and August showed the greatest rates of temperature increase. October showed a small but non-significant decrease in temperature over the time series.

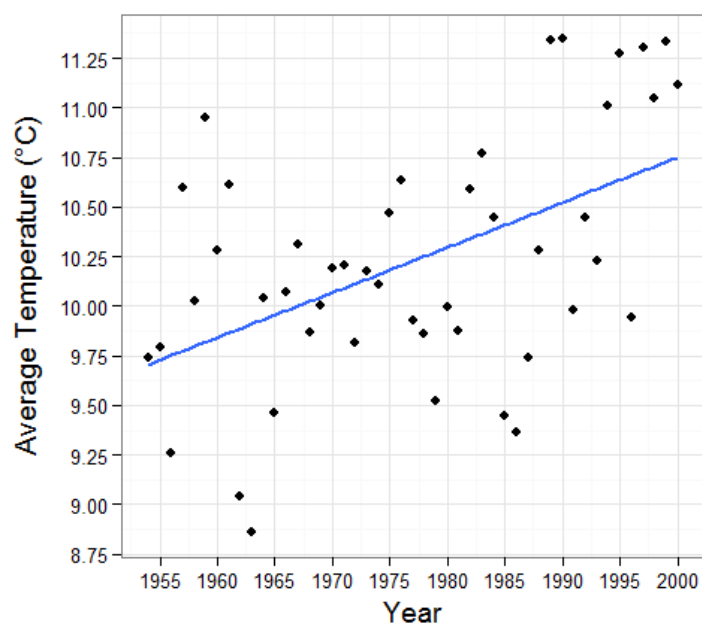


Figure 2.1: Average Temperature change at a Met Office weather station in Oxford (4509E, 2072N), from 1954-2000 (Met Office 2013).

Table 2.2: Results of regressions between average temperature and year, for the overall yearly average temperature and for the average temperature of each month in turn. Data are from a Met Office weather station in Oxford (4509E, 2072N) from 1954-2000 (Met Office 2013).

Trend	Estimate	S.E.	t-value	P-value
Year round	0.023	0.006	3.855	<0.001***
January	0.038	0.021	1.819	0.076 †
February	0.042	0.024	1.762	0.085 †
March	0.039	0.016	2.372	0.022*
April	0.006	0.010	0.587	0.560
May	0.017	0.012	1.431	0.159
June	0.005	0.013	0.363	0.718
July	0.035	0.014	2.523	0.015*
August	0.042	0.014	3.058	0.004**
September	0.013	0.011	1.262	0.213
October	-0.001	0.014	-0.103	0.919
November	0.020	0.013	1.587	0.120
December	0.018	0.018	0.986	0.329

† $0.05 \leq P \leq 0.10$, * $0.01 \leq P \leq 0.05$, ** $0.001 \leq P \leq 0.01$, *** $0.0001 \leq P \leq 0.001$

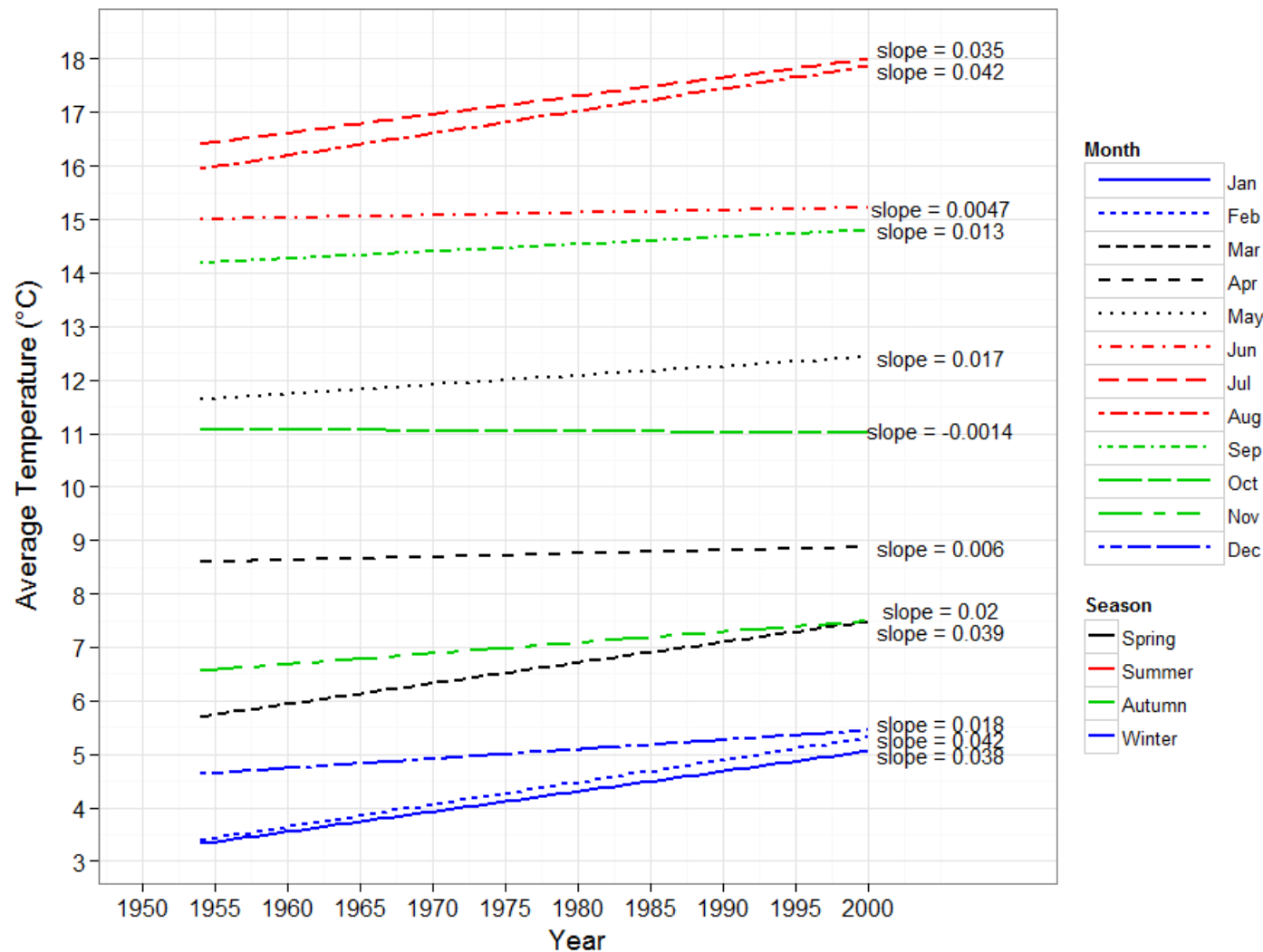


Figure 2.2: Regression slopes for the relationship between monthly average temperature and year, from 1954-2000. Data are from a weather station in Oxford (4509E, 2072N, Met Office 2013). The slope of each relationship is printed by each line. January, February, March, July and August show the greatest rates of temperature change.

2.3.3 Creating the responsiveness data

The phenological shift of a species under climate change represents an important indicator of that species' responsiveness to the changing climate. Here, what I term phenological responsiveness refers to the extent of change in a specific phenological event a species exhibits over a set period of time, ostensibly but not explicitly due to warming temperatures. Species experiencing large amounts of phenological change over this time period have high phenological responsiveness. Species experiencing small amounts of phenological change over this time period have low phenological responsiveness.

To calculate responsiveness for each species in my analysis, I took the raw FFD data from Fitter and Fitter (2002) and ran a linear regression of FFD against time, for each species (e.g. Figure 2.3). The slope of each regression was used as a measure of each species' responsiveness to climate change. Slopes were recorded for every species, regardless of significance, as I am interested in all varying magnitudes of phenological change. Species with negative slopes are *advancing* their FFD with time. Species with positive slopes are *delaying* their FFD with time.

Across species, there was variation in the number of years for which FFD was recorded. The uncertainty of an estimated slope increases with increasing variance and when the range of x values plotted is small, and declines with the number of data points plotted (Crawley 2007). As a result of this, the regression slopes for some species showed greater uncertainty than others. In order to standardise them, I created a vector of weights using the reciprocal variance of each slope, which were then used in my model selection analyses (see below).

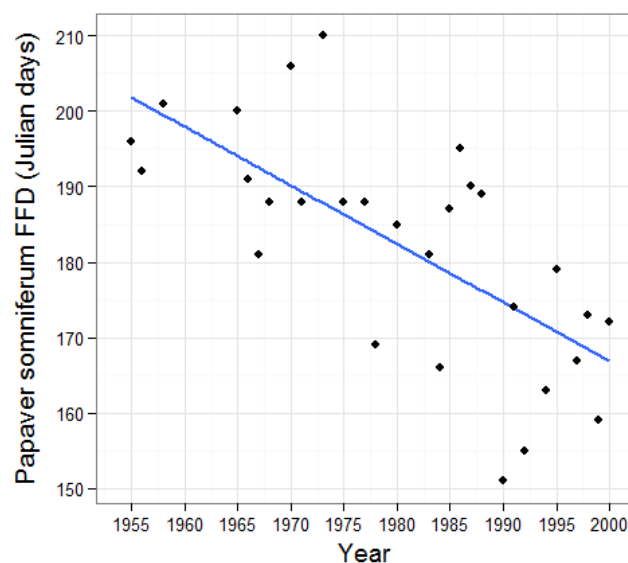


Figure 2.3: Plot of First Flowering Date (FFD) over time for *Papaver somniferum*.

2.3.4 Trait data

Table 2.1 details the ten traits used in this analysis. Where data for all traits were not available or attainable for a species, that species was excluded from the dataset. Any species that was the only representative of a particular level of a categorical trait was also excluded, resulting in that factor level being excluded. A total of 31 species were removed from the dataset at this stage. A further 3 were removed during the phylogeny generation process (see below). This left a final dataset of 351 species. There were 240 species showing FAD advances and 111 showing FAD delays.

Appropriate transformation of the three continuous traits in this analysis (Plant Height, Total Range in Britain and Habitat Breadth) was guided by inspection of plots of the relationship between species responsiveness and each continuous trait, and of the residuals of these fitted relationships. The range of the Plant Height and Total Range data also permitted the use of the R package *mgcv* (Wood 2004) to inspect the data. Using *mgcv*, a generalised additive model (gam) with a non-parametric smoother was plotted for the relationship between responsiveness and each trait. Plant Height was subsequently logged to improve its distribution with responsiveness. Appendix 1.1 details the null distribution of the responsiveness data and the graphical relationships between this data and each trait, before and after transformation.

Specific methodological details on trait compilation are detailed below. The main data sources are mentioned. Missing values were filled in from other sources (see Table 2.1). Data were also cross-checked between sources.

Plant Height

This data was taken directly from the PLANTATT database. It is in units of metres.

Native Status

PLANTATT gave the Native Status of plant species in this analysis in the categories of native, archaeophyte (plants that became established in the UK before 1500) and neophyte (plants that became established in the UK after 1500). For simplicity, I combined the categories of archaeophyte and neophyte to create a non-native category, leaving Native Status as a factor with two levels: native and non-native.

Perennation Strategy

PLANTATT provided the perennation strategy of plants in the categories: Annual, Biennial and Perennial. I collated these for each species and subsequently ordered the factor levels, thus: Annual<Biennial<Perennial.

Woodiness

PLANTATT classed species as woody, semi-woody or herbaceous. For simplicity, factor levels were recategorised as woody and non-woody. All plants originally classed as “woody” by PLANTATT remained in the woody category. Those classed as herbaceous went into the non-woody category. The 5 species PLANTATT had classed as semi-woody were inspected, and categorised as appropriate. All fitted into the non-woody category.

Biome

The plants in my dataset represented seven of the PLANTATT biome categories. These were simplified into four new categories: Temperate, Broad, Northerly and Southerly (Table 2.3).

Total Range in Britain

This data was taken directly from PLANTATT and was in units of km². The estimates include the area of the Isle of Man, but exclude Ireland and the Channel Islands.

Flowering Time

Some studies have looked at earliest flowering month as a predictor of plant responses to climate change (e.g. Sherry *et al.* 2007, Molnar *et al.* 2012). They demonstrate that earlier flowerers are more responsive than later flowerers. In this study, I take a different approach to the analysis of flowering time. Using the regressions of monthly temperature with time detailed earlier (Figure 2.2, Table 2.2) I calculated the average slope across all months. Those months that had slopes greater than this average value were considered as those showing the greatest temperature increase over the course of the study. These included January, February, March, July and August. Next, I compiled the earliest flowering month for each study species from Plants For a Future (<http://www.pfaf.org>). Species were then classified as flowering during months of greatest temperature increase (“peak” species), or not (“sub-peak” species), resulting in a two level factor.

Northern Range Limit in Britain?

This is a simple Yes/No factor compiled from PLANTATT, representing whether a species has its northern range limit in Britain or not.

Table 2.3: The PLANTATT Biome categories represented by species in my study, and their subsequent reclassification categories.

PLANTATT Biome	Description	Reclassification
Temperate	Present in the Broadleaf zone	Temperate
Wide Temperate	Present from Mediterranean region to coniferous forest zone	Broad
Wide Boreal	Present from the temperate zone to the tundra	Broad
Boreo-Temperate	Present in coniferous and broadleaf zones	Northerly
Boreo-Montane	Mostly present in coniferous forest zone	Northerly
Mediterranean	Present in the Mediterranean region	Southerly
Mediterranean-Atlantic	Present in the Mediterranean and extending north through the Atlantic zone of temperate Europe	Southerly
Southern Temperate	Present in the Mediterranean and broadleaf forest zones	Southerly

Pollen Vector

These data were compiled from Ecoflora (<http://www.ecoflora.co.uk>, Fitter and Peat 1994). Each species was classified as being insect-, wind- or self-pollinated. I created an additional category for non-angiosperms.

Habitat Breadth

PLANTATT provided a list of the different UK habitat categories a species was present in. These were summed for each species, providing a continuous measure of each species' habitat breadth.

2.3.5 Phylogeny data

In order to test for phylogenetic non-independence in my dataset, I needed to generate a phylogeny for the species in my analyses. Using the online tool Phylomatic (Webb and Donoghue 2005), I generated a Newick-format species phylogeny based on the provided base-phylogeny: "Phylomatic tree R20120829". Branch lengths were set to one and single dependent nodes stripped. Three species were not recognised by Phylomatic and were subsequently removed from the dataset. Details of testing for a phylogenetic signal in my dataset using this phylogeny follow.

2.3.6 Statistical analyses

Testing for a phylogenetic signal in the trait data

R 3.0.1 was used to perform all statistical analyses. The R package *Caper* (Orme *et al.* 2012) was used to generate a phylogenetic comparative dataset using the original data and the plant phylogeny generated by Phylomatic. Specifically, information on the phylogenetic relationships between species is used to incorporate the expected covariance among them into the comparative dataset. This controls for data non-independence due to species common descent. Lambda is a measure of the phylogenetic signal in a dataset, with a lambda value of 1 implying a strong phylogenetic signal in line with a Brownian model of evolution, and a lambda value of 0 implying phylogenetic independence. Setting lambda to its maximum likelihood (ML) value within the bounds of 0 and 1 provides the best estimate of the phylogenetic structure of a dataset (Freckleton *et al.* 2002). Based on this, I used my comparative dataset with the function *pgls* to run linear regression analyses for the relationships between the responsiveness data and each trait (Responsiveness~Trait), setting lambda to ML (bounded by 0 and 1). This provided the ML lambda value for each of these relationships (Table 2.4). Appendix 1.2 presents the ML profile for lambda estimation in each relationship. Phylogenetic signal was found to be negligible for all traits. Consequently, future analyses did not need to control for phylogenetic non-independence.

Table 2.4: Maximum likelihood estimates for lambda of each relationship of Responsiveness~Trait, estimated using *pgls* linear regressions. Lambda is bounded by 0 and 1, thus any lower confidence interval listed as NA is valued at 0 and any upper confidence interval listed as NA is valued at 1.

Trait	Lambda
log (Plant Height) (m)	0 (NA,0.036)
Native Status	0 (NA, 0.029)
Perennation Strategy	0 (NA, 0.036)
Woodiness	0 (NA, 0.037)
Biome	0 (NA, 0.032)
Total range in Britain (km ²)	0 (NA, 0.035)
Flowering time	0 (NA, 0.035)
Northern Range Limit in Britain?	0 (NA, 0.033)
Pollen Vector	0 (NA, 0.037)
Habitat Breadth	0 (NA, 0.035)

Model selection analyses

Following Burnham and Anderson (2002) I used a model selection approach to generate a candidate set of models looking at the relative effects of my ten selected traits on species phenological responsiveness. Variance weights were included at all stages to account for FFD sample size variation between species (see earlier). A maximal linear regression model including all traits as dependent variables and responsiveness as the independent variable was produced. Using the R package *Car* (Fox and Weisberg 2011), Variance Inflation Factors (VIFs) were calculated to assess the level of multicollinearity in the data. If VIF values are too large (>6), the information a model provides about individual predictors can be inaccurate. No predictor in my study had a VIF value >6 so analyses could proceed using all of them.

Using the R package *MuMIn* (Barton 2013), linear models representing all possible combinations of the predictor variables were generated (1024 possible models). These models were ranked according to their calculated Akaike Information Criterion adjusted for small sample sizes (AICc). AICc was used rather than AIC to account for the small ratio of sample size to model parameters in this analysis. AICc differences (delta AICc) between the model with the smallest AICc value (the model with the greatest amount of empirical support) and all other models were calculated, and a candidate model set identified. Following common practice, a delta AICc cut-off of <2 was used for identifying the candidate models. Model weights were then calculated, representing the relative likelihood of each model, given the data: these weights are normalised across the set of candidate models so as to sum to one (Johnson and Omland 2004). The significance of each term in each candidate model was calculated using sum to zero contrasts.

Any candidate model that has a weight of >0.9 is usually identified as the top model in a model selection analysis (Johnson and Omland 2004). Here, no candidate model had a weight of >0.9 . Consequently, model averaging of the coefficients in the candidate models was carried out, in line with Burnham and Anderson (2002), to produce a final average model that represented the data well. The model selection parameters for the candidate models were all checked by hand to ensure the automated analysis run in *MuMIn* was correct. All candidate model calculations were found to be accurate.

R package *lsmeans* (Lenth 2013) was used to calculate pairwise differences between the least squares means of factor levels for each categorical variable. Any categorical variables that

could be simplified by combining factor levels subsequently were, and the model selection analysis repeated using these new, simplified variables. For the purposes of this analysis I define significance as $P \leq 0.05$ and marginal significance as $P \leq 0.1$.

Interactions between traits were not included in models for several reasons:

- 1) A prohibitively large number of models would have to be run if all two-way interactions were to be included ($\sim 2^{55}$).
- 2) VIF values for models containing interactions were often >6 .
- 3) Interaction matrices were often incomplete due to the categorical nature of the trait data. Specifically, potential combinations of factor levels were not represented by any species, for example there are no annual woody species.
- 4) Sample size was restrictive (only $n/10$ parameters should be fit).

One potential solution to these problems is to run individual linear regressions of every potential trait combination and their interaction as a function of Responsiveness, and then include only those that are significant in subsequent multiple regressions, conditional on assessments of multicollinearity. This technique however, risks data dredging, and so was avoided.

Finally, it is worth considering five types of information that are important in interpreting the results of this trait-based model selection analysis:

- 1) Model rank *i.e.* the importance of a particular model relative to the other candidate models in the analysis
- 2) The inclusion of traits across candidate models
- 3) The statistical significance of traits across candidate models
- 4) The explanatory power of candidate models (R^2)
- 5) Trait relative importance in the overall averaged model

Points 1, 2, 3 and 5 give a good indication as to the importance of particular traits in explaining variation in phenological responsiveness. Point 4 gives an indication as to the amount of variation traits can explain in the response variable, and thus alludes more to their broader, practical relevance. Although the use of Akaike Information Criterion moves the analysis away from the concept of statistical significance, P-values and model explanatory power are also considered, in order to provide a little more inference.

2.4 Results

Pairwise comparisons between levels of each categorical variable in each candidate model in the model selection analysis revealed that the factor levels in Biome could be simplified (Figure 2.4). Southerly was kept as a level, and the other three levels (Temperate, Northerly and Broad) were combined. The model selection analyses were then repeated with this simplified version of Biome. Twelve candidate models predicting plant phenological responsiveness were generated (Table 2.5). Residual distributions for all of these models were good. No model had a weight of >0.9 and so model averaging was carried out (Table 2.6). Figure 2.5 displays the model averaged regression coefficients for this average model.

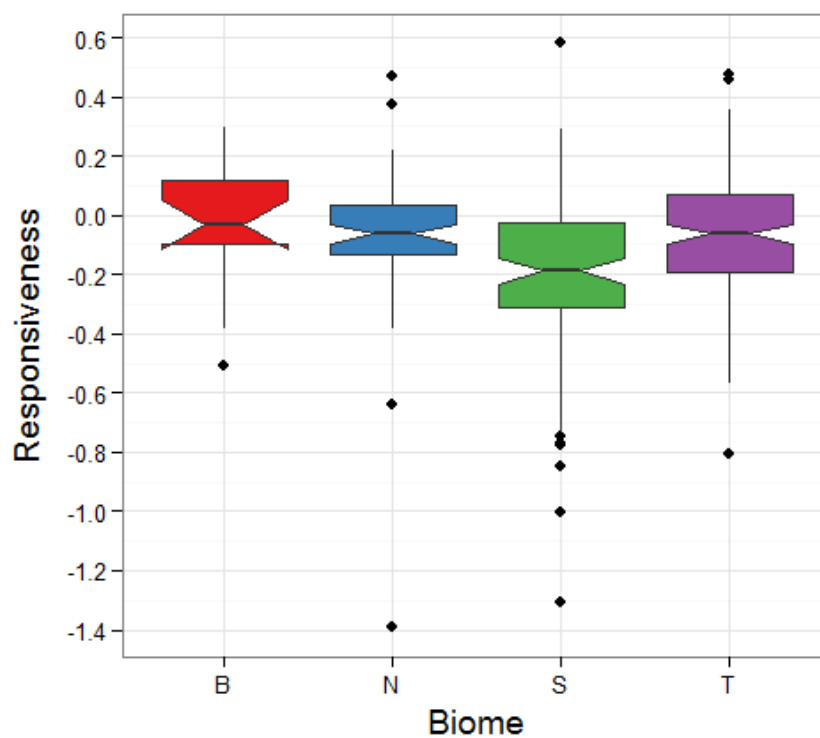


Figure 2.4: Notched box-plot of the relationship between Biome and Responsiveness, prior to factor level simplification. If notches do not overlap between two levels, this provides strong evidence that the medians for those levels differ (Chambers *et al.* 1983). Responsiveness represents the extent of change in first flowering date of a species from 1954-2000. Southerly species show different responses from other species types, advancing their first flowering date more. Black dots represent outliers. B = Broad; N = Northerly; S = Southerly; T = Temperate.

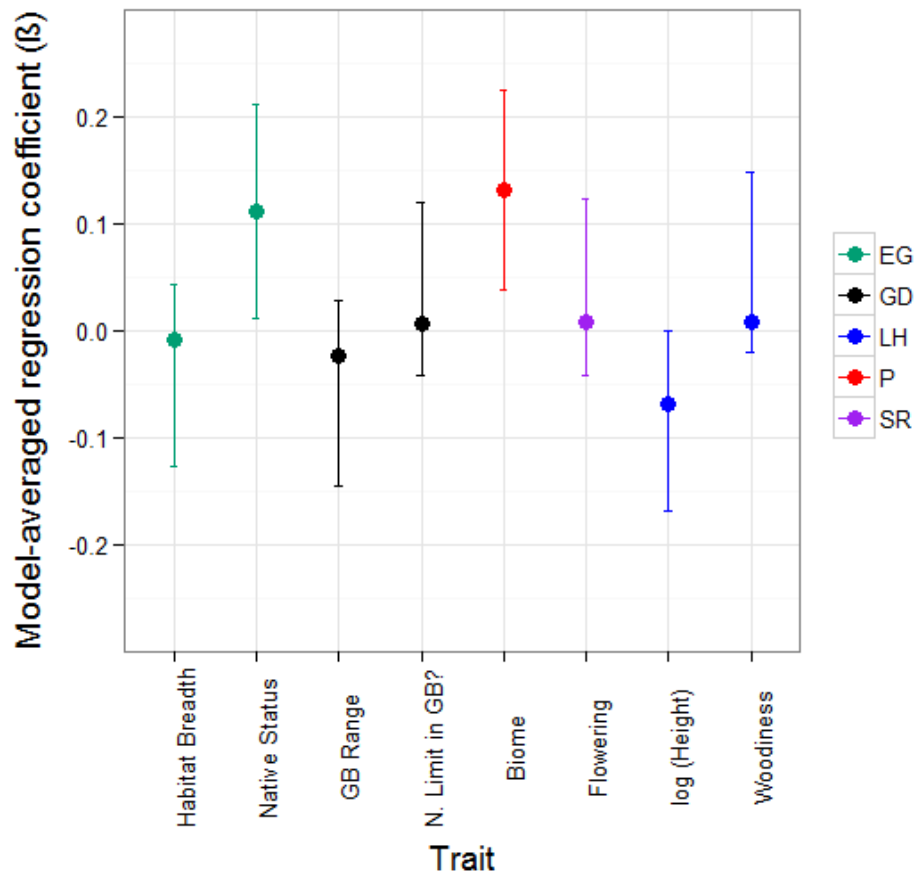


Figure 2.5: Model average regression coefficients (β) with 95% confidence intervals for each trait in the average trait model predicting phenological change in first flowering date for plants over time. Trait categories are denoted EG, GD, LH, P and SR for Ecological Generalism, Genetic Diversity, Life History, Preadaptation and Seasonal Readiness, respectively. GB Range = Total Range in Britain; N. Limit in GB? = Northern Range Limit in Britain?; Flowering = Flowering time.

Both Native Status and Biome were significant or marginally significant in all candidate models, and had a model average variable importance of 1.00. Least squares means comparisons between levels of Native Status in each candidate model revealed that Non-native species showed significantly greater advances in first flowering date than Native species over time, with a greater number of Native species tending to show delays than Non-native species (Figure 2.6). Similar comparisons for Biome revealed that Southerly species showed significantly greater advances than species from other Biome types, with a greater number of species from other Biome types tending to show delays than species from Southerly biomes (Figure 2.7). Appendix 1.3 details the least squares means comparisons performed.

Plant Height was significant or marginally significant in all candidate models it was present in (9/12, including the top ranked model). It had a model average variable importance of

0.82. Larger species showed greater advances in first flowering date with time. Further, there appeared to be a greater number of smaller species showing FFD delays than there were larger species (Figure 2.8). R^2 values were consistently low across all candidate models (0.06-0.08).

Table 2.5: Results from model selection on the relationship between species traits and change in plant flowering phenology with time. AICc differences (Δ_i) are calculated for each model as the difference between that model's AICc value and that of the model with the lowest AICc value. Models are then ranked according to Δ_i . Those with $\Delta_i < 2$ are considered as the candidate model set. Every candidate model from this analysis is presented in this table. Akaike weights (w_i) are then calculated for these candidate models as a measure of the likelihood of a model given the particular candidate model set.

Model Rank	Biome	log (Plant Height) (m)	Native Status	Total Range in Britain (km ²)	Habitat Breadth	Northern Range Limit in Britain?	Flowering time	Woodiness	R^2	AICc	Δ_i	w_i
1	+ **	+ †	+ *						0.071	-126.24	0.00	0.150
2	+ **	+ *	+ *	+					0.076	-126.00	0.24	0.133
3	+ **	+ †	+ †		+				0.074	-125.43	0.82	0.099
4	+ **	+ *	+ *	+		+			0.079	-125.22	1.02	0.090
5	+ **	+ *	+ *				+		0.074	-125.19	1.05	0.088
6	+ **	+ *	+ *	+			+		0.078	-124.74	1.51	0.071
7	+ **	+ †	+ *			+			0.072	-124.70	1.54	0.069
8	+ **		+ *					+	0.067	-124.56	1.68	0.065
9	+ **		+ *						0.061	-124.47	1.77	0.062
10	+ **	+ †	+ *	+	+				0.077	-124.47	1.77	0.062
11	+ **		+ *	+				+	0.071	-124.30	1.94	0.057
12	+ **	+ *	+ *		+		+		0.077	-124.25	2.00	0.055

+ $0.05 \leq P \leq 0.10$, * $0.01 \leq P \leq 0.05$, ** $0.001 \leq P \leq 0.01$, *** $0.0001 \leq P \leq 0.001$

Table 2.6: Given no candidate model from Table 2.5 had overwhelming importance (an Akaike weight (w_i) of >0.9), model averaging was carried out to calculate a weighted estimate (w_{ip}) of the parameters in the candidate model set. Methods follow Burnham and Anderson (2002). Results are presented in this table. β represents the model averaged regression coefficient for each explanatory variable. β values are presented here with 95% Confidence Intervals (CI) around each estimate. Figure 2.5 provides a graphical representation of these β values and their CIs.

Trait	Biome	log (Plant Height) (m)	Native Status	Total Range in Britain (km ²)	Habitat Breadth	Northern Range Limit in Britain?	Flowering time	Woodiness
w_{ip}	1.00	0.82	1.00	0.41	0.22	0.16	0.21	0.12
β	0.131	-0.069	0.111	-0.024	-0.009	0.006	0.009	0.008
95% CI	0.037 to 0.225	-0.168 to -0.0004	0.011 to 0.211	-0.145 to 0.028	-0.128 to 0.043	-0.042 to 0.119	-0.043 to 0.122	-0.020 to 0.147

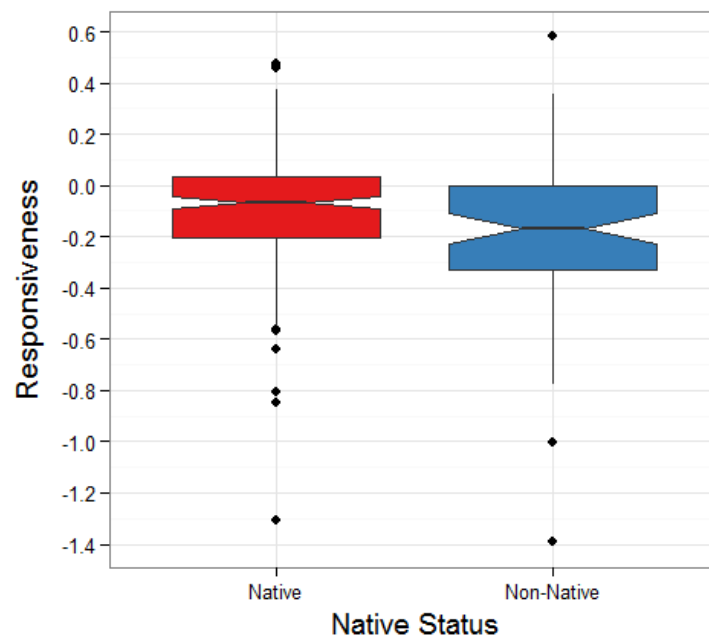


Figure 2.6: Notched box-plot of the relationship between Native Status and Responsiveness. If notches do not overlap between two levels, this provides strong evidence that the medians for those levels differ (Chambers *et al.* 1983). Responsiveness represents the extent of change in first flowering date of a species from 1954-2000. Non-native and Native species have different responses, with Non-natives advancing their first flowering date more. Black dots represent outliers.

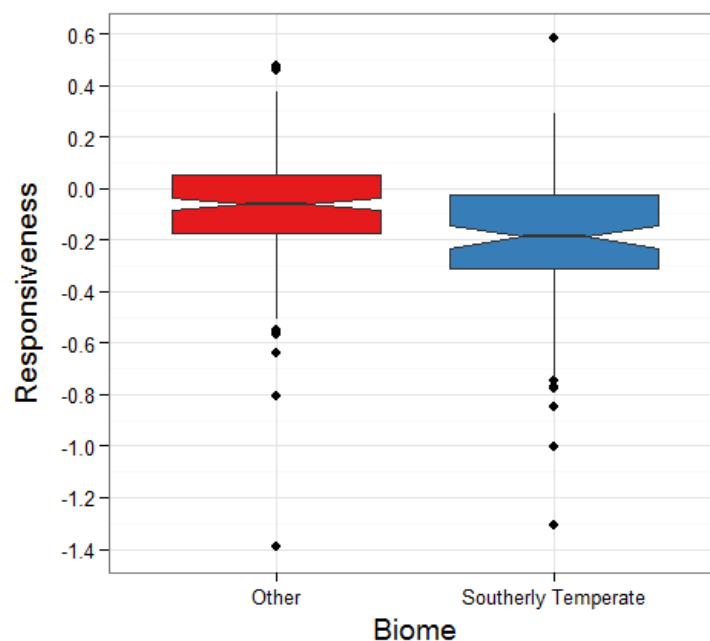


Figure 2.7: Notched box-plot of the relationship between Biome and Responsiveness, after factor level simplification. The category “Other” contains the original Biome levels of Northerly, Broad and Temperate. If notches do not overlap between two levels, this provides strong evidence that the medians for those levels differ (Chambers *et al.* 1983). Responsiveness represents the extent of change in first flowering date of a species from 1954-2000. Species from Southerly biomes show different responses from species from other biome types, advancing their first flowering date more. Black dots represent outliers.

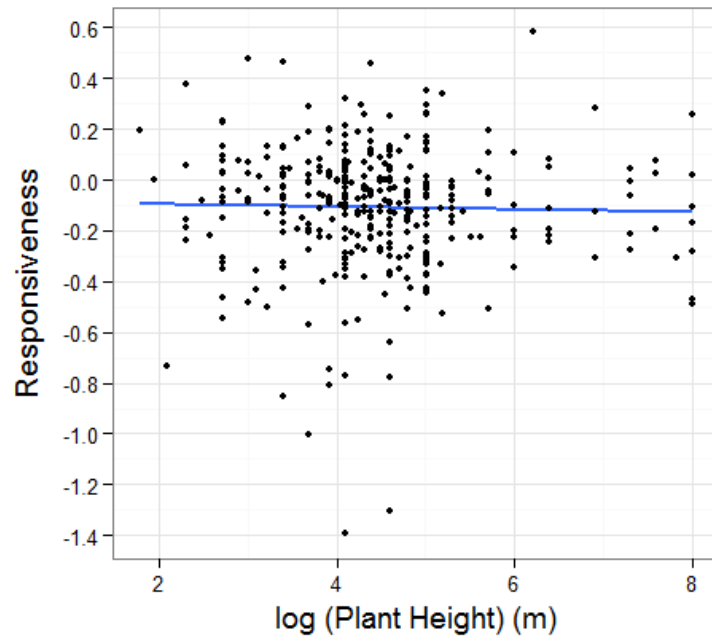


Figure 2.8: Scatterplot of the relationship between log (Plant Height) and Responsiveness. Responsiveness represents the extent of change in first flowering date of a species from 1954-2000. Larger plants are showing greater advances in flowering date than smaller plants.

2.5 Discussion

Changes in climate have important though often complex effects on ecological communities and their constituent species. Developing tools to predict variation in these effects based on species biology seems logical, since differences in biology should underpin differences in species responses to a shared climatic change. When the present research began, there were six studies demonstrating that traits could explain some of the variation in the phenological responses of species to climate change. None of these analysed multiple traits using an appropriate phylogenetically controlled model selection framework. There are now 15 studies providing evidence for traits as predictors of phenological change. Only four use the appropriate form of analysis. The analysis I present here adds to this body of work, using appropriate methods to identify traits that help to explain variation in plant phenological shifts across a 47-year time series.

These analyses identified three traits that were significant predictors of plant phenological responsiveness: Native Status, Biome, and Plant Height. Native Status and Biome were the most important predictors, followed by Plant Height. Native Status refers to the timing of a species' introduction to the UK. Species classed as native have always been present in the UK, whilst those classed as non-native are established species that were introduced from elsewhere. In line with hypothesis two, this analysis demonstrated that non-native species showed greater shifts towards earlier flowering than native species, suggesting that non-natives are keeping better pace with changes in climate. This may be due to the fact that non-native species, having originated from outside of the UK, are adapted to a wider range of habitats and diets than native species i.e. display greater ecological generalism. Consequently, they will be less dependent on tracking specific pollinators and habitats through time, allowing them to respond without constraint to the changing climate and to survive better at new temporal locales. In line with this finding, a recent study by Wolkovich *et al.* (2013) demonstrated that non-native species across four US sites showed greater advances in mean annual flowering time than native species. More broadly, 3 studies have demonstrated that other traits related to ecological generalism are important predictors of phenological responsiveness, with generalists showing greater advances than specialists across a variety of phenophases (birds: Vegvari *et al.* 2010; Hurlbert and Liang 2012; plants: Molnar *et al.* 2012).

Biome represents a classification of the biome types that each species is present in (Table 2.3). It gives an indication of the biogeographical distribution of each species. In support of hypothesis five, Southerly species (species present from the Mediterranean through to Temperate regions) were shown to display greater shifts towards earlier flowering than species of other Biome types. Being able to exist in warmer areas, Southerly species may have some level of preadaptation to the warmer temperatures that have been experienced in Chinnor in recent years. As such, they may be able to respond more rapidly to temperature increases across the timeframe. In keeping with this, Molnar *et al.* (2012), in their study of terrestrial orchids, showed that Mediterranean orchid species present in Hungary showed greater advances in mean flowering time than species from other areas.

Plant Height showed a small but significant trend with change in FFD. Larger plants showed greater advances in flowering date than smaller ones. This is in contrast with hypothesis one, where it was suggested that species with faster life histories (usually attributable to smaller species) would show greater advances in FFD. Specifically, species with faster life histories tend to see greater selective pressure on current breeding attempts rather than future ones (Sandvik and Erikstad 2008). One might thus expect them to be under stronger selective pressure to respond quickly to changes in climate. A similar result to that presented here was again obtained by Molnar *et al.* 2012, where orchid species with longer lifespans were shown to advance their flowering date more than shorter lived species. They suggested that this could indicate greater importance of phenotypic plasticity than of evolutionary adaptation in the climate change responses of their test species. Given the Plant Height trend displayed in my analysis was weak, and the other life history traits tested did not show similar significant trends (Perennation Strategy and Woodiness), it would be unwise to draw the same conclusion based on this specific instance. That being said, considering the role of evolutionary adaptation versus phenotypic plasticity is pertinent here. Neither of the tested trait related to Genetic Diversity was a significant predictor in my analysis. This adds fuel to the idea that in plants, evolutionary adaptation may have been less of a driving force in phenological responses to climate change than phenotypic plasticity. It is difficult to say how this might apply to future responses: there may be a tipping point beyond which plasticity is exhausted and rapid ability to evolve becomes crucial in adapting phenology appropriately to climate change.

The relationships between traits and species responsiveness did not show any phylogenetic signal. As such, whilst there is species to species variation in extent of response, this may be

more influenced by adaptation (perhaps to climate change) than by common descent (Vegvari *et al.* 2010). The explanatory power of all the candidate models in this analysis was low ($R^2 = 0.06-0.08$). This suggests that, in this case, traits are not particularly useful tools for predicting phenological changes in plant species in isolation. A similar conclusion was drawn in a range shift study by Angert *et al.* (2011), across plants, birds, butterflies and mammals. Three other studies also showed relatively low explanatory power of traits as predictors of species phenological responses to climate change (birds: Altermatt 2010; Rubolini *et al.* 2010; cross-taxa: Thackeray *et al.* 2010). Interestingly, some other phenological trait studies produce models with much higher explanatory power, including Molnar *et al.* (2012), who also looked at plant species. Molnar's study however, was over a longer time span than the present study, and used a more phylogenetically constrained group of plant species (all orchids) that might be more likely to be united by the same traits. This partly explains the higher explanatory power of models in their analysis. It is also worth noting that the spatial scale over which the raw data for this chapter was taken (Oxfordshire covers an area of $\sim 46\text{km}^2$, and Chinnor is just a small part of it) is much smaller than that of Molnar's study, which was carried out across Hungary. Given that biotic and abiotic idiosyncrasy increase as geographic scales narrow (Wiens 1989), the relatively low explanatory power of the models presented in this study is to be expected. That being said, these results caution that, despite being significant predictors at small spatial scales, traits may not be practical predictors in their own right, suggesting a multifaceted approach to prediction may be required at these scales.

Since this study was intended partly as a proof of concept for trait-based predictions of species responses to climate change, it is worth commenting on its methodological weaknesses. Firstly, the phylogeny used to test for a phylogenetic signal in the trait data should be considered. This phylogeny, generated using Phylomatic, was the only one available to me. Unfortunately, Phylomatic cannot produce trees with branch lengths that represent the amount of character change: all branch lengths were set to one in the phylogeny used in this study. This suggests some level of error in the subsequent tests of phylogenetic non-independence in my data. Further, I only used one phylogenetic tree in this analysis. In future studies it would be worth surveying the phylogenetic landscape of the species set by using a variety of generated trees, moving to use a consensus tree only if there is little to no variation between the various candidate phylogenies.

Another issue with this study is the variation between species in the sample size of the raw FFD data. By creating a vector of variance weights, I was able to overcome this issue in the non-phylogenetic model selection analyses. In the current implementation of pglS however, there is no option to weight data beyond that which is provided by a phylogeny. Consequently, there could have been some error in my lambda estimations for each trait. Finally, it would have been interesting to fit at least two-way interactions in the model selection process, as this could have produced more powerful models and revealed masked variable effects. As discussed earlier however, restrictions due to processing time, multicollinearity, incomplete interaction matrices and overparametrisation meant fitting a complete interaction set was not possible. I wanted to avoid only fitting interactions that were significant in simpler models due to risks of data dredging.

More generally, assumptions that species respond to climate change in isolation from other species in their communities could be an issue when drawing conclusions from these analyses. The indirect effects of biotic interactions are extremely important influences on the responses of species to the changing climate. They can even overturn the direct effects of climate on a species in some instances (e.g. Suttle *et al.* 2007). Phenological shifts may well represent a balance among multiple, sometimes conflicting or synergistic pressures, both physiological and ecological (e.g. Visser and Holleman, 2001). In a similar vein, assuming that climate is changing in isolation from other environmental factors is also risky (Tylianakis *et al.* 2008). Species may be responding to changing forcings from nitrogen deposition, land use, invasive species and other global change factors, in addition to climate. Ultimately, the goal in making predictions about species responses to climate change is to balance a model that well represents the complexity of abiotic change and biotic response, while being simple enough to fit and broad enough to apply across different groups.

Overall, the models presented here indicate that biological traits are significant predictors of species responses to climate change. Despite this, the low explanatory power of these models suggests that, at least at the small spatial scale of this study, a multifaceted approach may be required to provide practical predictions. That being said continued work into traits as predictive tools is well warranted. For one thing, the calculus may change with a stronger and stronger climate signal, additional basic ecological data on species traits and their responses to climate change may become available, and refinements in the modelling tools under development for this research are already occurring. Part of the appeal of a trait-based approach is its potential as a broad-brush, abstractable method: it is possible that the traits

elucidated as being important predictors in this analysis could help us to understand likely shifts in phenology of similar species across different geographies. Trait predictors may also be useful in improving the scalability of other methods of prediction. Additional investigation is required before abstraction of trait-based rule sets to other species or geographic locations can be carried out, but it is encouraging to see that the approach works and may one day be a useful component of the tool kit we use in planning for climate change impacts.

Chapter Three

Traits as predictors of phenological change in birds across different geographic areas

3.1 Abstract

Biological traits have been demonstrated to be useful predictors of species responses to climate change in a number of studies. One of the proposed values of using trait as predictors in this regard is that they may be applicable between collections of species at different sites. As such, trait-based predictors may combine site-level applicability with range-level generality. The broad applicability of trait-based predictors has yet to be formally tested, although there are many consistencies in the independent trait predictions generated for different species and sites. This chapter tests the transferability of trait-based predictions of bird phenological changes between two neighbouring US states, Minnesota and South Dakota. Difficulties in the estimation of a phylogenetic signal for the data used in these analyses restricted inferences. However results do indicate that traits are important predictors of phenological changes by birds in each state. Only one trait (Mass) shows good transferability between regions. Further research into the site-to-site transferability of trait-based tools is required before more concrete inferences can be drawn.

3.2 Introduction

The value of biological traits in explaining the responses of species to climate change has been examined in a number of recent studies. Many show traits to be statistically significant predictors of species responses. The explanatory power of these relationships is varied however, and there is a high level of methodological inconsistency between studies. One of the values of using traits as predictors of species responses to climate change is that they may be transferable between sites. Indeed, if species responses can be predicted on the basis of the traits they possess, and as such if traits are acting as functional respondents to climate change, then trait-based insights should carry over from region to region. There have been no tests concerning the transferability of trait-based inferences among regions, yet this is an important measure of their usefulness. In the analyses presented here, I address questions of the geographic transferability of trait predictions across two adjacent, climatically similar areas. Specifically, I analyse variation in first arrival date (FAD) over several decades in 44 species of North American migratory bird in the neighbouring US states of South Dakota and Minnesota.

The previous chapter and other studies (see Table 1.3) have established that species traits may predict their responses to climate change. A critical test that remains is to determine whether the traits that predict responses to a certain kind of climate change in one area among one set of species also predict responses in other areas among other sets of species. When we look across the earlier work of this thesis and at the results of the trait studies detailed in Table 1.3, we see great diversity in the species and sites studied, and in the scale of these studies. In spite of this variation, there are many inter-study consistencies in the predictions that are made, supporting the proposition that traits confer predictive generality. Broadly, characteristics related to high ecological generalism (Vegvari *et al.* 2010; Angert *et al.* 2011; Betzholtz *et al.* 2013; Hurlbert and Liang 2012; Molnar *et al.* 2012; Wolkovich *et al.* 2013; Chapter 2) and an ability to respond rapidly to changes in climate (Fitter and Fitter 2002; Butler *et al.* 2003; Hassall *et al.* 2007; Rubolini *et al.* 2007; Rubolini *et al.* 2010; Vegvari *et al.* 2010; Diamond *et al.* 2011; Betzholtz *et al.* 2013; Buskirk *et al.* 2012; Hurlbert and Liang 2012; Molnar *et al.* 2012) are commonly related to greater shifts in distribution or phenology in relation to climate change. More specifically, particular traits are shown to be consistent predictors of response in several studies. For example, five studies demonstrate that short distance migrant birds advance their breeding ground arrival date to a greater extent than long distance migrant birds in relation to changes in climate

(Butler 2003; Rubolini *et al.* 2007; Vegvari *et al.* 2010; Buskirk *et al.* 2012; Hurlbert and Liang 2012), whilst two demonstrate that earlier flowering plants are advancing their flowering phenology to a greater extent than later flowerers (Fitter and Fitter 2002; Molnar *et al.* 2012). Some studies carry out cross-site analyses demonstrating consistency in trait-predictions between these sites, although they don't formally test whether a trait-model from one area can effectively predict responses from another. For example, Thackeray *et al.* (2010) demonstrated a consistent signal in the effect of trophic level on advancement of spring phenophases across a variety of European sites, where secondary consumers were typically showing the slowest responses. Furthermore, Wolkovich *et al.* (2013) demonstrated that non-native plant species were advancing their flowering phenology to a greater extent than natives across four US sites. These results indicate that biological traits may be the site relevant, broadly applicable predictors we are looking for.

Despite the common findings of many of these studies, there are some opposing results to both the general trait trends and the specific trait results discussed above. For example Diamond *et al.* (2011) demonstrated that more specialist UK butterfly and moth species were showing greater phenological advances than generalist species, which is in stark contrast to the findings on ecological generalism detailed above. Furthermore, Jonzen *et al.* (2006) showed that long distance migrant birds in Scandinavia are advancing their spring migration to a greater extent than short distance migrants, which contrasts with the four studies detailed above that show the opposite for bird species in other areas. It is clear that further research is required into the geographic transferability of trait-based predictions; including a formal test of whether established relationships between traits and species responses to climate change in one area can be used to successfully predict responses in another area.

This study aims to provide such a test by examining the concordance of traits in predicting changes in bird migratory phenology across two adjacent, climatically similar areas: Minnesota and South Dakota. Altering the timing of migration is a major way in which birds respond to climate change. The general trend is for birds to show advancement in migratory timing (e.g. Cotton 2003; Barbraud and Weimerskirch 2006; Swanson and Palmer 2009) in an attempt to keep pace with advancing offspring food peaks. Migratory distance has already been demonstrated as an important trait predicting the phenological responses of birds to climate change at a variety of sites in Europe and the USA (see earlier), but the direct transferability of these predictions is yet to be tested.

Minnesota and South Dakota provide an excellent study locale. They are adjacent US states of similar sizes (199,905 km² and 225,181 km², respectively) and with similar climates: they are both classified as group D and subgroup f in the Koppen Climate classification. Group D is characterised by humid, microthermal climates where the minimum temperature is < -3°C and the maximum is ≥ 10°C, whilst subgroup f denotes wet weather throughout the winter (Lohmann *et al.* 1993). Being climatically similar, they provide a good baseline test for geographic transferability: if traits are not transferrable between state-wide populations in such proximal and climatically similar locations, they are unlikely to be broadly relevant predictors across more diverse geographies at narrower scales. Birds, in turn, are an exemplary group for analysis. Some of the foundational studies examining the impacts of climate change on ecological systems examined phenological changes in birds (e.g. Crick *et al.* 1997; Dunn and Winkler 1999). As such, changes in bird phenology are particularly well documented. The biology of birds is also extremely well characterised. Finally, the first complete, dated phylogeny of all 9993 extant species of bird was recently published by Jetz *et al.* (2012), which will be very useful for this analysis.

Data on the changes in breeding ground yearly FAD over time were obtained for 44 migratory bird species in both South Dakota and Minnesota, across 35 years (from Swanson and Palmer 2009). Using this data, I first examine which traits are important predictors of the extent and direction of bird phenological change in each state. I then compare whether these predictions are transferrable between the two states. Comparing predictions between the same species in different locales provides a good baseline test: if predictions are not broadly relevant between distinct groups of the same species, then hope for transferability between different groups may be limited. Six traits were selected for analysis (Table 3.1). These represent three of the trait groups laid out in Chapter One: Life History Speed, Ecological Generalism and Seasonal Readiness. For the purposes of my analyses, I once again defined traits in accordance with McGill *et al.* (2006) as “well-defined, measurable properties of organisms that are...defined at the level of the individual and [can be] used comparatively across species”.

The following hypotheses will be tested:

- 1) Based on reasoning laid out in Chapter One, species with faster life histories (smaller masses, lower longevity), greater seasonal readiness (shorter migration distance) and broader ecological generalism (broader diet and habitat usage) should show the greatest responsiveness in both states.

- 2) Those traits that are significant predictors of change in FAD will be consistent between South Dakota and Minnesota
- 3) Established relationships between traits and migratory phenology in one state will be effective predictors of migratory phenology in the other state.

Results will be compared in detail to these hypotheses.

Table 3.1: A summary of the traits analysed as predictors of bird phenological responsiveness to climate change in this chapter.

Trait	Trait Category*	Nature of variable	No. of factor levels	Transformation	Primary Data Source**
Mass (g)	LH	Continuous	N/A	logged	The Sibley Guide to Birds (Sibley 2008)
Maximum Clutch Size	LH	Continuous	N/A	logged	All About Birds/Birds of North America Online (Poole 2005; Cornell Laboratory of Ornithology 2013)
Maximum Longevity	LH	Continuous	N/A	logged	AnAge (Tacutu <i>et al.</i> 2013)
Diet Breadth	EG	Continuous	N/A	none	The Birder's Handbook (Ehrlich <i>et al.</i> 1988)
Habitat Breadth	EG	Continuous	N/A	logged	Bird Life International Data Zone (Birdlife International 2013)
Migration Distance	SR	Categorical	2 (Short/Long)***	N/A	Bird Life International Data Zone (Birdlife International 2013)

* LH=Life History; EG = Ecological Generalism; SR= Seasonal Readiness

** Gaps in the data for a particular trait were filled in from the other sources in this table.

*** *n* value for factor levels: Long=21; Short=23.

3.3 Methods

3.3.1 Raw bird response data

All analyses were performed in R 3.0.1. FAD is the most commonly studied metric of migration phenology (Swanson and Palmer 2009). It represents the early tail of the Gaussian curve for migration timing. On this basis, some critics have suggested that it could be more variable than other measures of migration timing and thus might not be representative of changes in this timing (Moller and Merila 2004). Specifically, it is susceptible to sampling error and population fluctuations, where increases in population size can cause earlier arrivals to occur simply by chance, or by virtue of the greater amount of genetic variation carried by a larger population (Rubolini *et al.* 2007). It may also be based solely on male singing activity (Rubolini *et al.* 2007). Despite this, because FAD is correlated with other measures of migration timing (e.g. medians and quartiles), it has been argued by Sparks *et al.* (2001) that it can be an effective measure of the impact of climate change on migration phenology, as long as caution is applied (Swanson and Palmer 2009). FAD also carries advantages over other measures of migration timing: FAD records are easy to collect and therefore abundant and diverse (Rubolini *et al.* 2007). FAD also has a stronger dependence on climatic conditions than later measures of migration such as mean or median arrival dates (MEDs), and thus FAD temporal trends tend to be stronger than those based on other measures of migration timing (Lehikoinen *et al.* 2004). Given the above, I decided that FAD was an acceptable metric of phenology to investigate.

Swanson and Palmer (2009) studied the change in FAD phenology with time for 44 species of North American birds across two US states: South Dakota and Minnesota. Data on FAD was available from 1971-2006 in South Dakota and from 1964-2005 in Minnesota. Swanson and Palmer numbered FAD values for each species between 1 and 365 (representing each day of the year chronologically) and ran regressions of FAD with year for each species in each state. The raw data they used were collected by the South Dakota and Minnesota Ornithologist's Unions across the entirety of both state areas. There were a large number of observers collecting both datasets. Due to this, Swanson and Palmer tested for observation bias in their analyses. It was found to be limited. I used the raw FAD data from this study (provided to me by David Swanson) as the phenological response to investigate in my trait-based analyses.

3.3.2 Creating the responsiveness data

Species responsiveness was defined as the extent of change in a specific phenological event a species exhibits over a set period of time, ostensibly but not explicitly due to warming temperatures. Swanson and Palmer (2009) provided regression slope values for the relationship between each species' FAD and time, per state. The recording period for each state was different: 36 years in South Dakota (from 1971-2006) and 42 years in Minnesota (from 1964-2005). In order to standardise timeframes between states, I reran these regressions across the common recording period for the two: 1971-2005 (e.g. Figure 3.1). Slopes were recorded for each species in each state, regardless of significance, as I was interested in all magnitudes of phenological change. These slopes represent the phenological responsiveness of a species to the changing climate. In Minnesota, 23 species (8 significantly) showed advances in their FAD phenology with time and 21 species (3 significantly) showed delays (Figure 3.2). In South Dakota, 39 species (13 significantly) showed advances in their FAD phenology and 5 (all significantly) showed delays (Figure 3.2). Across species and between states, there was variation in the number of years for which FAD was recorded. The range of data points across species for South Dakota was $n=16$ to $n=27$ per species. The range for Minnesota was $n=29$ to $n=35$. In order to standardise these slopes, I created a vector of weights using the reciprocal variance of each, which were then used in my statistical analyses, where possible (see below).

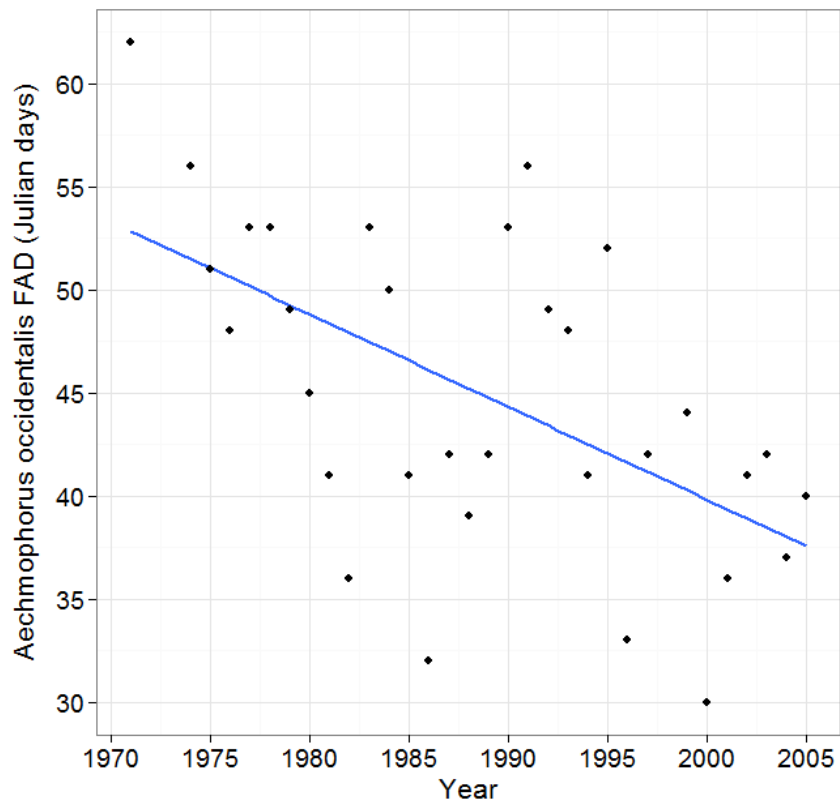


Figure 3.1: Plot of First Arrival Date (FAD) over time for *Aechmophorus occidentalis* in Minnesota.

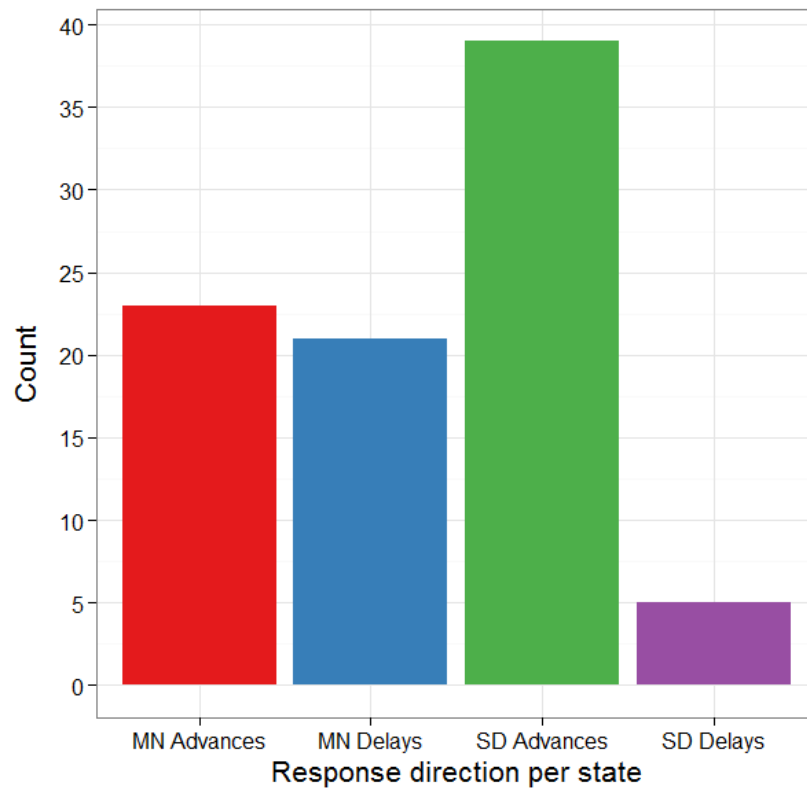


Figure 3.2: The number of species showing advances in first arrival date versus delays in first arrival date, for each state. MN = Minnesota; SD = South Dakota.

3.3.3 Trait data

Six traits were used in this analysis (Table 3.1). These represented three of the important trait categories (laid out in Chapter One) that might affect species' phenological responsiveness: Life History, Ecological Generalism and Seasonal Readiness. The trait data was identical between states. For each state, appropriate transformation of continuous trait data was guided by inspection of plots of the relationship between species responsiveness and each continuous trait, and of the residuals of these fitted relationships. Body Mass, Maximum Longevity, Clutch Size and Habitat Breadth were subsequently logged for both states, linearising the residuals of their relationship with responsiveness. Appendix 2.1 shows the graphical relationships between the responsiveness data and each trait with and without appropriate transformations. All traits were lifted directly from the data sources listed in Table 3.1 except for Habitat Breadth, Diet Breadth and Overwintering Location, which were modified. Specific methodological details for these three modified traits follow.

Habitat Breadth

BirdLife International provided detailed breeding habitat types for each species in my dataset. Using this data, I summed the number of different breeding habitats a species occupied, resulting in a continuous measure of Habitat Breadth.

Diet Breadth

Following the methods of Angert *et al.* (2011), the number of diet categories listed in The Birder's Handbook (Ehrlich *et al.* 1988) was summed for each species (ranging from 1-4). Omnivorous species were given a diet breadth of 5. This resulted in a continuous variable for Diet Breadth.

Migratory Distance

Birdlife International provided range maps for each species in my dataset. Breeding, overwintering and resident ranges were detailed. Using these maps I classified any species that had an overwintering or resident range within the continental USA as a short distance migrant. Species overwintering elsewhere were classed as long distance migrants.

3.3.4 Phylogeny data

Closely related taxa often share characteristics due to common descent. If analyses on evolved traits do not take account of this, pseudoreplication is introduced as differences

between major groups are counted multiple times (Purvis 2008). Consequently, when analysing traits across a number of species, it is essential to examine data for phylogenetic non-independence and to control for it in analyses, if present. In order to test for a phylogenetic signal in my trait data, I needed to generate appropriate phylogenies for my 44 bird species. Jetz *et al.* (2012) provides the first complete dated phylogeny for all 9993 extant bird species. This phylogeny remains the most up to date available. Using birdtree.org (the phylogeny tool linked to Jetz *et al.* 2012), 100 pseudo-posterior sample trees were generated for my 44 bird species via the Jetz *et al.* (2012) method. Hackett *et al.* (2008) was chosen as the backbone phylogeny for this task. The 100 phylogenies generated were used in subsequent analyses to test and control for phylogenetic signal in all Responsiveness~Trait relationships (see later).

3.3.5 Statistical analyses

Temperature trends in South Dakota and Minnesota

In line with Chapter Two, this study examines phenological change in units of time. If we are to link results with climate change however, the strength of the climate signal should be examined and compared for each state. Temperature data was obtained from the NOAA Climate Division database (<http://climate.sdstate.edu/ClimateDivisions/Seasonal.cfm>). This database broke each state down into 9 regions. Average temperature data for each year of my study (1971-2005) was collected for each region, and a state-wide average calculated, per year. For each state this combined temperature data was regressed with time to show the strength of the climate signal.

In order to compare the two state trends, both sets of temperature data were combined. Coding Area as a two level factor (Minnesota/South Dakota), I ran three regressions:

- 1) Temperature ~ Year*Area (i.e. fitting two separate slopes for Area)
- 2) Temperature ~ Year+Area (i.e. fitting one common slope for Area)
- 3) Temperature ~ Year

The fit of these models was then compared using Akaike Information Criterion (AIC). A difference of >2 between the AIC values of two compared models suggests a difference in fit, with the model with the smaller AIC value fitting better. An improved fit by model 2 over model 3 indicates that the intercept of the temperature trend with time varies between states (i.e. there is a magnitude difference in the state temperature trends). An improved fit of

model 1 over model 2 indicates that the slope of the temperature trend with time varies between states (i.e. the rate of change in temperature with time varies between states).

Are the responses of individual species different between states?

Before investigating how consistent traits are in predicting FAD change between states, it is instructive to first examine the geographical consistency in the phenological responses of the individual study species between them. Following the method for comparing temperature trends between states (see above) I combined the raw response data for Minnesota and South Dakota. Coding Area as a two level factor (Minnesota/South Dakota), I ran three regressions for each species:

- 1) $FAD \sim Year * Area$ (i.e. fitting two separate slopes for Area)
- 2) $FAD \sim Year + Area$ (i.e. fitting one common slope for Area)
- 3) $FAD \sim Year$

As with the state temperature comparisons, the fit of these models was compared using AIC. An improved fit by model 2 over model 3 indicates that the intercept of a species' response varies between states (i.e. there is a magnitude difference in the FAD trends with time between states). An improved fit of model 1 over model 2 indicates that the slope of a species' response varies between states (i.e. the rate of change in FAD with time varies between states). The comparison between models 1 and 2 (i.e. whether there is a difference in the rate of change in FAD a species shows between states) is of particular relevance here, as it is the rate of change that I take to represent a species "Responsiveness" to climate change, and that I am in turn trying to use traits to predict. However the intercept results are included here for completion.

To compare the interstate responses further, a *pgls* regression analysis was run between the entire set of species slopes in Minnesota and the entire set of species slopes in South Dakota and the correlation between the two examined. A consensus phylogeny was used (see below).

Testing for a phylogenetic signal and examining the importance of trait-based predictors

By generating 100 phylogenetic trees I have ensured that the phylogenetic landscape of my 44 species can be reliably surveyed. That being said, running iterations of every desired analysis using each phylogenetic tree in turn is time consuming, and can make synthesising

meaningful interpretations difficult. The use of a tree that represents a consensus version of a variety of phylogenies can avoid these two issues. It may not, however, provide a reliable embodiment of the phylogenetic relationships between species since it only represents an average of the sample phylogenies in question. The only time the use of a consensus tree is justified is when there is little to no variation in the estimated phylogenetic relationships that a variety of individual phylogenetic trees provide. In this analysis, I wanted to examine the consistency of my 100 phylogenetic trees in their estimations of the relationship between each trait and the responsiveness data (Responsiveness~Trait), for each state, to see if a consensus phylogeny could be used to make inferences from.

The R package *Caper* (Orme *et al.* 2012) provides a simple, reliable way of using *pgls* to execute phylogenetically controlled analyses on multiple dependent variables. At present however, it has no method for weighting data. This could prove problematic for my dataset because of the aforementioned variability in FAD sample size between species and states. “*Brutal*” (Freckleton 2013) is code containing the function *pglmspatialfit*. This allows datasets to be weighted by both phylogeny and their spatial or sample size relationships. Unfortunately, *pglmspatialfit* cannot be used in analyses with multiple dependent variables at present. The flexibility and ease of implementation that *Caper* and *pgls* provide should make them the tools of choice for trait-based analyses. However I wanted to be sure that sample size variation did not have a confounding effect on the *pgls* model estimations. In consequence, in testing for consistency in estimations across each of my phylogenetic trees, I also tested the consistency of the estimations of *pgls* and *pglmspatialfit*, for each state.

Using both *pgls* and *pglmspatialfit* in turn, regressions of responsiveness as a function of each individual trait were run for each of the 100 phylogenetic trees, for each state. Lambda was set to maximum likelihood (ML) between the bounds of 0 and 1. Slope estimates, standard errors and lambda estimates were recorded for each of these models. Subsequently, a plot of all the slopes estimated using *pgls* against all the slopes estimated using *pglmspatialfit* was produced, for each model in each state. This was repeated for each model’s standard error and lambda value in turn. These plots enabled visualisation of the consistency in estimation across trees and methods. Results showed very little variation between trees or methods within both states (e.g. Figure 3.3). Based on these results, for ease of implementation and interpretation, future analyses used a consensus tree generated from the original phylogenies using the *sumtrees* program of the *DendroPy* package (Sukumaran and Holder 2010). *pgls*

was chosen as the function for which any further phylogenetic analysis was carried out due to its compatibility with more complicated statistical analyses and its ease of use via *Caper*.

Using *pgls* and the consensus tree, and setting lambda to ML (bounded by 0 and 1), I ran a regression of responsiveness as a function of each trait in turn (Responsiveness~Trait), for each state. The ML values of lambda for each relationship are noted in Table 3.2. Lambda values were consistently zero for South Dakota. In Minnesota, these estimates were much larger, varying from 0.914-0.955. For many of the relationships in both states, confidence intervals surrounding lambda estimates were large. This indicates that lambda is difficult to estimate (e.g. Figure 3.4). In consequence, these trait analyses were repeated setting lambda first to 0, and then to 1. The results of these models showed the state-specific importance of each trait as a predictor of changes in FAD with time, with and without control for a phylogenetic signal. Pairwise differences between the factor levels of Migration Distance were calculated using treatment contrasts. Sample size restrictions prohibited the use of a model selection analysis investigating the predictive power of multiple traits on responsiveness.

Table 3.2: Maximum likelihood estimates for lambda of each relationship of Responsiveness~Trait, estimated using *pgls* linear regressions with a consensus phylogenetic tree. Lambda is bounded by 0 and 1, thus any lower confidence interval listed as NA is valued at 0 and any upper confidence interval listed as NA is valued at 1.

Trait	Lambda MN	Lambda SD
Mass (g)	0.926, (0.357, 0.997)	0, (NA, 0.445)
Maximum clutch size	0.952, (0.730, 0.998)	0, (NA, 0.700)
Maximum longevity	0.926, (NA, 0.994)	0, (NA, 0.256)
Diet Breadth	0.955, (0.735, NA)	0, (NA, 0.695)
Habitat Breadth	0.948, (0.721, 0.996)	0, (NA, 0.725)
Migration Distance	0.920, (0.520, 0.993)	0, (NA, 0.571)

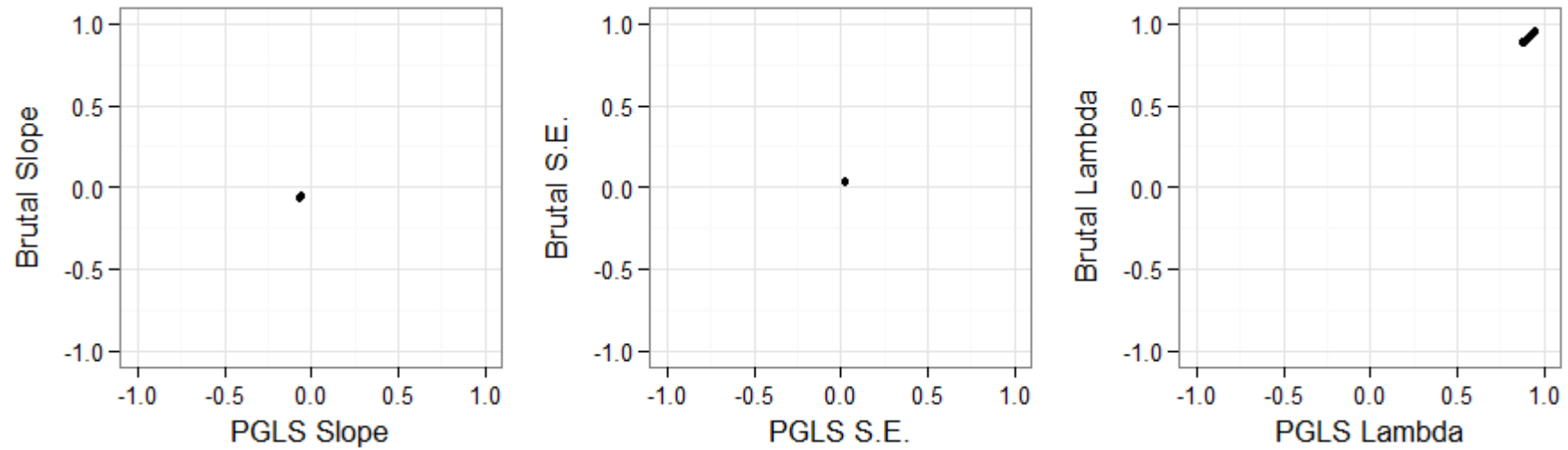


Figure 3.3: Plots of the *Brutal* and *pgls* estimates for the slope, standard error and maximum likelihood (ML) lambda estimate of the relationship of Responsiveness $\sim$ log (Mass) for Minnesota, for each of 100 different phylogenetic trees. Deviation from a one to one relationship indicates variation in the statistics provided by the two methods. Deviation between data points indicates differences between the phylogenetic trees.

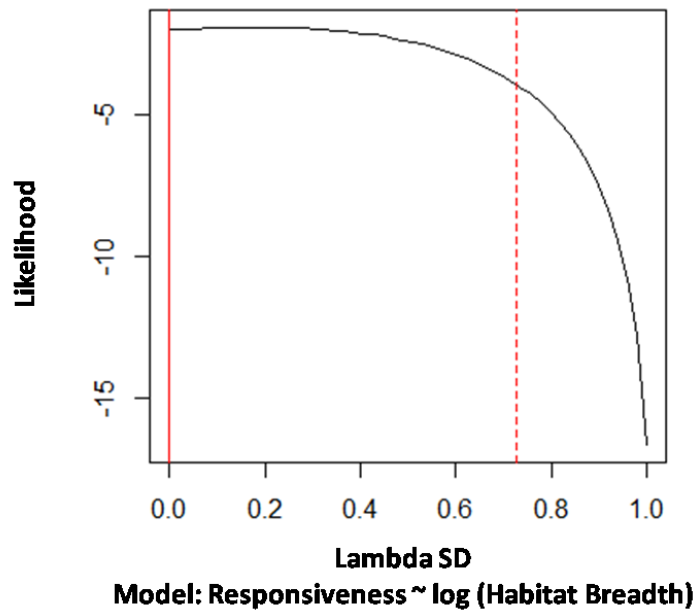


Figure 3.4: A plot of the likelihood profile for the estimation of the maximum likelihood (ML) value of lambda (bounded by 0 and 1), for the relationship of Responsiveness~Habitat Breadth, in South Dakota. Solid line represents the estimated ML lambda value. Dotted lines represent the 95% confidence intervals around the ML lambda estimate. The lower interval here does not display as it is at the lower bound of lambda (0).

Comparing the geographic transferability of trait-based predictors between states

Those traits that showed a significant or marginally significant relationship with responsiveness in at least one of the state-specific *pgls* analyses were selected. Using the *predict* function with *pgls*, I predicted Responsiveness values for the South Dakota species from the Minnesota model of Responsiveness~Trait, for each of the selected traits in turn. An offset of the actual and predicted South Dakota Responsiveness values was then calculated for each trait, by subtracting the predicted Responsiveness values from the actual Responsiveness values for South Dakota. Using *pgls*, each offset was then regressed with its respective trait, and the significance of the intercept and slope estimates of this offset model recorded. A significant intercept in the offset model indicates that the predicted and actual trait model intercepts are different. A significant slope indicates that the predicted and actual trait model slopes are different. If the intercept and/or slope are significant in this offset model, it indicates that predicting species responsiveness in one state using the relationship between responsiveness and traits in the other, has not been successful i.e. that the transferability of trait-based predictions from one state to the next is poor.

Due to the aforementioned difference in lambda values between states for the majority of the Responsiveness~Trait relationships, and the difficulty in its estimation for many of these relationships, lambda was first set to 1 during these analyses. The process was then repeated setting lambda to 0. Fixing lambda at a set value facilitated more accurate assessment of the transferability of predictions between the two states. The two levels of lambda investigated allowed assessment of the importance of phylogenetic control in these analyses. The whole process was repeated using South Dakota models to predict the Minnesota data.

3.4 Results

3.4.1 Temperature trends in South Dakota and Minnesota

Both states showed an upwards trend in temperature over the time series (Figure 3.5). This trend was significant for Minnesota ($t_{(1,33)}=2.294$, $P=0.028$) but not for South Dakota ($t_{(1,33)}=1.653$, $P=0.108$). South Dakota was generally warmer than Minnesota, however Minnesota showed a greater rate of temperature increase than South Dakota. As such, the temperature gap between the two closed slightly as time went on.

There was no difference in the slopes of the temperature-time relationship between states (AIC difference between models fitted with and without an individual slope for state were <2), however the intercepts were significantly different (AIC difference between models fitted with and without an individual intercept for state were >2 , where the model with individual intercepts had the lower AIC value) i.e. the rate of temperature change between states did not vary, however there was a magnitude difference in temperature between them.

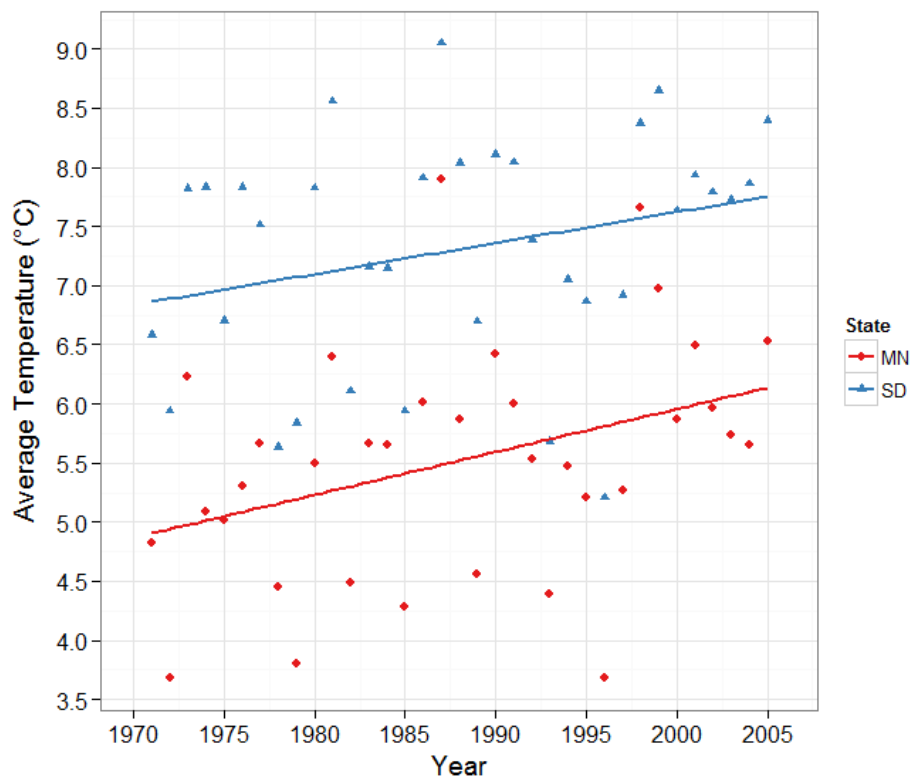


Figure 3.5: Average Temperature change in °C for Minnesota and South Dakota from 1971-2005. MN = Minnesota; SD = South Dakota

3.4.2 Are the responses of individual species different between states?

When comparing the FAD response of each species between states, 11 species showed a significant difference in their rate of FAD advance with time between Minnesota and South Dakota (AIC for the model of FAD with time fitted with individual slopes for each state was lower than that fitted with a common slope, for each of these 11 species). 30 species showed a magnitude difference between states i.e. the actual day of first arrival in each year was significantly different between states over time (AIC for the model of FAD with time fitted with individual intercepts for each state was lower than that fitted with a common intercept, for each of these 30 species). Appendix 3.3 details the results of these AIC model comparisons.

A *ppls* regression analysis between the entire set of species slopes for Minnesota and the entire set of species slopes for South Dakota showed a significant relationship between the two ($t_{(2,42)}=2.205$, $P=0.033$). The R^2 of this relationship was quite low (0.104). More species are showing FAD advances in South Dakota than in Minnesota, and these advances also tend to be more extensive (Figure 3.6).

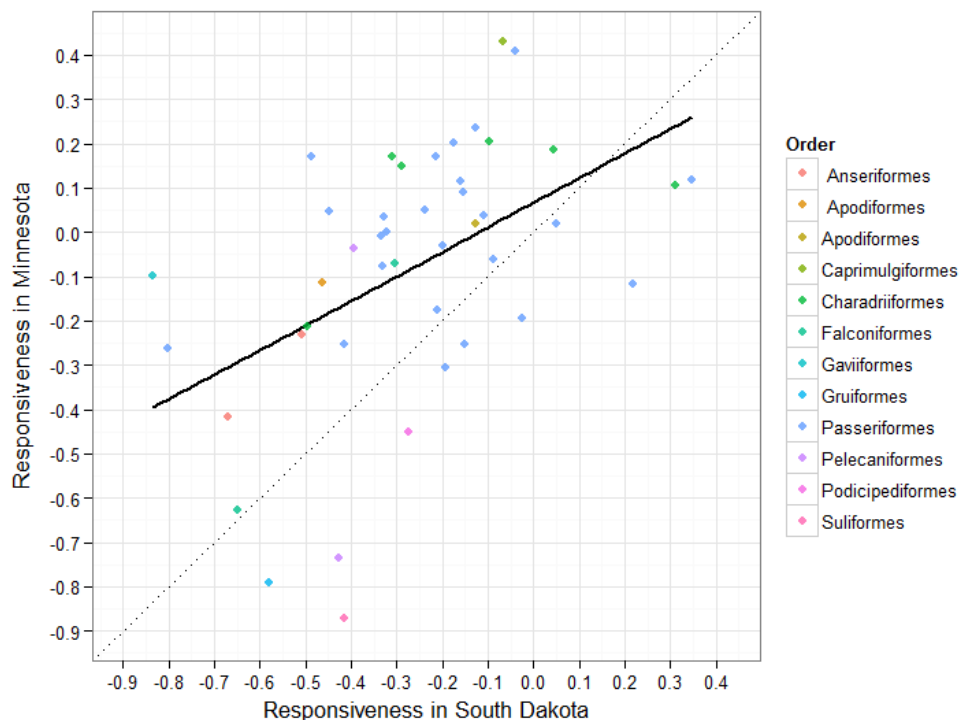


Figure 3.6: Plot of each species' Responsiveness in Minnesota versus its Responsiveness in South Dakota. Responsiveness represents the extent of change in FAD of a species from 1971-2005. The filled line represents the slope of the relationship between the Minnesota Responsiveness values and the South Dakota Responsiveness values. The dotted line represents a 1:1 gradient. More species are showing FAD advances in South Dakota than in Minnesota, and these advances tend to be more extensive.

3.4.3 The importance of trait-based predictors in each state

Body Mass was a consistently significant predictor of Responsiveness across all tested levels of lambda (ML, 0, 1) in both states. Specifically, species with larger masses showed greater advances in FAD with time (Figure 3.7). Maximum longevity was a significant predictor in South Dakota under an ML lambda value, and significant in both states when lambda was 0: longer-lived species showed greater advances in FAD with time (Figure 3.8). Maximum Longevity showed no significance in either state when lambda was 1. Checking the ML confidence intervals of this relationship for both states indicates that there is relatively high confidence in South Dakota that lambda = 0, but low confidence in Minnesota that lambda = 1. This suggests that the Maximum Longevity results when lambda is fixed at 1 are not particularly relevant. Migration distance was a significant or marginally significant predictor in both states under an ML and a 0 value of lambda. Specifically, short distance migrants showed greater advances in FAD (Figure 3.9). Migration Distance showed no significance as a predictor of responsiveness when lambda was 1. Table 3.3 details the results for all trait models run under the three values of lambda. Residual distributions for all these models were good.

Table 3.3: Results of the regressions of Responsiveness~Trait, for each trait in each state at three different levels of lambda (0, 1 and Maximum Likelihood). P-values highlighted in red represent traits that are significant or marginally significant predictors of Responsiveness in Minnesota for a particular level of lambda. P-values highlighted in blue represent traits that are significant or marginally significant predictors of Responsiveness in South Dakota for a particular level of lambda.

Trait	Lambda	State	Estimate	Standard Error	t-value	P-value	R ²
log (Mass)	ML	MN	-0.063	0.030	-2.122	0.040 *	0.097
log (Mass)	ML	SD	-0.052	0.018	-2.985	0.005 **	0.175
log (Max clutch size)	ML	MN	0.055	0.117	0.467	0.643	0.005
log (Max clutch size)	ML	SD	0.086	0.091	0.942	0.352	0.021
log (Max longevity)	ML	MN	-0.091	0.081	-1.114	0.272	0.029
log (Max longevity)	ML	SD	-0.259	0.070	-3.722	<0.001 ***	0.248
Diet Breadth	ML	MN	0.015	0.033	0.465	0.645	0.005
Diet Breadth	ML	SD	-0.013	0.038	-0.354	0.725	0.003
log (Habitat Breadth)	ML	MN	0.008	0.052	0.154	0.878	0.001

Continued...

Trait	Lambda	State	Estimate	Standard Error	t-value	P-value	R ²
log (Habitat Breadth)	ML	SD	0.029	0.059	0.499	0.620	0.006
Migration distance	ML	MN	0.122	0.071	1.725	0.092 †	0.066
Migration distance	ML	SD	0.149	0.075	1.985	0.054 †	0.086
log (Mass)	0	MN	-0.071	0.019	-3.769	<0.001 ***	0.253
log (Mass)	0	SD	-0.052	0.018	-2.985	0.005 **	0.175
log (Max clutch size)	0	MN	0.060	0.104	0.572	0.570	0.008
log (Max clutch size)	0	SD	0.086	0.091	0.942	0.352	0.021
log (Max longevity)	0	MN	-0.292	0.079	-3.703	<0.001 ***	0.246
log (Max longevity)	0	SD	-0.259	0.070	-3.722	<0.001 ***	0.248
Diet Breadth	0	MN	-0.003	0.043	-0.060	0.952	<0.0001
Diet Breadth	0	SD	-0.013	0.038	-0.354	0.725	0.003
log (Habitat Breadth)	0	MN	0.032	0.066	0.488	0.628	0.006
log (Habitat Breadth)	0	SD	0.029	0.059	0.499	0.620	0.006
Migration distance	0	MN	0.258	0.079	3.248	0.002 **	0.201
Migration distance	0	SD	0.149	0.075	1.984	0.054 †	0.086
log (Mass)	1	MN	-0.078	0.033	-2.351	0.024 *	0.116
log (Mass)	1	SD	-0.105	0.045	-2.322	0.025 *	0.114
log (Max clutch size)	1	MN	0.140	0.121	1.161	0.252	0.031
log (Max clutch size)	1	SD	0.238	0.165	1.448	0.155	0.048
log (Max longevity)	1	MN	-0.045	0.086	-0.519	0.607	0.006
log (Max longevity)	1	SD	-0.010	0.118	-0.848	0.401	0.017
Diet Breadth	1	MN	0.049	0.030	1.628	0.111	0.059
Diet Breadth	1	SD	-0.008	0.042	-0.187	0.852	0.001
log (Habitat Breadth)	1	MN	-0.015	0.053	-0.275	0.785	0.002
log (Habitat Breadth)	1	SD	0.094	0.071	1.310	0.197	0.039
Migration distance	1	MN	0.083	0.070	1.178	0.245	0.032
Migration distance	1	SD	0.143	0.095	1.497	0.142	0.051

† 0.05 ≤ P ≤ 0.10, * 0.01 ≤ P ≤ 0.05, ** 0.001 ≤ P ≤ 0.01, *** 0.0001 ≤ P ≤ 0.001

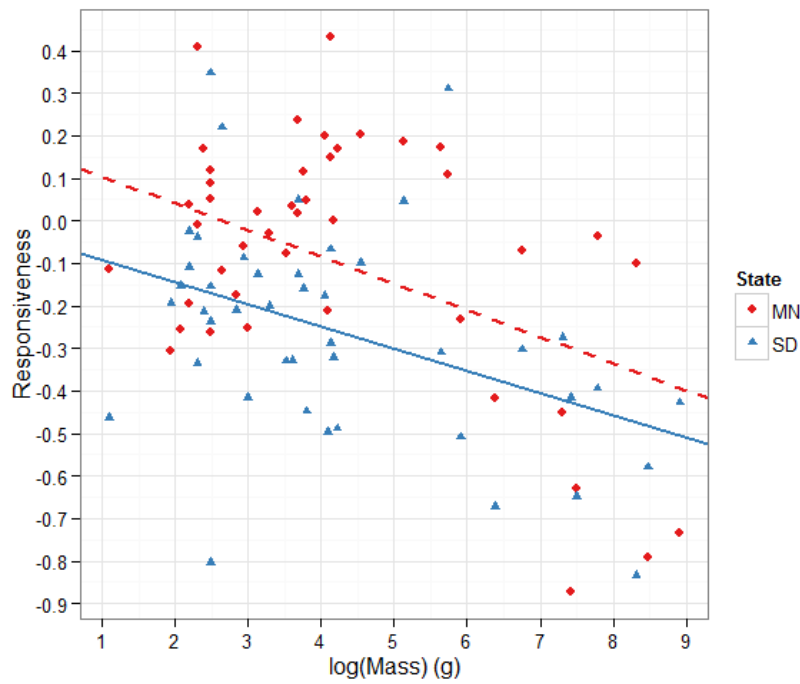


Figure 3.7: Scatterplot of the relationship between log (Mass) and Responsiveness for each state. Responsiveness represents the extent of change in FAD of a species from 1971-2005. In both states, species with larger masses are showing greater advances in FAD with time. MN = Minnesota; SD = South Dakota. Regression lines are taken from the pgls analysis at maximum likelihood lambda.

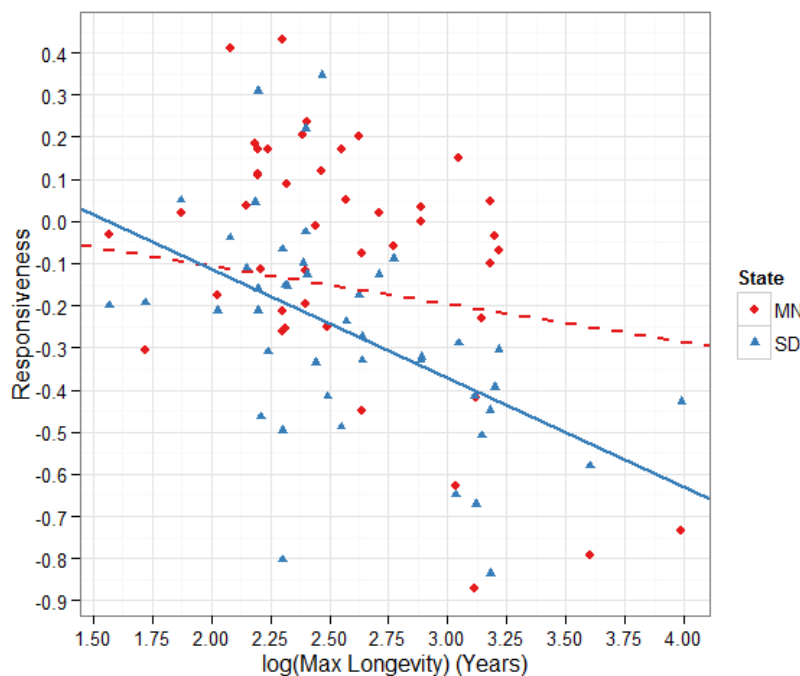


Figure 3.8: Scatterplot of the relationship between log (Maximum Longevity) and Responsiveness for each state. Responsiveness represents the extent of change in FAD of a species from 1971-2005. In both states, longer lived species are showing greater advances in FAD with time. MN = Minnesota; SD = South Dakota. Regression lines are taken from the pgls analysis at maximum likelihood lambda.

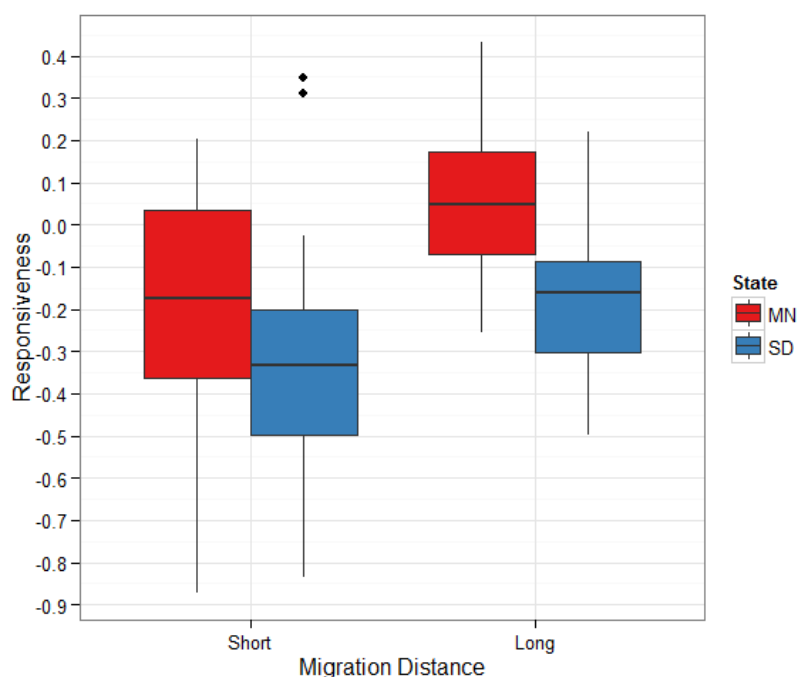


Figure 3.9: Boxplot of the relationship between Migration Distance and Responsiveness for each state. Responsiveness represents the extent of change in FAD of a species from 1971-2005. In both states, short distance migrants are showing greater advances in FAD with time than long distance migrants. Black dots represent outliers. MN = Minnesota; SD = South Dakota.

3.4.4 Comparing the geographic transferability of trait-based predictors between states

The efficacy of the Minnesota predictions about South Dakota are consistent with that of the South Dakota predictions about Minnesota, in all analyses. When lambda was set to 1, all tested Responsiveness~Trait relationships were shown to be transferable both ways between states ($P > 0.05$ for the intercept and slope of the relevant offset model). When lambda was set to 0, the Responsiveness~Maximum Longevity relationship was shown to be transferable both ways between states ($P > 0.05$ for the intercept and slope of the relevant offset model), however only the slope of the relationship between Responsiveness and Mass/Migration Distance is transferable ($P > 0.05$ for the slope of the relevant offset model; $P < 0.05$ for the intercept of the relevant offset model). Table 3.4 details the results of these offset models.

Table 3.4: Results of the *pgls* offset models for each state, indicating whether trait models are transferrable between states at two levels of lambda (0, 1). A significant value for either intercept or slope indicates poor transferability of trait-based predictions between states.

Trait	State being predicted	Lambda	Intercept Estimate	Slope Estimate	Intercept S.E.	Slope S.E.	Intercept t-value	Slope t-value	Intercept P-value	Slope P-value
log (Mass)	SD	0	-0.264	0.019	0.083	0.018	-3.193	1.076	0.003 **	0.288
log (Mass)	MN	0	0.264	-0.019	0.089	0.019	2.963	-0.998	0.005 **	0.324
log (Mass)	SD	1	-0.030	-0.028	0.353	0.045	-0.086	-0.612	0.932	0.544
log (Mass)	MN	1	0.030	0.028	0.257	0.033	0.118	0.841	0.907	0.405
log (Maximum longevity)	SD	0	-0.268	0.033	0.182	0.070	-1.472	0.475	0.148	0.637
log (Maximum longevity)	MN	0	0.268	-0.033	0.207	0.079	1.299	-0.419	0.201	0.677
log (Maximum longevity)	SD	1	-0.028	-0.055	0.424	0.118	-0.066	-0.468	0.948	0.642
log (Maximum longevity)	MN	1	0.028	0.055	0.311	0.086	0.090	0.639	0.929	0.527
Migration Distance	SD	0	-0.131	-0.109	0.052	0.075	-2.537	-1.453	0.015 *	0.154
Migration Distance	MN	0	0.131	0.109	0.055	0.079	2.397	1.373	0.021 *	0.177
Migration Distance	SD	1	-0.208	0.060	0.260	0.095	-0.798	0.630	0.429	0.532
Migration Distance	MN	1	0.208	-0.060	0.192	0.070	1.085	-0.857	0.284	0.396

+ 0.05 ≤ P ≤ 0.10, * 0.01 ≤ P ≤ 0.05, ** 0.001 ≤ P ≤ 0.01, *** 0.0001 ≤ P ≤ 0.001

3.5 Discussion

Management implementations of predictions on how species will respond to climate change are faced by numerous constraints, most notably those of time and scale. Range level predictions may give a good indication of large-scale trends, but their coarse predictions are not necessarily applicable at site level, where biotic and abiotic idiosyncrasy is high (Wiens 1989). Site-level predictions may be locally effective, but can be inefficient, as they are not necessarily broadly applicable between areas (Buckley and Kingsolver 2012). The use of biological traits as predictors of species responses to climate change could reconcile these two approaches. This is because trait-based predictors, by their probabilistic nature, transcend some of the local variability in context than confronts species-focused approaches, allowing individual species to behave idiosyncratically while responses at the level of shared traits are more consistent. Thus, certain fundamentals of species biology that govern responses at one site should be important determinants of responses for populations or species at other sites, given similar levels of climate change. As such, trait-based predictions may combine site-level relevance with broad applicability. The work presented here develops a formal test of the geographic transferability of trait-based predictions. Specifically I investigate the importance of traits in predicting FAD changes for North American migratory birds in two climatically similar but geographically distinct areas: the adjacent US states of South Dakota and Minnesota. I next assess the transferability of trait predictions between these two areas. Results demonstrate that Body Mass is a consistent predictor of species responsiveness in both states. Furthermore, Mass-based predictions are accurately transferable between both states, provided phylogenetic non-independence is controlled for.

When examining the consistency of the raw FAD trends over the timeframe of this study (1971-2005), there appeared to be reasonable concordance in species responses between states: 33/44 species showed no significant difference in the rate of change in FAD with time between the two areas. We might thus expect some level of consistency in trait-based predictions between states. This was indeed the case. Analyses revealed that Body Mass was the most consistent and effective predictor of changes in first arrival date with time both within and between states. Due to difficulties in estimating the phylogenetic signal in the data, all trait models were run at three levels of lambda (ML, 0 and 1) and in both states, Body Mass was a significant predictor of responsiveness at all three levels.

The direction of the relationship between Body Mass and Responsiveness contrasts with hypotheses however. I expected that bird species with smaller masses would show greater FAD advances in response to temperature increases over time. This is because species with smaller masses tend to have faster life histories and thus are often under stronger selective pressure to maximise current breeding attempts rather than their future ones (Sandvik and Erikstad 2008). Since advances in phenology are a way of keeping pace with changing conditions and so maintaining immediate fitness, species with faster life histories might be expected to show greater phenological advances under climate change than those with slower life histories. This was not the case however: species with larger masses (slower life histories) actually showed greater advances in FAD over the course of this study than species with smaller masses (faster life histories).

Molnar *et al.* (2012) and Chapter Two demonstrated similar trends in plant species. In both these studies, taller plants (which tend to have slower life histories) showed greater advancement in flowering date with temperature increases. They suggested that this trend may indicate a greater importance of phenotypic plasticity (which may be higher in species with slower life histories) than of evolutionary adaptive capacity (which may be higher in species with faster life histories) in the responses of species to climate change. The results from the bird species analysed in this chapter provide further evidence for this suggestion.

The explanatory power of each Body Mass model was low to moderate, and showed some variation between states at certain lambda levels. Specifically, under phylogenetic control, Body Mass showed consistent power in explaining variation in responsiveness in both states. In models where phylogenetic non-independence was not controlled for however, and when a ML estimate of lambda was used for each model, explanatory power was much more variable between states. This provides an initial caution that site-specific factors are an important determinant of trait-based responses, at least when phylogenetic non-independence is unaccounted.

The established models of Body Mass as a predictor of responsiveness were accurately transferrable between states under phylogenetic control (i.e. the relevant offset models showed no significant effect of slope or intercept). Specifically, species responsiveness in one state was accurately predicted by the established relationship between Body Mass and responsiveness for the other state, when phylogenetic non-independence was controlled for. When the phylogenetic relationships between species were not accounted for, predictions

were not transferable between states. This result again highlights the importance of controlling for phylogenetic non-independence in trait analyses.

Both Migration Distance and Maximum Longevity also showed some site-specific importance as predictors of FAD changes over the course of this study. They further showed accurate transferability as predictors between states under phylogenetic control, although neither was a significant predictor in either state under $\lambda=1$, suggesting that these results are practically irrelevant. Despite this, it is worth discussing the site-specific importance of these two traits further. Migration Distance was a marginally significant predictor of responsiveness in both states under ML and 0 λ . Short distance migrants showed greater FAD advances than long distance migrants. The direction of the relationship between Migration Distance and responsiveness accords with hypotheses that species displaying greater Seasonal Readiness will show greater advances in FAD. Specifically, short distance migrants spend most of the year in conditions similar to those at their breeding ground, being exposed to the local warming climate all year round. Long distance migrants, by contrast, overwinter at locations remote from the breeding area and may only be exposed to local warming temperatures in late Spring (Lehikoinen *et al.* 2004). As such, short distance migrants may be more attuned to temperatures at their breeding grounds and thus have better cues to match their phenology to (Vegvari *et al.* 2010), so showing greater responsiveness to changes in these temperatures (i.e. they will show greater Seasonal Readiness). Long distance migrants may be responding to conflicting conditions between their distant overwintering grounds and their breeding grounds, further explaining their less extensive responses to climatic changes at the breeding ground. In addition, the phenology of long distance migrants tends to be more constrained by endogenous circannual rhythms than that of short distance migrants, limiting their ability to adapt plastically to local changes in climate (Both and Visser 2001; Wilkelski *et al.* 2008). The results for Migration Distance thus fit in with the earlier discussion that phenotypic plasticity may be more important in the responses of species to climate change than evolutionary adaptive capacity. Supporting these site-specific findings, a variety of other studies also demonstrate the same relationship between Migration Distance and changes in FAD, in different areas (Butler 2003; Rubolini *et al.* 2007; Vegvari *et al.* 2010; Buskirk *et al.* 2012; Hurlbert and Liang 2012).

Maximum Longevity was a highly significant predictor of responsiveness in South Dakota under an ML λ value, but not in Minnesota. It was significant in both states when λ was set to 0. Specifically, longer lived species showed greater advances in FAD than

shorter lived species. Similar to the result for Body Mass, this contrasts with hypotheses that species with faster life histories (often short lived) will show greater responsiveness to climate change, and further supports the importance of phenotypic plasticity in determining these responses (see earlier). The lack of significance of both Migration Distance and Maximum Longevity under phylogenetic control indicates further research is required before we can assert these site-specific interpretations with confidence however.

The establishment that Body Mass models show good predictive transferability between states under phylogenetic control suggests that some trait models can show both broad applicability and site-level relevance. The lack of clear transferability for more of the trait-based predictions in this analysis however, is a cause for concern – if several trait predictions cannot readily be transferred between populations at locations as proximal and climatically similar as South Dakota and Minnesota, hope for transferability further afield is diminished. The difficulties in lambda estimation for these datasets (and the resulting inconsistencies between models run at different levels of lambda) make it hard to draw concrete conclusions of success or failure. What is clear from these analyses however, is the large impact phylogenetic relationships can have on the results of such trait models.

Some methodological considerations are important here. It is possible that the use of larger datasets may facilitate more accurate assessment of phylogenetic relationships, which would make analyses such as these much easier to implement and interpret. Such large datasets would also facilitate the comparative analysis of multiple traits at once, which the models presented here could not do. These types of comparative analyses ensure that model predictors are independent of each other, and not just simple correlates. Furthermore, such multi-trait models are likely to have greater explanatory power and thus practical relevance. More generally, transferability needs to be assessed between more sites and species, and at a broader variety of scales. This will help to affirm whether there is a lower bound of geographical scale to such predictive transferability, perhaps determined by biotic idiosyncrasy, and if so, where this bound lies. Making known such biological unknowns will be a crucial element of managing for the consequences of a changing climate.

Overall, this study provides further evidence that traits are important predictors of the site-specific responses of species to climate change. Akin to Chapter Two, it suggests that species' phenotypic plasticity may be a more important determinant of response than evolutionary adaptive capacity. It has further demonstrated some consistency in the specifics of predictive traits between sites (similar to Thackeray *et al.* 2010 and Wolkovich *et al.*

2013). Despite this, the direct transferability of established relationships between traits and responses to climate change between areas remains unconfirmed. One trait (Body Mass) shows predictive transferability when phylogenetic non-independence is controlled for, however other trait results are less clear. More evidence than is available from this Chapter is required before traits can be asserted as effective cross-site predictors.

Chapter Four

Traits and changes in species abundances under climate change

4.1 Abstract

Earlier chapters established that traits have some value in predicting the phenological responses of species to climate change. Abundance is a metric of species response that is very relevant to conservation practitioners. As such, it would be useful if the trait-based models applied to phenological data could be used to predict abundance responses. Here, I address this idea by attempting to develop trait-based correlates of plant abundance changes observed in response to alternative experimental precipitation regimes. Contrary to expectation, observed changes in species abundance are highly idiosyncratic and do not appear to break down according to shared traits. Furthermore, community reorganisation observed in response to water-addition appears to be attributable to the response of a specific nitrogen-fixing species. This contrasts with the expectation of previous researchers in this system that the mechanism of observed community change was driven by the response of nitrogen-fixers as a trait-linked functional group. Further analyses presented in this chapter do, however, support a clear mechanism of change that is resolved to a single proximal trait driver.

4.2 Introduction

This thesis has so far explored species responses to climate change at the level of shared traits. The intention has been to gain additional, scalable insights to predicting species responses on the ground, and to help extend existing predictions to new groups of species. If responses to climate change break down by species traits, then species that are well studied with respect to climate change may guide our expectations for others, for which we have information on phylogeny and biology, but not on responses to climate. To test the value of trait-based predictors, Chapters Two and Three used alternative datasets of phenological observations across broad groupings of species. Phenological changes are a useful measure of the impact of climate change on species (IPCC 2007) however changes in abundance represent a more conservation-relevant metric of species response, and thus may be of greater practical relevance. This chapter makes use of a ten-year dataset of species responses to alternative climate regimes in natural grassland communities, and examines whether changes in abundance can be understood in terms of shared traits.

A key value of interpreting ecological responses to climate change in terms of species traits is that traits may respond to environmental change more predictably than species do (Fukami *et al.* 2005; Eronen *et al.* 2010). *Community Assembly* theory describes how environmental conditions act as filters determining what traits can exist in what places (Kraft and Ackerly 2010). Members of a regional species pool are sorted into local communities according to the action of those environmental filters, which allow some traits to “pass through” and exclude others. This environmental filtering of traits however, may be more predictive of species absence than of species presence. Indeed, community ecology suggests that in local communities, biotic interactions determine species abundances and persistence, with changes in climate affecting species through effects on resources, competitors, mutualists, consumers, or pathogens (e.g. Schiel *et al.* 2004, Biro *et al.* 2007). So while traits limit the pool of prospective occupant species in a given ecological community, actual presence and local abundance can depend on a series of complex interactions among biotic and abiotic factors.

The effects of biotic interactions on changes in species phenologies (the metric studied in earlier chapters) are likely to be less extensive than on their abundances. Generally organisms use cues that correlate with future conditions to determine the timing of phenophases. The most common of these are temperature and photoperiod (van Asch and Visser 2007). Phenological changes are not immune to biotic forcings however. If

interacting species show different extents of plastic response to changes in climate, the synchrony of phenophases between species can be disrupted, leading to trophic mismatches (e.g. Visser and Holleman 2001; Both *et al.* 2009). Such mismatches can result in fitness consequences for the species involved, and thus directional selective pressure upon resynchronisation (van Asch and Visser 2007). In terms of short term responses to climate change however, it is fair to suggest that phenologies are less affected by biotic interactions than are species abundances, and more tied to abiotic conditions.

Changes in abundance are an important currency in conservation and natural resource management, representing measureable consequences of climate for local populations. They are thus a major focus of predictive research in climate change ecology. As such, if the trait-based tools I established for predicting phenological responses could be extended to abundances as well, traits would be a more valuable addition to our predictive toolkit. This process may be complicated by the aforementioned influence of biotic interactions on climate driven changes in species abundances. As such, the process of identifying trait-based predictors of these responses may be more difficult than it was for the phenological responses analysed in earlier chapters. Phenology and abundance however, will be related in many ways in actual communities, with changes in phenology carrying implications for population abundances. Indeed, the trophic mismatches discussed earlier often have consequent effects on population abundance (e.g. Winder and Schindler 2004; Both *et al.* 2006). As such, a theoretical link between traits and climate driven changes in species abundance does exist, if indirectly via phenological responses. What this chapter looks to do is to develop a direct link between species traits and changes in abundance under climate change.

There are scattered signs in the literature that this direct link may exist, with evidence that changes in species abundances break down along trait lines. In a long term study of rodents in the US Great Basin, 8000 years of climate fluctuations were shown to have effects on rodent abundance according to diet. Under drier conditions, seed-eating species showed increases in abundance whilst plant-eating species showed decreases. Under wetter conditions, the reverse was true (Terry *et al.* 2011). In the Siskyou Mountains, climate-induced dicot population declines between an original sample (1949-1950) and a modern resurvey (2007) varied according to geographic affinity. More northerly distributed species showed stronger declines in population size than more southerly distributed species (Damschen *et al.* 2010). Changes in ocean phytoplankton abundances under warming were shown to be correlated with body size: smaller species are showing increases and larger

species declines (Daufresne *et al.* 2009; Li *et al.* 2009). Among demersal fishes in the English Channel between 1911 and 2007, body size was identified as an important determinant of abundance changes as a result of climate change and fishing. Smaller species have varied non-directionally across the time series, while larger species have shown steady declines (Genner *et al.* 2010). Studies of breeding birds across Europe (Jiguet *et al.* 2010) and ants in Eastern US deciduous forests (Diamond *et al.* 2012) likewise show that changes in abundances under directional changes in climate vary according to identifiable traits.

Several of the above examples represent patterns detected in the data by study authors: they were not the result of systematic statistical models analysing responses by species traits akin to those implemented in earlier chapters (e.g. Li *et al.* 2009; Genner *et al.* 2010; Terry *et al.* 2011). The challenge confronting such a formalised statistical approach is that species-resolved abundance data for whole communities are rare. Most population monitoring efforts target key species within a community, such as a potentially invasive exotic or a species of key conservation or functional value. The scarcity of population abundance datasets as comprehensive as the phenology datasets of Fitter and Fitter (2002) analysed in Chapter Two, and to a lesser extent of Swanson and Palmer (2009) analysed in Chapter Three, is probably attributable to the relative difficulty of collecting abundance versus phenology data. It is easier to see a bird or flower than to count all such birds or plants. As such, when a researcher does count abundances, it is usually with a more targeted focus than ‘all species in the community’. The exception comes in certain experimental contexts. Here, species composition is tracked in terms of relative abundances of each species in the community, albeit over a more abbreviated timeframe.

The Angelo Reserve Rainfall Addition Experiment (Mendocino County, California, 39°43’45’’ N, 123°38’40’’ W) provides an exemplary experimental system in which to test for a direct link between traits and climate driven changes in species abundances. Beginning in 2001, it documents the responses of an entire California grassland plant community to experimentally imposed changes in the seasonality and intensity of rainfall, from the level of individual species to that of ecosystem function. Following five years of dynamics under alternative precipitation regimes, researchers highlighted leguminous plants associated with nitrogen-fixing bacteria (hereafter ‘nitrogen-fixers’) as the strongest respondents to an experimental extension of the rainy-season (Suttle *et al.* 2007). They proposed that the strongly positive response to late rainfall in this group drove subsequent changes in diversity, energy flow, and production throughout the food web. Specifically, early in the experiment,

many plants saw directly positive responses to late rainfall. A particularly strong direct response by nitrogen-fixers led to an abundance of nitrogen-rich litter at the end of each growing season. This litter decomposed at the onset of each subsequent rainy-season, resulting in increases in mineralised nitrogen in the soil. At this early-season stage, only exotic annual grasses are active in the system. Resultantly, the high mineralised nitrogen content of the soil fuelled large increases in the biomass of exotic annual grasses via a fertilisation effect. This subsequently caused competitive suppression of more broad-leaved forbs. Broadly, the short-term increases in plant diversity seen early in the experiment were overturned, leading to intermediate-term collapses in this diversity. With early-senescing exotic annual grasses ultimately dominating the plant resource base, initially high diversity and abundance of invertebrate consumers were also overturned in the intermediate-term. Thus the indirect effects of rainfall-extension mediated by the nitrogen-fixer response were proposed to reverse the direct effects of this extension on multiple aspects of community structure. The Angelo study thus demonstrated the importance of species interactions in driving community responses to climate change, even in contravention to the direct effects of climate.

The present chapter looks to this experimental dataset to determine whether plant traits correlate to changes in species abundance under alternative climate regimes. It takes a particular focus on the strongest responders in this system, as proposed by the original study authors: nitrogen-fixers. It then follows up the analyses of Suttle and colleagues with five additional years of data at the level of functional group and that of the whole plant assemblage, and data on nitrogen concentrations in the ecosystem. The intention is to test more mechanistically the proposed pathways of ecological change under this climate manipulation.

4.3 Methods

4.3.1 Natural history of the study system

The Angelo Coast Range Reserve is part of the University of California's Natural Reserve System, as one of a network of over 30 sites protected for ecological research across the state (Herring 1997). It encompasses the watersheds of three perennial streams in the headwaters of the South Fork Eel River, and consists primarily of old-growth conifer forest interspersed with grassy meadows, over a total area of 16.42km² (Herring 1997). The climate is Mediterranean, with hot dry summers and cool wet winters: average annual rainfall is 216cm and falls predominantly between the months of October and April (Suttle *et al.* 2007). There is little overlap between periods of high sunlight availability and warmth conducive to plant growth, and periods of high water availability (Major 1988). As such, the specific timing of the annual rainy-season is an important determinant of ecosystem productivity.

Grasslands on the reserve are found on strath terraces. These are river terraces that were abandoned during periods of high tectonic activity and became filled in with soil during landslides from adjacent slopes. The reserve grasslands host approximately 50 species of vascular plant (Suttle and Thomsen 2007). These include many California endemics that have lost most of their original habitat throughout the state to non-native species. California grasslands are heavily invaded by European and Asian exotic species (Heady 1977). Resultantly, they have suffered precipitous declines in native species over the last 150 years. It is only along the north coast of the state that many natives have been able to persist in large populations alongside these invaders (Hektner and Foin 1977; Hayes and Holl 2003).

The study meadow itself contains a well-mixed assemblage of grasses and forbs of both native and exotic origins. Sizable populations of the native forbs *Eschscholzia californica*, *Trichostemma lanceolatum* and *Eremocarpus setigerus*, and the native bunchgrasses *Danthonia californica*, *Elymus glaucus*, and *Elymus multisetus* exist alongside dominant cover by the exotic annual grasses *Bromus hordeaceus*, *Vulpia myuros* and *Bromus diandrus*.

4.3.2 Experimental design

The analyses presented in this chapter are based on data collected by earlier researchers at the Angelo Reserve Rainfall Addition Experiment. This experiment began in 2001, and tests the consequences for grassland production and diversity of alternative scenarios of precipitation

change projected for Northern California by the Hadley Centre for Climate Prediction and Research (HadCM2) and the Canadian Centre for Climate Modelling and Analysis (CCM1) (detailed experimental design in Suttle *et al.* 2007; Suttle and Thomsen 2007). Briefly, thirty-six circular plots ($\sim 70\text{m}^2$ each) have been subjected to one of three precipitation treatments each year, in a randomised block design:

- A wintertime addition of water (January through March) to simulate the Hadley model's proposed intensification of the Californian rainy-season.
- A springtime addition of water (April through June) to simulate the Canadian model's proposed extension of the Californian rainy-season.
- An un-manipulated ambient control.

Springtime-addition plots receive approximately 44cm of water over ambient precipitation each year, from April through June. Wintertime-addition plots receive the same amount from January through March. This water is delivered in increments of 14cm to 16cm two hours after dawn every three days during the aforementioned periods, regardless of ambient weather. Plant production, diversity and abundance have been sampled for 18 of these plots (*i.e.* 6 replicates in each of 3 precipitation treatments), while the remaining 18 were left undisturbed but for annual applications of the precipitation treatments (Figure 4.1). Wintertime-addition plots did not factor into the work presented here because researchers reported no conspicuous direct or indirect effects for this treatment in the first five years of experimentation (Suttle *et al.* 2007). This work focuses on springtime-addition plots (hereafter referred to as water-addition plots) and ambient control plots.

4.3.3 Response variables

Species abundances

Relative abundances of the different plant species in each experimental community are available from point-frame sampling undertaken from 2002 to 2010. A point-frame sampling device with 8 pins was placed in six permanent designated locations in each plot in late May of each year. This coincided with the period of peak production and diversity in the grassland. Data were recorded on the species first contacted by each pin as it was lowered vertically into the vegetation canopy, the species that pin landed on, and the species in contact with that pin at a marked location of 3cm above the ground surface. These measurements yielded a dataset of 144 'hits' for each plot in each year.

Raw hits for every species in the dataset were summed, for each plot in each year. Plot-specific relative abundance of each species in each year was then calculated as the total number of hits for that species hits as a proportion of the total number of trials (not including hits on the pointframe that were attributable to litter, bare ground and moss).

Plant production

Plant production was measured from biomass samples collected three times each growing season to capture each species at or near its individual annual peak. Two sampling quadrats measuring 900cm² each were placed at pre-designated locations in each plot in late May, early July, and late August/early September of each year. All plant material was clipped at the soil surface, sorted by functional group (i.e. spring annual grass, summer annual grass, perennial grass, spring annual forb, summer annual forb, late-summer annual forb, perennial forb, and nitrogen-fixers) and dried at 72°C for 48 hours. Samples were then weighed. Once harvested for biomass, each subplot was eliminated from future sampling.

I estimated yearly aboveground net primary production (ANPP), by summing the biomass of the different plant groups listed above at their annual peak production. I calculated an overall sum for annual forb and annual grass productivity in each year, by summing the biomass of their respective phenological subgroups (spring annual forbs, summer annual forbs and late-summer annual forbs, and spring annual grasses and summer annual grasses respectively) to go with the year sums for perennial grasses, perennial forbs and nitrogen-fixers.

Plant diversity

Plant diversity was measured as the mean species richness of two permanent 2500cm² subplots in each plot. It was sampled regularly over the growing season to account for phenological differences in the seasonal activity of different species.

Soil and plant nitrogen concentrations

Soil nitrogen availabilities were measured using specialised probes called Plant Root Simulators™ (Northern Innovations©). These use ion exchange membranes to bind molecules of nitrogen in mineralised form (NO₂⁻, NO₃⁻, NH₄⁺). Following pilot sampling to determine periods of peak nitrogen movement through soils, four replicate probes were placed in each plot on 1st January for 90 days, in 2006, 2007, and 2008. The amount of NO₂⁻,

NO₃⁻ and NH₄⁺ was recorded for each plot in each year. These values were summed to provide a value for total soil nitrogen in each plot for each year.

Plant leaf nitrogen concentrations were measured in the most widely and continuously distributed species in the study system (the annual grass *Bromus hordeaceus*). Leaves were collected in mid-May from 2001 through 2006. At this time of year, the plant is transitioning from vegetative growth to seed production. As such, sampling targeted the period just before the plant has begun to withdraw nutrients from vegetative tissues and put them towards developing seeds. Data were in the form of a Carbon to Nitrogen (C:N) ratio for *Bromus hordeaceus* in each plot for each year.

4.3.4 Statistical analyses

California grasslands are notably dynamic in their production, diversity and composition. Successional dynamics are largely absent and instead, composition and production vary year to year based on the specifics of climate, and in particular the timing and amount of precipitation (Pitt and Heady 1978; Stromberg and Griffin 1996; Hobbs *et al.* 2007). ‘Ambient’ conditions, as represented by control plots, therefore entail a high degree of interannual variability in all measured variables. Given this, a linear trajectory of response over time would not be expected in this system. Responses for each year are thus modelled separately. All analyses were performed in R 3.0.2.

Species abundances

With a view to examining whether species possessing particular traits showed strong or weak changes in abundance relative to treatment, I analysed treatment effects on the abundance of each individual species in the system in turn. This was done using generalised linear mixed effects models in the R package *afex* (Singmann 2013). The relative abundance of each species in turn was modelled as the dependent variable, for each year. Each model was weighted by the total number of trials. A binomial error distribution was specified. The following R syntax was used, where watering treatment is a fixed effect and block is a random effect:

Relative Abundance ~ Watering Treatment + (1|Block), family=binomial

The significance of watering treatment in each model was calculated using likelihood ratio tests. Model overdispersion was tested for using a function provided by

<http://glmm.wikidot.com> in conjunction with Bolker *et al.* (2009). In correcting model overdispersion in these analyses, an observation-level random effect was added to each overdispersed model.

Plant production

Production data from 2001-2010 were analysed using linear mixed effects models in *afex*. A Gaussian error distribution was used. ANPP and the productivity of each functional group (annual grass, perennial grass, annual forb, perennial forb and nitrogen-fixer) were used as the dependent variable in turn, for each year. The following R syntax was used:

```
Production ~ Watering Treatment + (1|Block)
```

The significance of the effect of Watering Treatment on each dependent variable was calculated using conditional F-tests with Kenward-Roger approximated degrees of freedom. ANPP and the responses for annual grasses and perennial forbs were logged to improve model residual distributions. Serious distributional issues remained for models of nitrogen-fixer production in 2001, in spite of attempts to resolve them. These data are not presented here. Perennial grass production models suffered from serious residual distribution problems for most years of the experiment and thus were not presented for any year.

Plant diversity

Plant diversity data from 2001-2010 were analysed in the same manner as the production data, with total plant diversity as the dependent variable, for each year. Although data are in the form of counts, a Gaussian error distribution fitted them much better than a Poisson error distribution, so Gaussian models were used.

Soil and plant nitrogen concentrations

Soil and plant nitrogen data were analysed in the same way as the plant production and plant diversity data. Total soil nitrogen content ($\mu\text{g}/10\text{cm}^2$) and the C:N ratio of *Bromus hordeaceus*, for each plot, were used as dependent variables in turn, for each year of data. A Gaussian error distribution was specified for both.

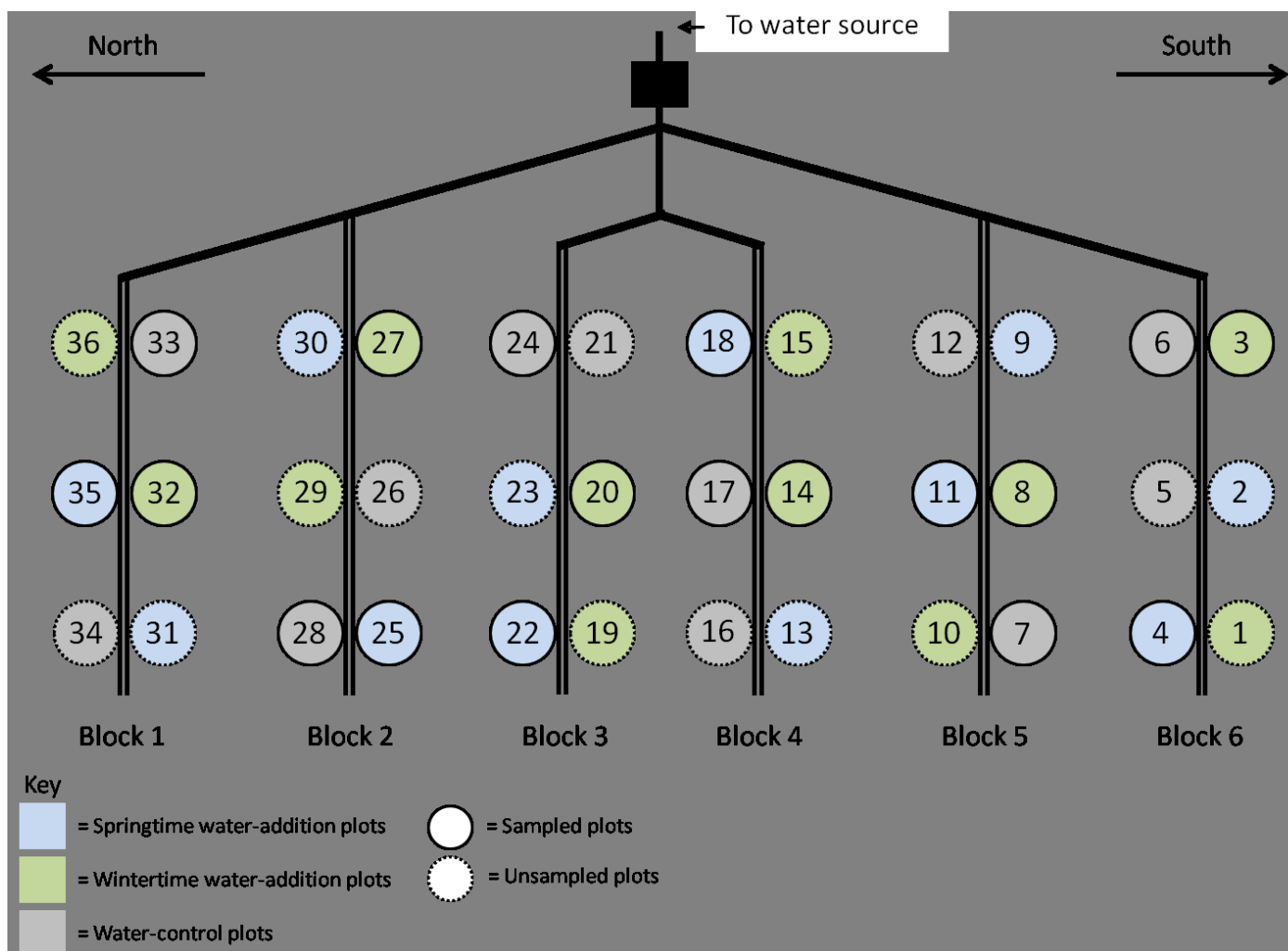


Figure 4.1: Design of the Angelo Reserve Rainfall Addition Experiment (Mendocino County, California, 39°43'45" N, 123°38'40" W) which tests the consequences for grassland production and diversity of two alternative scenarios of climate change projected for Northern California: a wintertime addition of water (from January through March) and a springtime addition of water (from April through June).

4.4 Results¹

4.4.1 Species abundances

Despite having comprehensive abundance data for the entire set of 38 species in this system, the employment of formalised trait analyses was negated by the lack of common traits shared between species showing similar abundance responses to water-addition. For example, there are few conceivable traits that could unify a deeply rooted native bunchgrass (*Danthonia californica*, Figure 4.2a), a shallowly rooted, annual invasive grass (*Bromus diandrus* Figure 4.2b) and a native nitrogen-fixing forb species (*Lotus micranthus* Figure 4.2c). Species that *do* share common traits show great variation in how their abundance changes with treatment. For example, whilst *Bromus diandrus* showed a strongly positive response to water-addition (Figure 4.3a), *Aira caryophyllea* showed the complete opposite response (Figure 4.3b), and *Bromus tectorum* also appeared to show the opposite response, but was at an abundance that was insufficient to facilitate accurate assessment (Figure 4.3c). Such low abundances were common for several species in the system, adding further complications of sample size to prospective trait analyses.

This high level of idiosyncrasy in the system was further apparent at the level of individual experimental plots, where there was large variation in abundance responses for specific species between plots and within treatments. Despite this inter-plot variation, some species still appeared to break down clearly by treatment (e.g. Figure 4.4a, Figure 4.4b) however others showed far less clear breakdowns (Figure 4.4c).

It appears that at the spatial scale of this experiment, there is too much complexity at the level of individual species responses to make much sense of them in terms of traits. Understanding the specific dynamics here will thus have to come by scaling out to the level of production and diversity. This observation lead me question the proposal by Suttle and colleagues that the mechanism of community reorganisation that they observed in this system was driven via a response by the trait-linked functional group, nitrogen-fixers. I thus examined the abundance changes of individual nitrogen-fixer species in this system. Response idiosyncrasy was, again, very high (Figures 4.5a, 4.5b, 4.5c and 4.5d). The strong nitrogen-fixer response that Suttle and colleagues had observed in production data actually appeared to be attributable to just one species of nitrogen-fixer: *Lotus micranthus*.

¹ Appendix 3.1 presents treatment means for every response variable modelled, in every experimental year

Models of the effects of watering treatment on the proportion of total nitrogen-fixers, the proportion of *Lotus micranthus* alone, and the proportion of other nitrogen-fixers in the system supports the evidence from Figure 4.5 (Table 4.1). Near the beginning of the Angelo experiment in 2002 and 2003, water-addition plots showed higher abundances of nitrogen-fixers as a functional group than water-control plots, with significance or marginal significance. After 2003, this effect was not significant again until 2008 and 2009, where the same directional difference was observed (Figure 4.6a).

Examining the effects of watering treatment on the relative abundance of *Lotus micranthus* demonstrated significant effects in every modelled year from 2002-2010. Water-addition plots had higher abundances of *L. micranthus* than water-control plots in every year (Figure 4.6b).

Watering treatment had a significant or marginally significant effect on the relative abundance of all other nitrogen-fixers in 2004, 2007 and 2010. Water-addition plots had lower abundances of these other nitrogen-fixers than water-control plots in these three years (Figure 4.6c). Clearly, the observed effects of watering treatment on the abundance of nitrogen-fixers apparent at the functional group level are in fact attributable *Lotus micranthus* alone.

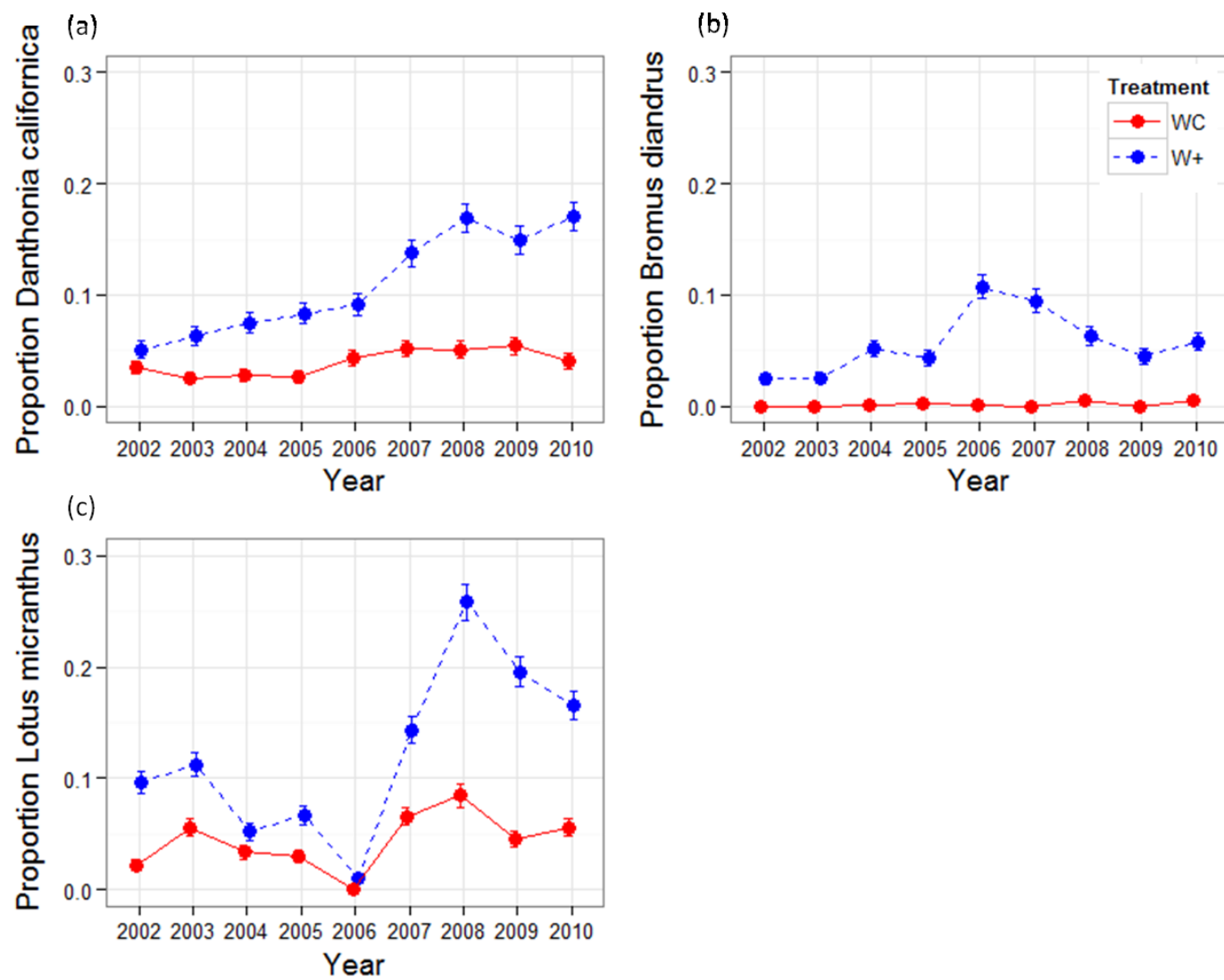


Figure 4.2: Effects of water-addition on the relative abundance (proportion) of (a) *Danthonia californica*; (b) *Bromus diandrus*; (c) *Lotus micranthus*. Data represent mean ± 1 standard error for each treatment application, from 2002-2010. W+ denotes water-addition; WC denotes water-control.

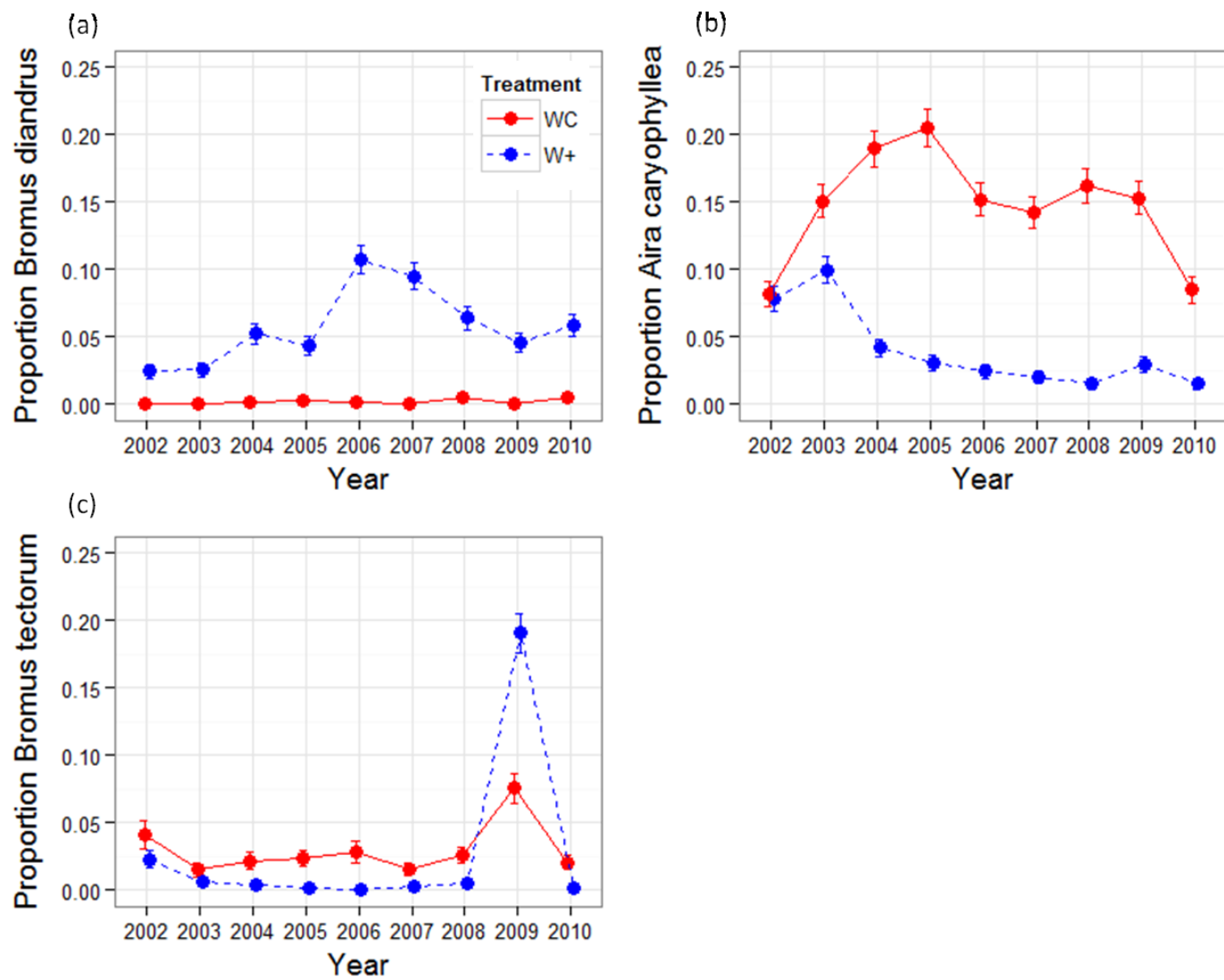


Figure 4.3: Effects of water-addition on the relative abundance (proportion) of (a) *Bromus diandrus*; (b) *Aira caryophyllea*; (c) *Bromus tectorum*. Data represent mean ± 1 standard error for each treatment application, from 2002-2010. W+ denotes water-addition; WC denotes water-control.

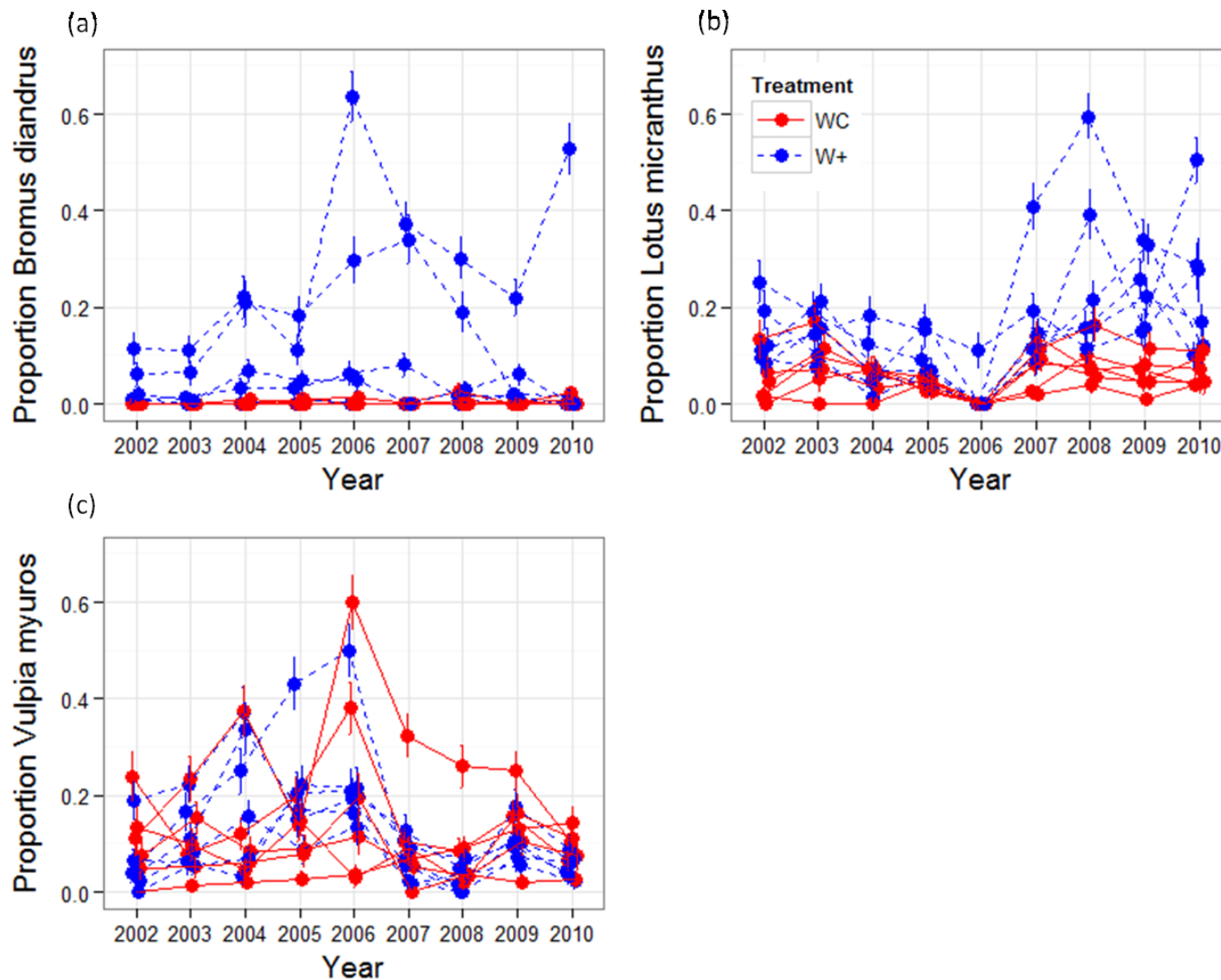


Figure 4.4: Effects of water-addition on the relative abundance (proportion) of (a) *Bromus diandrus*; (b) *Lotus micranthus*; (c) *Vulpia myuros* in each experimental plot. Each line represents one plot. Data represent mean ± 1 standard error for each treatment application, from 2002-2010. W+ denotes water-addition; WC denotes water-control.

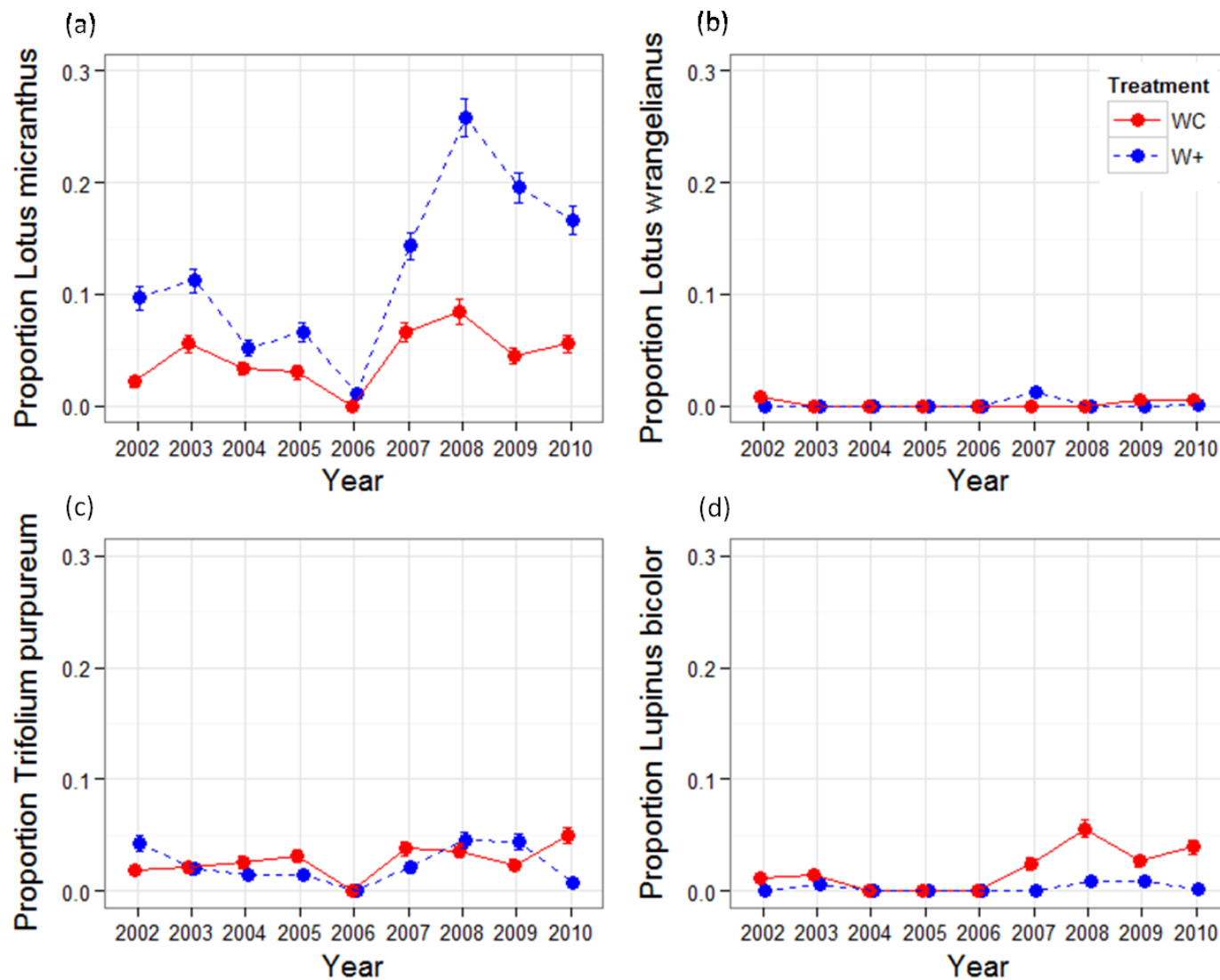


Figure 4.5: Effects of water-addition on the relative abundance (proportion) of the nitrogen-fixers (a) *Lotus micranthus*; (b) *Lotus wrangelianus*; (c) *Trifolium purpureum*; (d) *Lupinus bicolor*. Data represent mean ± 1 standard error for each treatment application, from 2002-2010. W+ denotes water-addition; WC denotes water-control.

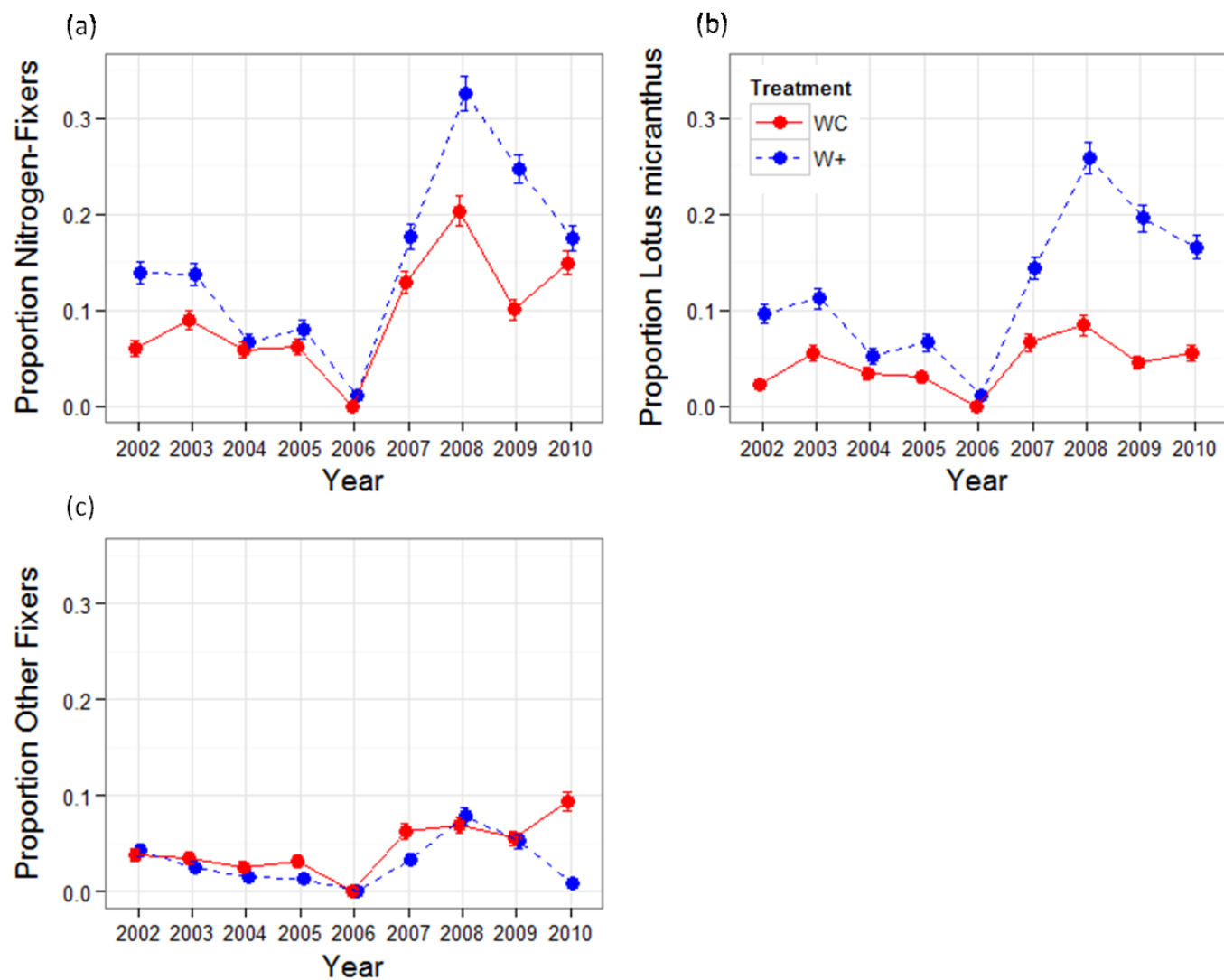


Figure 4.6: Effects of water-addition on the relative abundance (proportion) of (a) Total Nitrogen-Fixers; (b) *Lotus micranthus*; (c) Other-Nitrogen Fixers. Other Nitrogen-Fixers represent all nitrogen-fixing species excepting *Lotus micranthus*. Data represent mean ± 1 standard error for each treatment application, from 2002-2010. W+ denotes water-addition; WC denotes water-control.

Table 4.1: Effect of watering treatment on the relative abundance of nitrogen-fixers as a functional group, the relative abundance of *Lotus micranthus* alone, and the relative abundance of nitrogen-fixers excluding *L. micranthus* (denoted “Other Nitrogen-Fixers”), calculated using likelihood ratio tests on generalised linear mixed effects models. Degrees of freedom listed Large then Small. 2006 not presented due to uncorrectable issues with model residual distributions for all groups.

Year	Functional Group	Chi-squared	df	P-value
2002	Nitrogen-fixers	7.25	3, 2	0.007 **
	<i>Lotus micranthus</i>	8.06	4, 3	0.005 **
	Other Nitrogen-Fixers	2.47	3, 2	0.116
2003	Nitrogen-fixers	3.4	3, 2	0.065 †
	<i>Lotus micranthus</i>	3.88	4, 3	0.049 *
	Other Nitrogen-Fixers	0.61	4, 3	0.437
2004	Nitrogen-fixers	0.00	4, 3	0.966
	<i>Lotus micranthus</i>	4.37	3, 2	0.037 *
	Other Nitrogen-Fixers	2.96	4, 3	0.085 †
2005	Nitrogen-fixers	0.88	4, 3	0.349
	<i>Lotus micranthus</i>	16.71	3, 2	<0.001 ***
	Other Nitrogen-Fixers	2.31	4, 3	0.129
2006	Nitrogen-fixers	NA	NA	NA
	<i>Lotus micranthus</i>	NA	NA	NA
	Other Nitrogen-Fixers	NA	NA	NA
2007	Nitrogen-fixers	0.71	4, 3	0.399
	<i>Lotus micranthus</i>	4.59	4, 3	0.032 *
	Other Nitrogen-Fixers	4.89	4, 3	0.027 *
2008	Nitrogen-fixers	3.50	4, 3	0.062 †
	<i>Lotus micranthus</i>	7.80	4, 3	0.005 **
	Other Nitrogen-Fixers	0.23	4, 3	0.630
2009	Nitrogen-fixers	57.25	3, 2	<0.001 ***
	<i>Lotus micranthus</i>	89.92	3, 2	<0.001 ***
	Other Nitrogen-Fixers	0.08	4, 3	0.7831
2010	Nitrogen-fixers	1.46	4, 3	0.227
	<i>Lotus micranthus</i>	9.61	4, 3	0.002 **
	Other Nitrogen-Fixers	9.03	4, 3	0.003 **

† 0.05 ≤ P ≤ 0.10, * 0.01 ≤ P ≤ 0.05, ** 0.001 ≤ P ≤ 0.01, *** 0.0001 ≤ P ≤ 0.001

4.4.2 Plant production

Aboveground net primary productivity

Early in the Angelo experiment (2001-2003), there was a highly significant effect of watering treatment on plot ANPP. By 2004, watering treatment showed a marginally significant effect on plot ANPP. Water-addition plots had higher ANPP than water-control plots in these years. 2005 saw no significant effect of watering treatment on plot ANPP, however this effect returned as significant from 2006-2010, in the same direction as before (Figure 4.7, Table 4.2).

Functional group-specific productivity

Watering treatment had a significant effect on annual grass production in experimental plots from 2001-2006. This effect was marginally significant in 2007. Water-addition plots had higher annual grass production than water-control plots in all these years. From 2008-2010 there was no significant effect of watering treatment on annual grass production in experimental plots (Figure 4.8a, Table 4.3).

Watering treatment showed a significant effect on nitrogen-fixer production in every modelled year except 2007, with water-addition plots showing higher nitrogen-fixer production than water-control plots (Figure 4.8b, Table 4.3).

Near the beginning of the experiment in 2002, there was a significant effect of watering treatment on perennial forb production. Water-addition plots showed higher perennial forb production than water-control plots in this year. In 2003, this effect was marginally significant but the direction had reversed: water-addition plots showed lower perennial forb production than water-control plots. No significant effects on perennial forb production were then seen until 2010, where again water-addition plots showed lower perennial forb production than water-control plots (Figure 4.8c, Table 4.3).

Unlike perennial forbs, there was no effect of watering treatment on annual forb production in either of the first two years of the experiment. By 2003 there was a marginally significant effect of watering treatment on annual forb production, and in 2005 this effect was significant. In both years, annual forb production was lower in water-addition plots. This effect was not apparent later in the experiment (Figure 4.8d, Table 4.3).

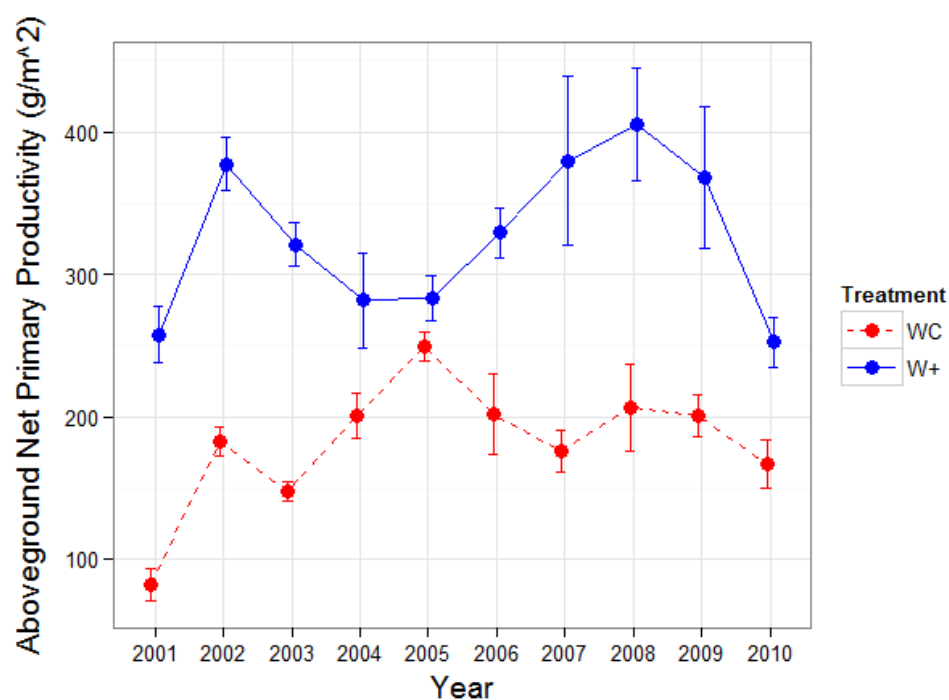


Figure 4.7: Effects of water-addition on aboveground net primary production (ANPP). Data represent mean dry mass in $\text{g/m}^2 \pm 1$ standard error for each treatment application, from 2001-2010. W+ denotes water-addition; WC denotes water-control.

Table 4.2: Effect of watering treatment on aboveground net primary production, calculated using conditional F-tests with Kenward-Roger approximated degrees of freedom on linear mixed effects models (df = 1, 5 for all models).

Year	F-value	P-value
2001	55.881	<0.001 ***
2002	282.324	<0.001 ***
2003	150.011	<0.001 ***
2004	5.433	0.067 †
2005	3.126	0.137
2006	13.775	0.014 *
2007	20.601	0.006 **
2008	21.047	0.006 **
2009	18.711	0.008 **
2010	13.570	0.014 *

† $0.05 \leq P \leq 0.10$, * $0.01 \leq P \leq 0.05$, ** $0.001 \leq P \leq 0.01$, *** $0.0001 \leq P \leq 0.001$

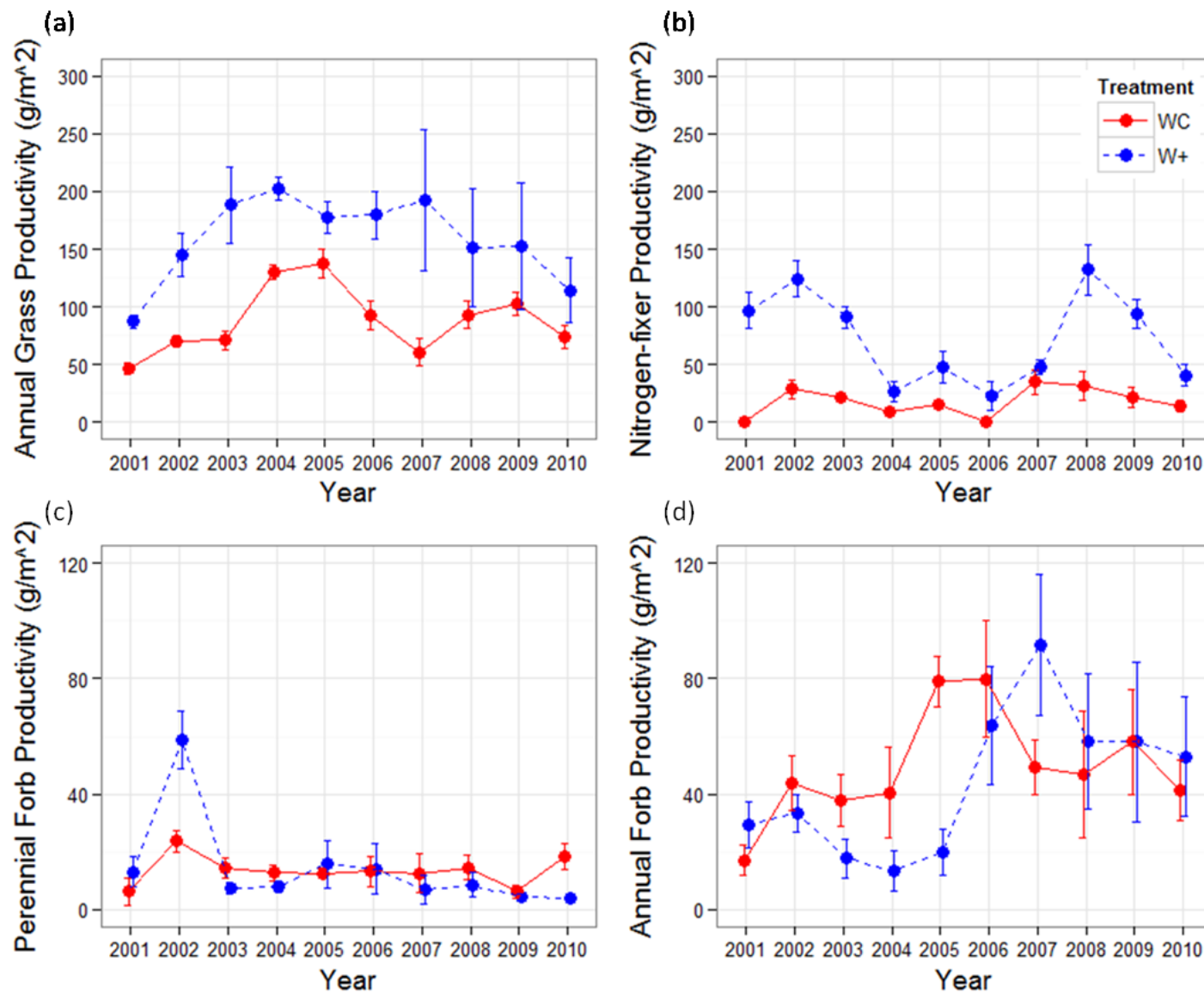


Figure 4.8: Effects of water-addition on functional group productivity: (a) Annual Grass; (b) Nitrogen-fixers; (c) Perennial Forb; (d) Annual Forb. Data represent mean dry mass in $\text{g/m}^2 \pm 1$ standard error for each treatment application, from 2001-2010. W+ denotes water-addition; WC denotes water-control. Note the difference in scale between the top and bottom row.

Table 4.3: Effect of watering treatment on the productivity of different functional groups, calculated using conditional F-tests with Kenward-Roger approximated degrees of freedom on linear mixed effects models (df = 1, 5 for all models).

Year	Functional Group	F-value	P-value
2001	Annual grasses	27.309	0.003**
	Perennial forbs	2.853	0.152
	Annual forbs	1.831	0.234
	Nitrogen-fixers	NA	NA
2002	Annual grasses	26.619	0.004**
	Perennial forbs	50.471	0.001***
	Annual forbs	1.117	0.339
	Nitrogen-fixers	91.878	<0.001***
2003	Annual grasses	18.830	0.007**
	Perennial forbs	5.038	0.075†
	Annual forbs	6.292	0.054†
	Nitrogen-fixers	69.944	<0.001***
2004	Annual grasses	50.984	<0.001***
	Perennial forbs	1.510	0.274
	Annual forbs	2.486	0.176
	Nitrogen-fixers	7.418	0.042*
2005	Annual grasses	13.276	0.015*
	Perennial forbs	0.495	0.513
	Annual forbs	25.572	0.004**
	Nitrogen-fixers	15.997	0.010*
2006	Annual grasses	96.905	<0.001***
	Perennial forbs	0.283	0.618
	Annual forbs	0.302	0.607
	Nitrogen-fixers	15.796	0.011*
2007	Annual grasses	6.032	0.058†
	Perennial forbs	1.040	0.355
	Annual forbs	2.624	0.166
	Nitrogen-fixers	1.894	0.227
2008	Annual grasses	1.171	0.329
	Perennial forbs	1.384	0.292
	Annual forbs	0.124	0.740
	Nitrogen-fixers	14.551	0.012*
2009	Annual grasses	0.188	0.683
	Perennial forbs	0.216	0.661

Continued...

Year	Functional Group	F-value	P-value
2010	Annual forbs	0.000	0.999
	Nitrogen-fixers	25.673	0.004**
	Annual grasses	1.672	0.253
	Perennial forbs	12.083	0.018*
	Annual forbs	0.252	0.637
	Nitrogen-fixers	6.686	0.049*

† 0.05 ≤ P ≤ 0.10, * 0.01 ≤ P ≤ 0.05, ** 0.001 ≤ P ≤ 0.01, *** 0.0001 ≤ P ≤ 0.001

4.4.3 Plant diversity

Early in the experiment in 2001 and 2002, there was a significant effect of watering treatment on plant diversity in experimental plots. Water-addition plots showed higher levels of plant diversity than water-control plots in both these years. There was no significant effect of watering treatment in 2003. By 2004, a significant effect of watering treatment on plant diversity in plots was again apparent, however the direction of this effect had reversed: water-addition plots showed lower levels of plant diversity than water-control plots. This same effect was seen in all years thereafter with significance or marginal significance, except in 2006 (Figure 4.9, Table 4.4).

Table 4.4: Effect of watering treatment on plant diversity, calculated using conditional F-tests with Kenward-Roger approximated degrees of freedom on linear mixed effects models (df = 1, 5 for all models).

Year	F-value	P-value
2001	35.843	0.002 **
2002	13.474	0.014 *
2003	0.890	0.389
2004	19.406	0.007 **
2005	38.232	0.002 **
2006	1.680	0.252
2007	9.771	0.026 *
2008	4.593	0.085 †
2009	8.055	0.036 *
2010	44.328	0.001 **

† 0.05 ≤ P ≤ 0.10, * 0.01 ≤ P ≤ 0.05, ** 0.001 ≤ P ≤ 0.01, *** 0.0001 ≤ P ≤ 0.001

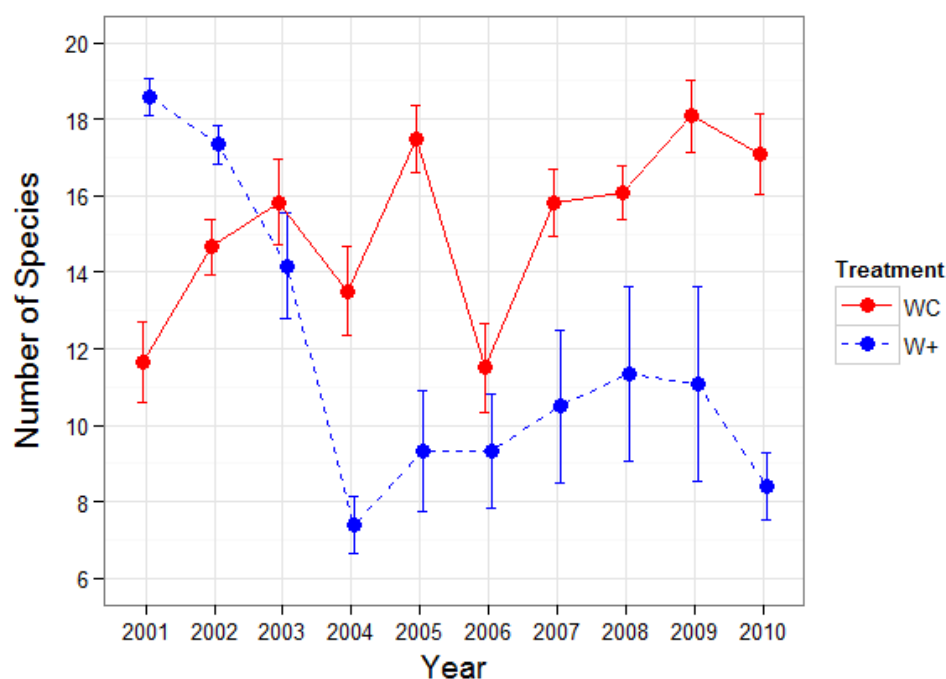


Figure 4.9: Effects of water-addition on plant diversity. Data represent mean number of species $\pm$ 1 standard error for each treatment application, from 2001-2010. W+ denotes water-addition; WC denotes water-control.

4.4.4 Soil and plant nitrogen concentrations

There was a significant effect of watering treatment on soil nitrogen content in 2007 and 2008. In both these years, soil nitrogen content was higher in water-addition plots than in water-control plots (Figure 4.10a, Table 4.5). There was a significant or marginally significant effect of watering treatment on the C:N ratio of *Bromus hordeaceus* in 2001, 2002, 2004 and 2006. In all these years, this C:N ratio was lower in water-addition plots compared with water-control i.e. the tissue-nitrogen content of *B. hordeaceus* was higher in water-addition plots (Figure 4.10b, Table 4.6).

Table 4.5: Effect of watering treatment on soil-nitrogen content, calculated using conditional F-tests with Kenward-Roger approximated degrees of freedom on linear mixed effects models (df = 1, 5 for all models).

Year	F-value	P-value
2006	0.922	0.381
2007	6.819	0.048 *
2008	11.703	0.019 *

+ $0.05 \leq P \leq 0.10$, * $0.01 \leq P \leq 0.05$, ** $0.001 \leq P \leq 0.01$, *** $0.0001 \leq P \leq 0.001$

Table 4.6: Effect of watering treatment on the C:N ratio of *Bromus hordeaceus*, calculated using conditional F-tests with Kenward-Roger approximated degrees of freedom on linear mixed effects models (df = 1, 5 for all models).

Year	F-value	P-value
2001	5.438	0.067 †
2002	180.202	<0.001 ***
2003	2.352	0.186
2004	41.637	0.003 **
2005	1.584	0.264
2006	16.520	0.010 **

† $0.05 \leq P \leq 0.10$, * $0.01 \leq P \leq 0.05$, ** $0.001 \leq P \leq 0.01$, *** $0.0001 \leq P \leq 0.001$

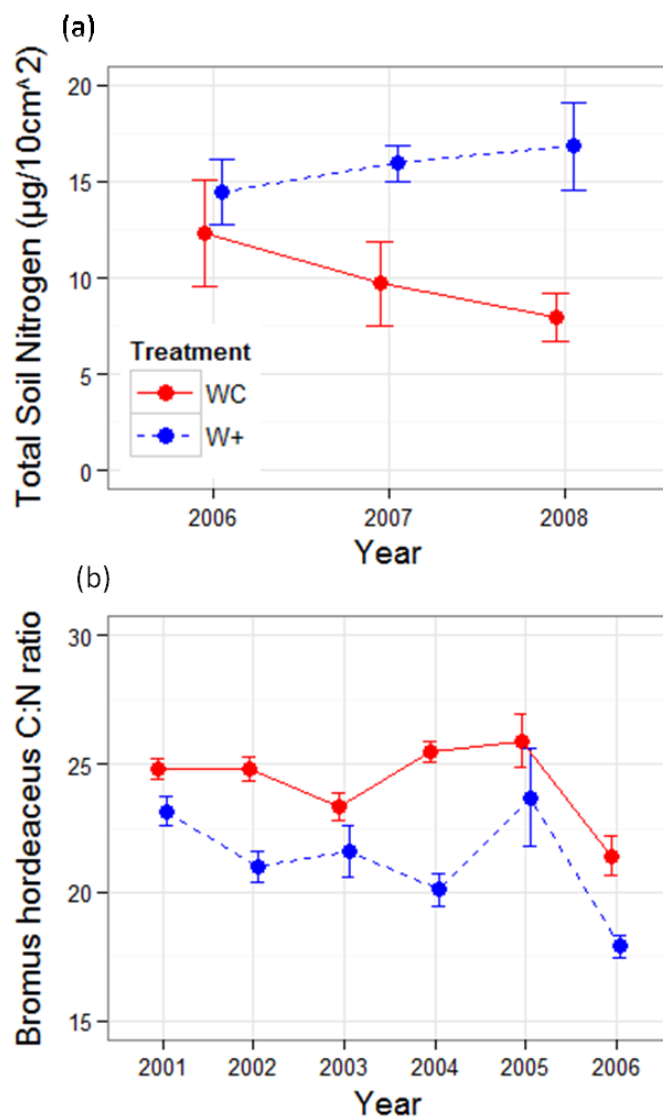


Figure 4.10: Effects of water-addition on (a) soil nitrogen content ($\mu\text{g}/10\text{cm}^2$); (b) the C:N ratio of *Bromus hordeaceus*. Data represent mean $\pm$ 1 standard error for each treatment application, for each year. W+ denotes water-addition; WC denotes water-control.

4.5 Discussion

Management of ecosystems under global change often deals in units of species presence or absence. Management plans are enacted when a species of particular conservation interest dips below a certain level of abundance for example, or when a population of an invasive species rises above a particular threshold level. As such, if the trait-based tools developed in earlier chapters could be turned to predicting abundance changes, they would be of greater practical relevance. The present chapter addresses this using previously unanalysed data from the Angelo Reserve Rainfall Addition Experiment. It tests whether changes in local plant abundances in response to alternative climate regimes can be understood in terms of shared traits. It also provides a more mechanistic test of the pathway of change for this system as proposed by the original researchers (Suttle and colleagues). Results indicate that, contrary to expectation, species-specific abundance responses to an experimental extension of the rainy season do not break down according to traits. They also indicate that, in contrast with hypotheses of previous researchers in the system, community reorganisation in response to this water addition was not driven by responses of nitrogen-fixers as a trait-linked group. In fact, the driver appeared to be one specific species of nitrogen-fixer: *Lotus micranthus*. Production, diversity and ecosystem nitrogen content data analysed here, however, do support the notion that these changes were likely driven proximally, by the process of nitrogen-fixation. As such, the mechanism of change observed in this system appears to be trait-linked, but enacted via the response of a single species.

Community Assembly theory predicts that traits may be more important determinants of community composition than species themselves (Kraft and Ackerly 2010). One might expect, therefore, that changes in species abundance under climate change might break down according to specific traits. This idea seemed a particularly logical expectation for the Angelo dataset analysed here, since previous researchers in this system had already described a strong production response to rainfall-extension by a particular trait-linked functional group: nitrogen-fixers (Suttle *et al.* 2007). Examination of treatment effects on the abundances of specific species however, countered these expectations. Those species that showed strongly positive responses to the rainfall-extension imposed in water-addition plots appeared to show no clear unifying traits. For example the deeply rooted native perennial grass, *Danthonia californica*, the shallowly rooted annual invasive grass, *Bromus diandrus*, and the native nitrogen-fixing forb, *Lotus micranthus*, all showed strongly positive abundance responses to rainfall-extension. It is hard to conceive of a set of traits that might unify these

species. Species abundance responses under this experimental manipulation, then, appear to be highly idiosyncratic, precluding formal comparative trait analyses and cautioning that trait-based predictions of these responses cannot be formulated.

One pertinent finding from these analyses with regards to the specific functioning of this system was the lack of consistency in the abundance changes of different nitrogen-fixing species in response to an extended rainy season. Following five years of dynamics in the Angelo Experiment, researchers highlighted nitrogen-fixers as the strongest positive respondents to an experimental extension of the rainy-season. This was based on observed production increases in this functional group over the course of the study (Suttle *et al.* 2007). They proposed that this nitrogen-fixer response drove subsequent changes in diversity, energy flow, and production throughout the food web (see earlier), effectively overturning the direct short-term, positive responses of most species in the system to rainfall-extension. Abundance data analysed here however, demonstrated that this nitrogen-fixer response was in fact attributable to one species alone: *Lotus micranthus*. Indeed in all study years, models of treatment effects on the abundance of *Lotus micranthus* demonstrate significantly higher abundance of this species in water-addition plots compared with water-control plots. Other nitrogen-fixers in the system generally showed negative abundance responses to the rainfall-extension imposed in water-addition plots across the timeframe of study. As such, the proposed driver of community reorganisation in this system was not nitrogen-fixers as a trait-linked group, but rather one specific species acting via nitrogen-fixation as a trait.

Failing to validate this aspect of Suttle and colleague's proposed mechanism of community reorganisation, I examined in more detail the rest of their proposed pathway. I took a specific focus on the role of nitrogen in driving this reorganisation since, as discussed earlier, nitrogen-fixation was proposed as the proximal driver of the changes that ensued in response to an extended rainy season. I analysed an additional 5 years of production and diversity data beyond that presented in Suttle *et al.* (2007), and also examined data on soil and tissue nitrogen content. In interpreting these results and their support for the proposed mechanism of change, I will consider each step in this mechanism in turn.

The present results, in demonstrating abundance increases in *Lotus micranthus* in response to an extension of the rainy-season, still indicate an important systemic role for nitrogen, as proposed by Suttle and colleagues. Functional group biomass results from recent years too, show that, consistent with the first three years of data already presented from this experiment, production of nitrogen-fixers was higher in water-addition plots than in water-control plots,

in 2006, 2008, 2009 and 2010. Suttle *et al.* (2007) proposed that this strongly positive response by nitrogen-fixers to rainfall-extension resulted in an abundance of nitrogen-rich litter at the end of each growing season. This litter was suggested to decompose at the onset of each subsequent rainy-season, resulting in increases in mineralised nitrogen in the soil. The soil nitrogen data analysed in this chapter support this proposition. Indeed soil nitrogen levels were higher in water-addition plots than in water-control plots in two of the three analysed study years.

At this early-season stage, only exotic annual grasses are active in the system. Resultantly, Suttle and colleagues proposed that the high mineralised nitrogen content of the soil under rainfall-extension conditions fuelled large increases in the biomass of exotic annual grasses, via a fertilisation effect. This resulted in high ANPP in water-addition plots. In support of this, the tissue-nitrogen content of a common exotic annual grass in this system (*Bromus hordeaceus*) was found to be higher in water-addition plots as compared to water-control plots in four of the six experimental years that were analysed. This indicates that some exotic annual grasses may indeed be taking advantage of the increased mineralised nitrogen content of the soil found under conditions of an extended rainy season. Furthermore, the high productivity of exotic annual grasses in water-addition plots that was reported for the first four years of the experiment was shown to persist in later years. Water-addition plots showed a significant or marginally significant increase in exotic annual grass production over water-control plots in all years from 2001-2007. Results also demonstrate that ANPP remained higher in water-addition plots in the more recent years of the experiment. These biomass results indicate that the increased nitrogen uptake by exotic annual grasses in water-addition plots is, as proposed, fuelling an increase in their productivity, although the idiosyncratic abundance data suggest that this response may be unique to just a few species of exotic annual grass.

As a result of increased exotic annual grass production, Suttle and colleagues then suggested that these grasses caused competitive suppression of broad-leaved forbs that had initially shown direct, positive responses to increased water availability. Indeed, the thatch of annual grasses is well known to suppress germination and regrowth of leafy forbs in California grassland systems (Bentley *et al.* 1958; Heady 1958; Hobbs *et al.* 1988; Huenneke *et al.* 1990). Plant diversity analyses provide some support for this. Plant diversity was higher in water-addition plots than in water-control plots in the first two years of the experiment, however by the fourth and fifth year, this effect had been reversed (Suttle *et al.* 2007). The results from

the present analysis show that these low levels of plant diversity persisted in water-addition plots in more recent years of the experiment.

Returning to the functional group biomass results, we can glean a little more information about the specifics behind this trajectory of plant diversity. Suttle *et al.* (2007) analyse the productivity of all forb species together, and show no significant treatment effects on forb production. The analyses presented in this chapter however, split forbs into separate annual and perennial groups. This split reveals that, in 2002, perennial forb productivity was higher in water-addition plots than in water-control plots. This could reflect that the early species richness increases seen under conditions of rainfall-extension were in part attributable to recruitment of perennial forbs that were responding directly to increased water availability. By 2003, the direction of this perennial forb response had switched, with perennial forbs showing lower productivity under such conditions. The same was true of annual forbs in this year, and in 2005. These results are in-keeping with the lower plant diversity observed in water-addition plots from 2004 onwards, and further support this final bit of Suttle and colleague's mechanism.

The results from these latter analyses confirm that, although the proximal driver of change in this system was a specific species of nitrogen-fixer and not nitrogen-fixers as a functional group, the mechanism that drove change was, as proposed by Suttle and colleagues, nitrogen-fixation as a trait. The trajectory of response that ensued in light of increased nitrogen availability appeared to consist of a complex series of indirect effects mediated by interactions between specific species.

These indirect effects may shed light on why the individual abundance responses of species discussed earlier were so idiosyncratic. Considering the ecology of California grasslands, responses to an extended rainy season might be expected to be predictable, and to break down according to particular traits. Specifically, California grasslands are heavily invaded by Eurasian exotic species (Heady 1977). Resultantly, they have suffered precipitous declines in native species over the last 150 years. It is only along the north coast of the state that many natives have been able to persist in large populations alongside these invaders (Hektner and Foin 1977; Hayes and Holl 2003). Native success in this region is most commonly attributed to its longer rainy-season relative to the rest of the state (Elliott and Wehausen 1974; Heady *et al.* 1977, 1992; Stromberg *et al.* 2001). The Mediterranean origin of the most important exotic species in the area has selected for a reproductive phenology that is tied to winter rainfall. These exotics are thus relatively unresponsive to late-season rainfall (Pitt and Heady

1978; Jackson and Roy 1986). By contrast, California native species are less hard-wired in their reproductive phenology, and thus can take better advantage of late rains to increase growth and produce larger seeds in greater numbers (Laude 1953; Jackson and Roy 1986). As such, late rains generally favour California natives relative to their non-native competitors. If this system were responding directly to the imposed water manipulation then, greater success would be expected for those species native to California over any exotic annual grasses in this system. In actuality, indirect effects in this system have overturned these direct responses, meaning that the expected results did not manifest: some exotic annual grasses showed strongly positive responses to the extended rainy-season in water-addition plots, indirectly mediated via the response of the nitrogen-fixer, *Lotus micranthus*.

The pervasiveness of indirect effects in this system may be attributable to the higher levels of biotic and abiotic idiosyncrasy present at such local scales (Wiens 1989), and makes sense in the knowledge that at these scales, an increasing number of factors may come to determine (and complicate) the responses of species to climate change (e.g. Pearson *et al.* 2004; Janowski *et al.* 2010). In light of our understanding of these indirect effects then, it perhaps makes sense that the changes species show in this system do not break down according to shared traits, and in fact show high levels of inter-species idiosyncrasy. These results indicate that, in line with expectations from Davis *et al.* (1998) and Petchey *et al.* (1999), considering the direct effects of climate alone can lead to spurious and misleading projections about how systems will change in response to it.

Overall, this chapter has provided clear evidence that the abundance responses of species to climate change at local levels are highly idiosyncratic and, in this system, do not break down according to traits. This cautions as to the value of trait-based predictions at local scales. It has made clear that the community reorganisation observed by Suttle and colleagues in their experimental system is proximally attributable to one specific species of nitrogen-fixer (*Lotus micranthus*), and is not driven by the response of this trait-linked functional group as a whole. That being said, analyses provide solid evidence to suggest that nitrogen-fixation was indeed the mechanism by which community reorganisation was driven in this system, mediated by the positive response of *Lotus micranthus* to an extension of the rainy season. This nitrogen mechanism drove a cascade of indirect responses to the rainfall manipulation, resulting in the reductions in plant diversity observed by Suttle and colleagues in 2007, and in the more recent data presented here. Despite a lack of the patent trait-unified responses that I expected for this system then, there does appear to be a clear mechanism of change that is resolved to a

single proximal trait driver. This knowledge could hold value for managing the responses of systems such as this to climate change in the future.

Chapter Five

Functional traits as mediators of community level responses to climate change

5.1 Abstract

Interactions between species can generate unforeseen complexities in the way that populations respond to a change in climate. These “indirect effects” of climate change challenge efforts at predicting future responses of species. It is possible that if we can identify key indirect effects that most strongly govern system dynamics, we may be able to simplify interactions into tractable terms that can improve predictive understanding. This chapter designs and implements an experiment to ask whether indirect effects throughout a community under precipitation manipulation can be resolved to the level of a single major driver. Furthermore, it assesses whether, if by selectively removing this driver, we can reduce the role of indirect relative to direct effects in community responses. The experiment is intended to run for five years in total, with results through the first three years presented here. With allowances for potential strengthening or weakening of inferences in the coming years, the results at three-years indicate that a single, trait-based driver does indeed play a crucial role in responses to rainfall manipulation throughout the community. Furthermore, they suggest that removal of this driver from the system leads to at least partial reversal of long-term changes in production and diversity.

5.2 Introduction

A likely trade off exists between the reliability of predictions around how species will respond to climate change and the spatial scale at which these predictions are made. As we narrow our focus from the scale of species ranges to the scale of individual sites, an increasing number of factors are likely to play a role in how a species responds to the changing climate (Pearson and Dawson 2003). Range-level shifts often accord with physiological tolerances to climate itself (e.g. Lindgren *et al.* 2000; Sobrino *et al.* 2001; Sanz-Elorza *et al.* 2003). More local-scale changes within species' ranges however, are shaped by many factors, such as habitat type, suitability or availability (Scheel *et al.* 1996; Pearson *et al.* 2004), human density (White and Kerr 2006), phenotype (Skelly and Freidenburg 2000; Skelly 2004; Pelini *et al.* 2009), and interactions with other species (Klein *et al.* 2004; Menge *et al.* 2009; Jankowski *et al.* 2010). These ecological interactions may be particularly impactful and complicating influences upon local scale responses.

Interactions complicate the responses of species to climate change because they create indirect effects. Specifically, because organisms interact in numerous ways in nature, the direct physiological response a species could have to climate may not translate clearly into effects at the level of communities, due to the ways that climate change affects those communities (e.g. reducing resources or increasing competitors and enemies). Actual responses therefore, can be contingent upon a multitude of intra- and inter-trophic level interactions (Harrington *et al.* 1999; Tylianakis *et al.* 2008). Consequently, the trajectory any species or community takes under climate change may be quite different from what we'd expect by considering only direct responses to climate (Davis *et al.* 1998; Petchey *et al.* 1999). Interactions and indirect effects may be particularly impactful at local scales. Where interactions are relevant at the level of species ranges, they can generally be defined in terms of presence or absence of a critical host species or a particular natural enemy (Pearson and Dawson 2003; Merrill *et al.* 2008; Schweiger *et al.* 2008). At site-level, interactions are generally more apparent and complex, with changes in climate affecting species through effects on resources, competitors, mutualists, consumers, or pathogens (e.g. Schiel *et al.* 2004, Biro *et al.* 2007). Accounting for biotic interactions in predictions of local-scale responses to climate change is imperative for understanding the ultimate consequences of this climate change.

The importance of these interaction-driven indirect effects has now been demonstrated in ecosystems throughout the world: fungus and beetles have driven forest responses to milder winters in western North America (Woods *et al.* 2005 and Berg *et al.* 2006 respectively), pathogens and parasites have driven lion responses to drought in the Serengeti (Munson *et al.* 2008), and phytoplankton have driven zooplankton responses to warming temperatures in lakes of the US Pacific Northwest (Winder and Schindler 2004). Such studies have been able to retrospectively disentangle the indirect drivers of responses to climate change in these systems. Projecting these responses into the future however, is more challenging. As community composition changes with climate, new interactions among species are formed and further chains of indirect effects are generated (Burns *et al.* 2003). Ecologists thus face considerable challenges when making predictions about the local-scale responses of species to climate change. Predictive models that do not take into account ecological interactions between species can lead to spurious and misleading results (Davis *et al.* 1998), however identifying and accounting for all possible influential interactions is a near impossible feat.

One solution to this problem could be to identify key indirect effects that most strongly govern system dynamics. As such, the ecological complexity these effects generate could be simplified into tractable terms. For certain communities, this may come in the form of keystone species that singularly govern system trajectories (Paine 1966, 1969). However such instances are likely to be a small minority of total cases (Power *et al.* 1996). More generally, it may be possible to simplify community dynamics into interactions among key groups assorted by traits or trophic level (Berg *et al.* 2010), or among a small number of particularly influential or representative species (Gilman *et al.* 2010). Such simplifications would not provide for detailed predictive understanding for a community of species, but may sufficiently capture dynamics that allow for predictive understanding of aggregate properties or functions, like diversity or production. Theoretically, knowledge of key drivers of change could also be of value in managing for the consequences of climate change in ecological communities. Absent preventing climate change itself, we may still prevent many negative consequences of it if we can pre-empt major surprises and unforeseen regime shifts by identification and management of key drivers of indirect effects. A useful focus in climate change research should thus be identifying these key drivers of indirect effects, and evaluating management options for them.

This chapter describes experimental research into identifying and managing indirect effects of climate change via the identification and management of their key drivers. It asks whether

indirect effects throughout a community can be resolved to the level of a single major driver, and whether by targeting this driver we can reduce the role of indirect relative to direct effects in a community under climate change. This research is carried out within the context of a long-running rainfall manipulation in northern California. The Angelo Reserve Rainfall Addition Experiment demonstrated the pervasiveness of indirect effects in the responses of species to climate change, by showing how such effects can reverse trajectories in diversity and food chain length initiated by direct effects (Suttle *et al.* 2007). The previous chapter identified a strong role for nitrogen-fixation in these changes, likely mediated by the effects of climate on the nitrogen-fixing forb *Lotus micranthus*. Specifically, changes in seasonal water availability initially resulted in strongly positive, direct responses by many species in the system, particularly *Lotus micranthus*. Resulting increases in mineral nitrogen levels apparent in leaf tissues and soils drove increased exotic annual grass production. The thatch of annual grasses is well known to suppress germination and regrowth of leafy forbs in California grassland systems (Bentley *et al.* 1958; Heady 1958; Hobbs *et al.* 1988; Huenneke *et al.* 1990). As such, the increased exotic annual grass production was thought to lead to the observed reductions in species diversity in the system. The present study tests the role of climatic effects on nitrogen-fixation in driving system dynamics via a controlled removal of this trait from experimental communities. It asks whether removal of this major driver that fuelled and likely maintains the changes in this community actually reverses the changes themselves. In doing this, it also provides further empirical testing of the mechanisms for community change proposed in Suttle *et al.* 2007 and broadly supported in Chapter 4.

The specific hypotheses to be tested are as follows:

- 1) Removal of nitrogen-fixing forbs will reduce aboveground net primary productivity (ANPP) in water-addition plots, beyond that amount accounted for by nitrogen-fixer biomass itself (as the nitrogen fertilisation effect attenuates).
- 2) Removal of nitrogen-fixing forbs will increase species diversity in water-addition plots (as the decreased overall production relaxes competitive dominance and allows leafy forbs to re-establish).

This study represents, to my knowledge, the first empirical test of the mechanistic importance of a key driver of indirect effects in a system subject to a climate manipulation. It is also the first attempt at unwinding chains of indirect effects of experimental climate change by targeting not climate itself, but the first shared link in these chains.

5.3 Methods

5.3.1 Experimental design

As previously discussed, the experiment described in this chapter was developed in the context of a longer-term experiment that has been running continuously since 2001 (Suttle *et al.* 2007). This longer term experiment tests consequences of alternative scenarios of precipitation change projected for Northern California by the Hadley Centre for Climate Prediction and Research (HadCM2) and the Canadian Centre for Climate Modelling and Analysis (CCM1) for grassland production and species composition. Briefly, thirty-six circular plots ($\sim 70\text{m}^2$ each) have been subjected to one of three precipitation treatments each year, in a randomised block design:

- A wintertime addition of water (January through March) to simulate the Hadley model's proposed intensification of the Californian rainy season.
- A springtime addition of water (April through June) to simulate the Canadian model's proposed extension of the Californian rainy season.
- An un-manipulated ambient control.

Sampling of plant and invertebrate communities was restricted to 18 of these plots (*i.e.* 6 replicates in each of 3 precipitation treatments). The remaining 18 were left undisturbed except for annual applications of the precipitation treatments.

The present manipulation was undertaken in the six replicates of the springtime-addition treatment (hereafter water-addition treatment) and the six replicates of the control treatment. Wintertime-addition plots did not factor into the design, because there were no conspicuous direct or indirect effects reported for this treatment by Suttle *et al.* (2007). The experiment implements orthogonal treatment combinations of extended seasonal rainfall, nitrogen-fixer-removal, and their respective controls, using a split-plot design. The pre-existing design of the watering experiment had watering treatments replicated into blocks along a north-south gradient through the meadow. This accounted for background patterns in perennial grass abundance, shading, and any other factors that could influence plant productivity, diversity and composition in plots. Resultantly, my twelve experimental plots were arranged into six blocks in a randomised complete block design (Figure 5.1).

In 2010, I established plots for a nitrogen-fixer-removal experiment (hereafter NITROX experiment), imposed on the template of the watering experiment. I established two square 4m² subplots within each water-addition and water-control plot. These were marked out at the corners with PVC pipe in adjacent quadrants of the larger watering plots. I randomly assigned each subplot as either “nitrogen-fixer-removal” or “nitrogen-fixer-control”. Subplots within the larger water-addition plots were positioned to the inside of a 1m-wide buffer ring from the outside of the watering radius, to ensure that the entire subplot surface area was safely within this radius. The split-plot design thus consisted of six plots in each of four treatments in total: a water-addition/nitrogen-fixer-removal (shorthand W+N-); a water-addition/nitrogen-fixer-control (W+NC); a water-control/nitrogen-fixer-removal (WCN-); and a water-control/nitrogen-fixer-control (WCNC) (Figure 5.2).

5.3.2 Watering treatments

The watering treatments in this study are exact continuations of the springtime-addition (water-addition) and control treatments that have been applied since 2001. Water-addition plots receive approximately 44cm of water over ambient precipitation each year, from April through June (*i.e.* a simulated extension of the rainy season). This is delivered in increments of 14cm to 16cm two hours after dawn every three days, regardless of ambient weather. This water is collected from a mountain spring to the south east of the study meadow, and has been shown to contain nitrogen concentrations within the range of that naturally present in rainwater at the site (Suttle *et al.* 2007). This water is delivered via a RainBird® RainCurtain™ sprinkler present at the centre of each water-addition plot, over a radius of 5m.

5.3.3 Nitrogen treatments

The nitrogen-fixer treatments were initiated in May 2011 and maintained across the growing season over three consecutive years. Each year in late May, all nitrogen-fixing plants (technically, plants associated with nitrogen-fixing bacteria within specialised nodules in their roots) were removed by hand at the soil surface. Follow-up checks and removal of any newly established tissues were carried out in early July and again in late August. All nitrogen-fixers in the study system were removed. These included several species of clover

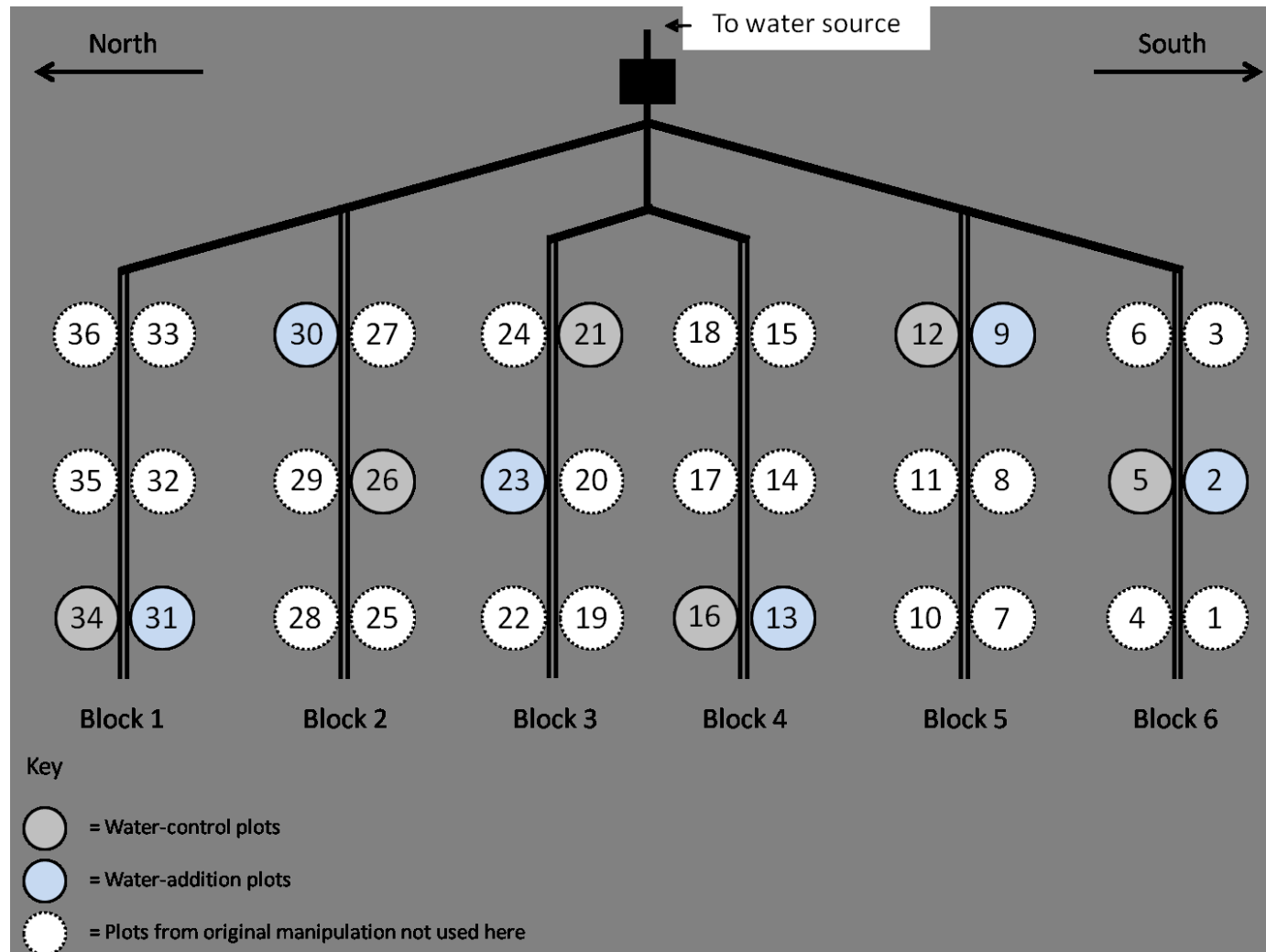


Figure 5.1: Schematic representation of the randomised complete block design of the NITROX experiment. Each block contains a water-addition and a water-control plot, both containing a nitrogen-fixer-removal plot, and a nitrogen-fixer-control plot. Note that these are different plots from those for which data were analysed in the previous chapter. Schematic is not to scale.

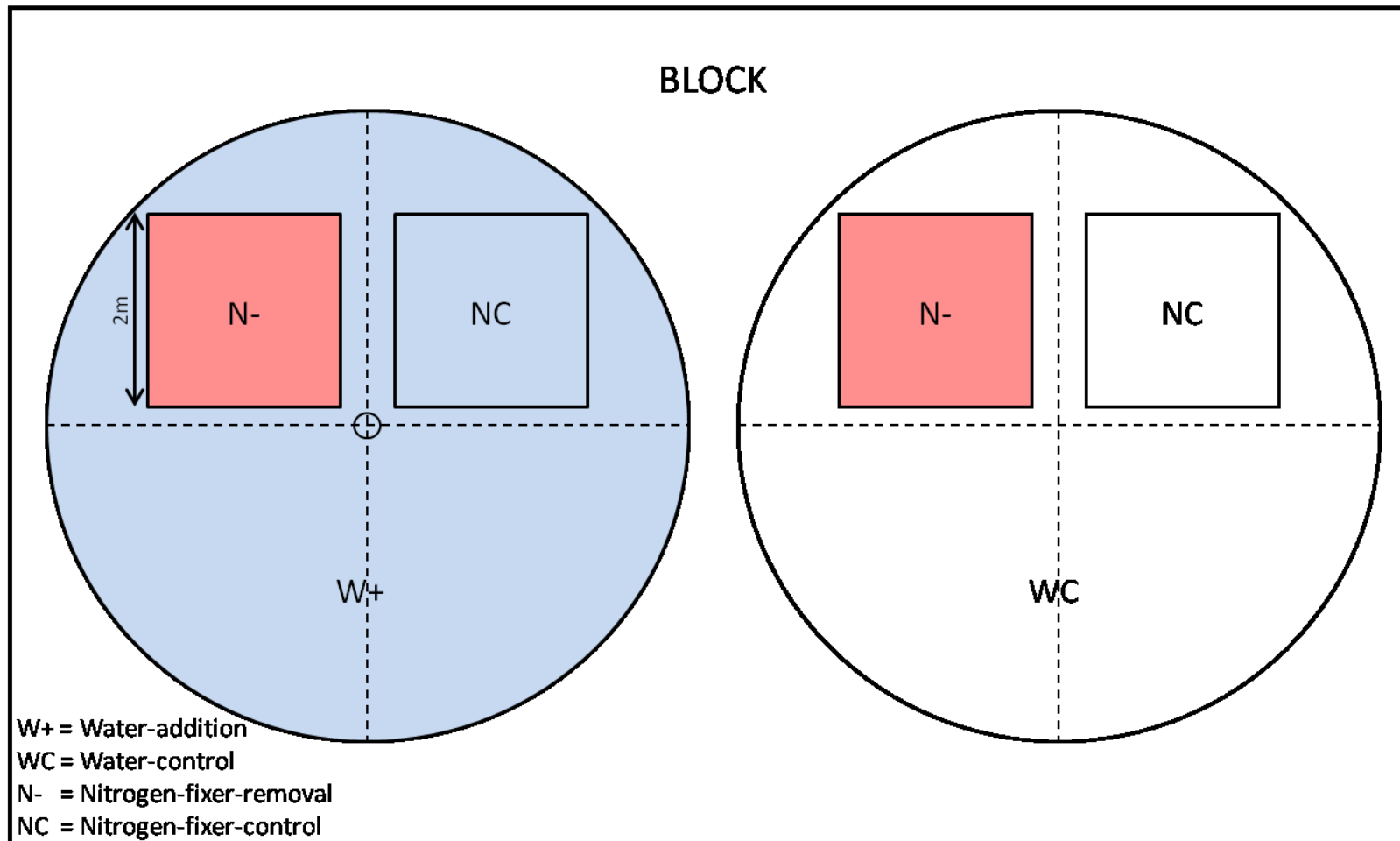


Figure 5.2: Schematic representation of the split-plot design of the NITROX experiment. The largest area represents a single block in the experiment. The two circles represent the water-addition (W+) and water-control (WC) plots. The two squares within each circle represent the nitrogen-fixer-removal (N-) and nitrogen-fixer-control (NC) plots. Schematic is not to scale.

(*Trifolium purpurea*, *Trifolium microcephalum*, *Trifolium albopurpureum*, and *Trifolium wildenovii*), one lupine (*Lupinus bicolor*), and vetch (*Lotus micranthus*). As shown in Chapter 4, *Lotus micranthus* most likely drove the indirect effects being investigated via nitrogen-fixation. In order to test this mechanism however, all nitrogen-fixing species need to be removed to stave off potential compensatory responses that would confound focal tests. Nitrogen-fixer-removal extended over the entire surface area of each nitrogen-fixer-removal plot, and through a 0.10m buffer strip on all sides. This avoided nitrogen “leakage” into plots.

5.3.4 Data collection

For each treatment plot, plant production, diversity and relative abundance were measured in pre-designated subplots in 2011, 2012, and 2013 (Figure 5.3).

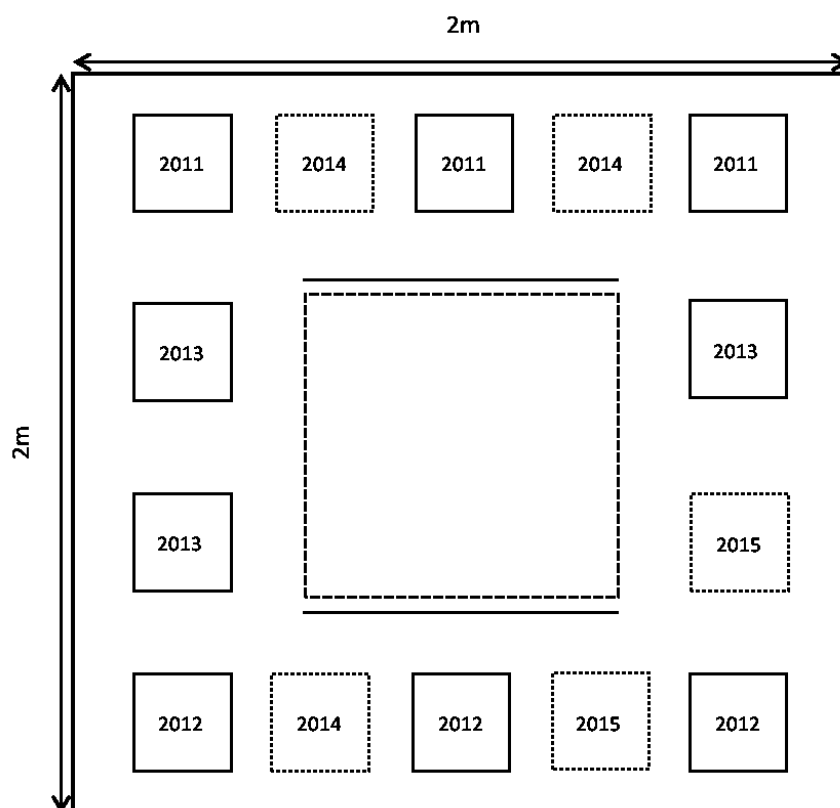


Figure 5.3: Schematic representation of an experimental plot. Small squares represent positioning of the three 900cm² biomass clips for each year. Dashed smaller squares represent the position of future clips. The final 2015 clip will be collected from the centre of the plot, after the conclusion of richness sampling in September 2015. The central dashed square represents the position of the permanent 2500cm² species diversity subplot. The solid lines above and below the species diversity subplot represent the position of the two pointframe samples used to measure relative species abundance. Schematic is not to scale.

Plant production

An important feature of the diversity of California grasslands is phenological diversity, or diversity through time. Distinct guilds of plants emerge into the system at different times during the overall growing season, with some reaching their peak biomass in late May, others in early July, and others in early September. Prior to initiation of the experiment, all plant species in the study system were categorised into one of eight functional and phenological groups: nitrogen-fixers, perennial forbs, perennial grasses, spring annual grasses, spring annual forbs, summer annual grasses, summer annual forbs, and late-summer annual forbs. Aboveground net primary production (ANPP) was estimated from a seasonal sequence of biomass measurements targeting these different plant groups at their annual peak production. Biomass was sampled with one-off harvests of all aboveground plant material within a 900cm² subplot, after which that subplot was excluded from future measurements. Samples were taken on or around 25 May, 2 July, and 1 September each year, timed to coincide with the peak biomass of the different groups such that all plant species could be sampled at their annual maximum. Vegetation was sorted according to functional and phenological group, placed into small paper bags, and dried in an oven at 70°C for 48 hours. Each plant group was weighed separately, and the biomass of each group at its peak was added together to derive an estimate of ANPP. Nitrogen-fixers were excluded from this calculation for all treatments, so that ANPP estimates would not be biased high in nitrogen-fixer-control plots relative to nitrogen-fixer-removal plots. The phenological subgroups for each functional group were also summed at their peak, to create biomass sums for each major functional group in each year (annual grasses, perennial grasses, annual forbs and perennial forbs). ANPP sums and individual functional group measures were converted to units of grams per square metre.

Plant diversity

Plant diversity was measured by visual inspection of a permanent 2500cm² subplot in the centre of each 4m² plot, on the same seasonal schedule as laid out for production measurements. This subplot was marked by metal pins at its corners. A PVC sampling frame was placed over these pins to clearly delineate the sample from the surrounding plot. No other work was conducted within this subplot to ensure that it remained untouched by observer interference over the course of the experiment. Total plant diversity was calculated for each sampled plot as the sum of all unique species identified across a particular year, with

nitrogen-fixers again excluded to avoid introduction of systematic biases among treatments. In the same way, diversity sums were calculated for two broad functional groups: forbs and grasses.

Plant abundance

The relative abundance of plant species was measured by point-frame sampling. This gave an indication of plant species composition for each plot. Specifically, a wooden frame is anchored at set locations within each plot and pins dropped from a suspended crossbar, vertically to the ground surface. The sampling device consists of 8 pins at 5cm intervals. Data were recorded as the first plant species a pin contacted as it was lowered into the vegetation, the plant species touching the pin at a marked height of 5cm above the ground surface, and the plant species on or in which the pin landed at the ground surface. This point-frame sample provides data in the form of 24 distinct “hits” through the plant assemblage, per sampled subplot. A minimum of 2 permanent subplots were sampled in this manner on or around 25th May each year, coinciding with the period of peak plant diversity in the study system (i.e. the period when most plant species that *will* be present in a plot in a given year *are* present, at some stage of their phenological development). As with the plant diversity subplot, these pointframe subplots were otherwise untouched throughout the experiment.

5.3.5 Statistical analyses

I designed the NITROX experiment to run for five years. This was to account for natural history based expectations of effect sizes and timescales, and for sample size constraints imposed by the structure of the larger watering manipulation in which the experiment is embedded. Analyses presented here are based on the first three years of collected data.

California grasslands are notably dynamic in their production, diversity and composition. Composition and production vary year to year based on the specifics of climate, and in particular the timing and amount of precipitation: successional dynamics largely absent (Pitt and Heady 1978, Stromberg and Griffin 1996, Hobbs *et al.* 2007). ‘Ambient’ conditions, as represented by control plots, therefore entail a high degree of interannual variability in all measured variables. Given this, a linear trajectory of response over time would not be expected in this system. Responses for each year are thus modelled separately. All analyses were performed in R 3.0.2.

Univariate responses

Production data were analysed using linear mixed effects models in the R package *lme4* (Bates *et al.* 2013). ANPP was used as the dependent variable, followed by annual grass, perennial grass, annual forb, and perennial forb production in turn. A Gaussian error distribution was specified. Watering treatment and nitrogen treatment were fixed effects. Watering treatment was also fitted as a random effect, nested within block. The following R syntax was used:

Production ~ Watering Treatment * Nitrogen Treatment + (1|Block/Watering Treatment)

In order to test the main effects of each model term and the effects of their interaction, the R package *afex* (Singmann 2013) was used to calculate sum to zero contrasts using conditional F-tests with Kenward-Roger approximated degrees of freedom. Any interaction terms with $P \geq 0.1$ were removed from models, but further model simplification was not performed due to the many problems inherent to stepwise multiple regression modelling (e.g. parameter estimation bias; model selection algorithm inconsistencies; inappropriate focus on a single “best” model (Whittingham *et al.* 2006)). If watering treatment and/or nitrogen treatment showed a significant main effect, or there was a significant interaction between these treatments, the R package *lsmeans* (Lenth 2013) was used to calculate pairwise differences between least-square means for different treatment combinations, again using Kenward-Roger approximated degrees of freedom. Perennial grass, perennial forb and annual forb response data for all years were log-transformed to improve residual distributions. In spite of these attempts there were some uncorrectable distribution issues in the 2011 model for perennial grass production. Results from this one model should be treated with caution.

Plant diversity data were analysed in the same manner as production data. Total plant diversity, forb diversity (not including nitrogen-fixing forbs) and grass diversity were all analysed as dependent variables in turn. Although data are in the form of counts, a Gaussian error distribution fitted these data much better than a Poisson error distribution, so Gaussian models were used. Grass diversity sums were logged for all years of the NITROX experiment to improve residual distributions. Distributional issues remained for the 2012 grass diversity model. Results from this model should be treated with caution.

For each univariate response, analyses were repeated for baseline plot data from 2010, using watering treatment as a fixed effect and block as a random effect. This provided a baseline indication of the state of the system prior to implementation of the NITROX experiment.

Multivariate responses

To investigate compositional similarities between plots, plant abundance data from pointframe sampling were analysed using non-metric multidimensional scaling (NMDS). This is a multivariate ordination approach that makes no assumptions about the distribution structure of the underlying data, and so is well suited for analyses of community-level proportion data in Ecology (Peck 2010). Resulting ordinations express overall patterns of similarity and dissimilarity in species composition, based on distances among plots.

Prior to analysis, point-frame hits were converted to proportions of total hits for each species, in each plot, in each year of sampling. Species below a defined rarity threshold were omitted. Specifically, species present in only 1, 2 or 3 total study plots *and* at proportions in those plots of ≤ 0.0625 were discarded. This ensured that biases of plot to plot similarity based on shared *absence* of rare species were not introduced, such that similarity was based predominantly on shared *presence* of species and common patterns of non-zero abundance. Hits for nitrogen-fixers, moss, litter and bare ground were not included in proportion calculations.

Using this proportion data, the function *isoMDS* in the R package *Vegan* (Oksanen *et al.* 2013) was used to calculate an optimal yearly 2-dimensional ordination solution out of 999 runs, based on Bray-Curtis distance measures. All final 2D solutions had low stress (< 0.2 for each year) and thus additional axes were not useful. To visualise these patterns of similarity and dissimilarity, the *Vegan* function *metaMDS* was used alongside package *ggplot2* (Wickham and Chang 2013) to plot the ordination for each year. An ellipsis representing each treatment's 95% confidence standard error limits ($\pm 1.96SE$) was added to each plot using the *Vegan* function *ordiellipse*.

To test for treatment-based differences in these patterns of species composition, distance-based MANOVA (PerMANOVA) analyses of the main effects of the two treatments and their interaction were carried out, using the *Adonis* function in *Vegan*. A Bray-Curtis distance measure was used. As with the mixed model methods, interaction terms showing less than marginal significance ($P=0.1$) were removed from models, but further simplification was not carried out.

Traditional MANOVA is not appropriate here for two reasons: firstly, it models relationships among data points using Euclidean distances, which have been shown to perform poorly with community data (Faith *et al.* 1987; McCune & Grace 2002); secondly, it assumes

multivariate normality, which is not appropriate for the binomial nature of the presence/absence community data being modelled (Anderson 2001, McCune & Grace 2002). By contrast, PerMANOVA is compatible with any distance measure and with non-Gaussian data. The main weakness of a PerMANOVA approach with respect to the present data is that there is no implementation that can account for the nested error structure from the employed split-plot design, and so water-addition and nitrogen-fixer-removal treatments are treated within *Adonis* as independent. Analyses can, however, at least be stratified by block.

All multivariate analyses were repeated for baseline data from 2010, using watering treatment as the explanatory variable and stratifying PerMANOVA analyses by block.

5.3.6 Multiple comparisons

Given the multiple year by year statistical tests performed in this Chapter (and indeed the multiple comparisons performed throughout the rest of this thesis), it is worth mentioning issues associated with these kinds of multiple comparisons. When undertaking several tests at the same P-value (α), the probability of achieving one statistically significant result is inflated above that of the significance level (Zaykin *et al.* 2002). As such, there is an increased probability of Type I error (Moran 2003). Some researchers contend with this by lowering the α -value and so reducing the Type I error rate (e.g. sequential Bonferroni corrections; Benjamini-Hochberg procedures) (Moran 2003). The more variables tested in a particular study under such corrections however, the less likely one is of obtaining statistical significance (Moran 2003). This can lead to publication bias towards simplified interpretations of ecological systems and discourage thorough investigation of these systems. This is a particular problem for the study of the responses of local communities to climate change, as impacts on these responses are multifaceted, and studying too few of them can lead to spurious and misleading interpretations (Davis *et al.* 1998). Here, I take the view of Moran (2003) in not applying sequential corrections for multiple comparisons. I instead present unadjusted P-values in the knowledge that my chosen explanatory variables are backed up by carefully considered hypotheses and my model structure and interpretation are based on close consideration on detailed model properties, rather than just statistical significance.

5.4 Results^{2,3}

5.4.1 Plant production

Aboveground net primary productivity

In 2010, before implementation of the NITROX experiment, water-addition plots showed higher productivity than water-control plots ($P=0.044$). This difference was not apparent early in the NITROX experiment. There were no significant main treatment effects in Year 1 (2011) or Year 2 (2012) of NITROX, although Year 1 saw a marginally significant effect of the interaction between watering treatment and nitrogen treatment ($P=0.057$). Least-square mean comparisons clarify this interaction. They reveal that nitrogen-fixer-removal plots had lower ANNP than nitrogen-fixer-control plots under water-control conditions ($P=0.094$), but that there was no significant difference under water-addition conditions ($P=0.258$). They also reveal production differences between the water-addition and water-control plots under nitrogen-fixer-control ($P=0.072$) but not under nitrogen-fixer-removal conditions ($P=0.258$): water-addition plots had lower ANPP than water-control plots under nitrogen-fixer-control conditions.

By Year 3 (2013), there was a borderline significant effect of watering treatment ($P=0.053$), a significant effect of nitrogen treatment ($P=0.034$), and a significant watering treatment by nitrogen treatment interaction ($P=0.020$). Least-square mean comparisons show that the production difference seen in Year 1 between water-addition and water-control plots under nitrogen-fixer-control conditions was reversed: water-addition plots had higher ANPP than water-control plots under nitrogen-fixer-control conditions ($P=0.013$). This effect was not apparent under nitrogen-fixer-removal conditions ($P=0.218$). They also reveal that by Year 3 of the experiment, nitrogen-fixer-removal plots had significantly lower ANPP than nitrogen-fixer-control plots under water-addition conditions ($P=0.004$). This difference was not apparent under water-control conditions ($P=0.833$).

Figure 5.4 illustrates the effect of each treatment combination on ANPP through time. Table 5.1 details the main effects watering treatment, nitrogen treatment and their interaction on ANPP through time.

² Appendix 4.1 presents treatment means for every response variable in every experimental year

³ Appendix 4.2 presents all the least square means comparisons for different treatment combinations carried out in this study

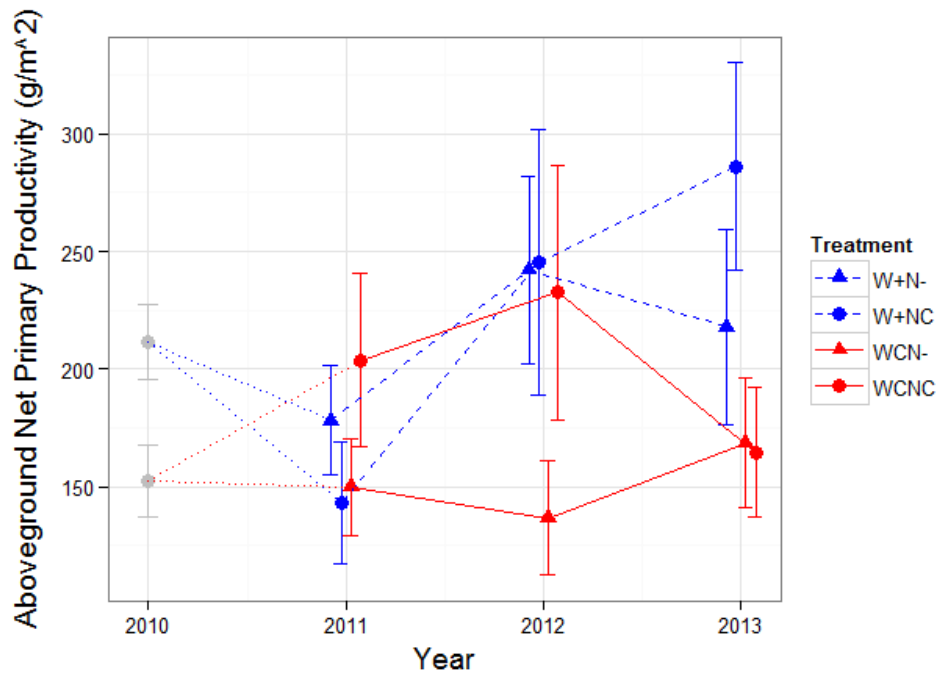


Figure 5.4: Effects of water-addition and nitrogen-fixer-removal on aboveground net primary production (ANPP). Data represent mean dry mass in $\text{g/m}^2 \pm 1$ standard error for each treatment application across three successive years. W+N- denotes water-addition/nitrogen-fixer-removal; W+NC denotes water-addition/nitrogen-fixer-control; WCN- denotes water-control/nitrogen-fixer-removal; and WCNC denotes water-control/nitrogen-fixer-control. In grey are 2010 baseline data from plots featured in Suttle *et al.* (2007), prior to imposition of the nitrogen-fixer treatments. Dotted lines link baseline data to experimental data.

Table 5.1: Effect of watering treatment, nitrogen treatment and their interaction on aboveground net primary production (ANPP), calculated using conditional F-tests with Kenward-Roger approximated degrees of freedom on linear mixed effects models. Dropped denotes a non-significant interaction term that was removed from the full model.

Year	Model Term	F-value	df	P-value
2010	Watering	7.137	1, 5	0.044 *
2011	Watering	0.500	1, 5	0.511
	Nitrogen	0.212	1, 10	0.655
	Watering x Nitrogen	4.649	1, 10	0.057 †
2012	Watering	3.168	1, 5	0.135
	Nitrogen	2.226	1, 11	0.164
	Watering x Nitrogen	<i>dropped</i>	<i>dropped</i>	<i>dropped</i>
2013	Watering	6.407	1, 5	0.053 †
	Nitrogen	6.031	1, 10	0.034 *
	Watering x Nitrogen	7.631	1, 10	0.020 *

0.05 ≤ P ≤ 0.10, * 0.01 ≤ P ≤ 0.05, ** 0.001 ≤ P ≤ 0.01, *** 0.0001 ≤ P ≤ 0.001

Functional group-specific productivity

In 2010, prior to implementation of the NITROX experiment, there was no effect of watering treatment on perennial grass, annual grass or annual forb production ($P=0.152$; $P=0.227$; $P=0.637$ respectively). Perennial forbs showed lower production in water-addition plots than in water-control plots ($P=0.024$).

In Year 1 of the NITROX experiment, there was a significant effect of watering treatment ($P=0.047$) and a marginally significant effect of nitrogen treatment ($P=0.096$) on perennial grass production. Year 1 saw no treatment effects on the productivity of any other functional group. Least-square mean comparisons provide more information on these treatment-based differences. Across both nitrogen treatments, water-addition plots had higher perennial grass production than water-control plots ($P=0.047$), whilst across both watering treatments, nitrogen-fixer control plots had higher perennial grass production than nitrogen-fixer removal plots ($P=0.096$).

This same significant effect of watering treatment on perennial grass production remained into Year 2 of the experiment ($P=0.001$), where water-addition plots again showed higher perennial grass production than water-control plots across both nitrogen-fixer treatments ($P=0.001$). Year 2 also saw a marginally significant effect of watering treatment on annual forb production ($P=0.076$), and of nitrogen-fixer treatment on perennial forb production ($P=0.093$). Across both nitrogen treatments, water-addition plots showed higher annual forb production than water-control plots ($P=0.07558$). Across both watering treatments, nitrogen fixer-control plots showed higher perennial forb production than nitrogen-fixer-removal plots ($P=0.093$). These effects were not apparent in Year 3.

By Year 3 of the NITROX experiment, a borderline significant interaction effect on perennial grass production ($P=0.054$) revealed that the same difference seen in previous years between water-addition and water-control plots remained under nitrogen-fixer-control conditions ($P=0.095$: water-addition plots had higher perennial grass production), but not under nitrogen-fixer-removal conditions ($P=0.751$). It further revealed that nitrogen-fixer-removal resulted in higher perennial grass production in water control plots as compared to these same plots under nitrogen-fixer-control conditions ($P=0.023$), but this difference was not apparent in water-addition plots ($P=0.701$).

There were no treatment effects on annual grass production until this third year, where nitrogen-fixer treatment and the interaction between watering and nitrogen treatments had

significant effects ($P=0.006$; $P=0.019$ respectively). Least-square mean comparisons revealed that these results reflect the annual grass production difference between nitrogen-fixer-removal and nitrogen-fixer-control plots under water-addition but not under water-control. In water-addition plots, the removal of nitrogen-fixers resulted in decreased annual grass production by Year 3.

Figure 5.5 illustrates the effect of each treatment on production of each functional group through time. Table 5.2 details the main effects of each treatment and their interaction on the production of each functional group through time.

Table 5.2: Effect of watering treatment, nitrogen treatment and their interaction on functional group production, calculated using conditional F-tests with Kenward-Roger approximated degrees of freedom on linear mixed effects models. Dropped denotes a non-significant interaction term that was removed from the full model.

Year	Functional Group	Model Term	F-value	df	p-value
2010	Perennial Grasses	Watering	2.856	1, 5	0.152
	Annual Grasses	Watering	1.892	1, 5	0.227
	Perennial Forbs	Watering	10.325	1, 5	0.024*
	Annual Forbs	Watering	0.252	1, 5	0.637
2011	Perennial Grasses	Watering	6.858	1, 5	0.047*
		Nitrogen	3.320	1, 11	0.096†
		Watering x Nitrogen	dropped	dropped	dropped
	Annual Grasses	Watering	0.243	1, 5	0.643
		Nitrogen	0.374	1, 11	0.553
		Watering x Nitrogen	dropped	dropped	dropped
	Perennial Forbs	Watering	0.006	1, 5	0.943
		Nitrogen	2.093	1, 11	0.176
		Watering x Nitrogen	dropped	dropped	dropped
	Annual Forbs	Watering	2.983	1, 5	0.145
		Nitrogen	2.916	1, 11	0.116
		Watering x Nitrogen	dropped	dropped	dropped
2012	Perennial Grasses	Watering	41.136	1, 5	0.001 **
		Nitrogen	1.813	1, 11	0.205
		Watering x Nitrogen	dropped	dropped	dropped
	Annual Grasses	Watering	0.763	1, 5	0.423

Continued...

Year	Functional Group	Model Term	F-value	df	p-value
	Perennial Forbs	Nitrogen	3.004	1, 11	0.111
		Watering x Nitrogen	dropped	dropped	dropped
		Watering	0.161	1, 5	0.705
	Annual Forbs	Nitrogen	3.384	1, 11	0.093 †
		Watering x Nitrogen	dropped	dropped	dropped
		Watering	5.001	1, 5	0.076 †
		Nitrogen	0.0001	1, 11	0.991
		Watering x Nitrogen	dropped	dropped	dropped
2013	Perennial Grasses	Watering	1.506	1, 5	0.274
		Nitrogen	2.624	1, 10	0.136
		Watering x Nitrogen	4.751	1, 10	0.054†
	Annual Grasses	Watering	0.106	1, 5	0.758
		Nitrogen	11.878	1, 10	0.006 **
		Watering x Nitrogen	7.753	1, 10	0.019 *
	Perennial Forbs	Watering	0.239	1, 5	0.646
		Nitrogen	0.320	1, 11	0.583
		Watering x Nitrogen	dropped	dropped	dropped
	Annual Forbs	Watering	0.060	1, 5	0.817
		Nitrogen	0.478	1, 11	0.504
		Watering x Nitrogen	dropped	dropped	dropped

0.05 ≤ P ≤ 0.10, * 0.01 ≤ P ≤ 0.05, ** 0.001 ≤ P ≤ 0.01, *** 0.0001 ≤ P ≤ 0.001

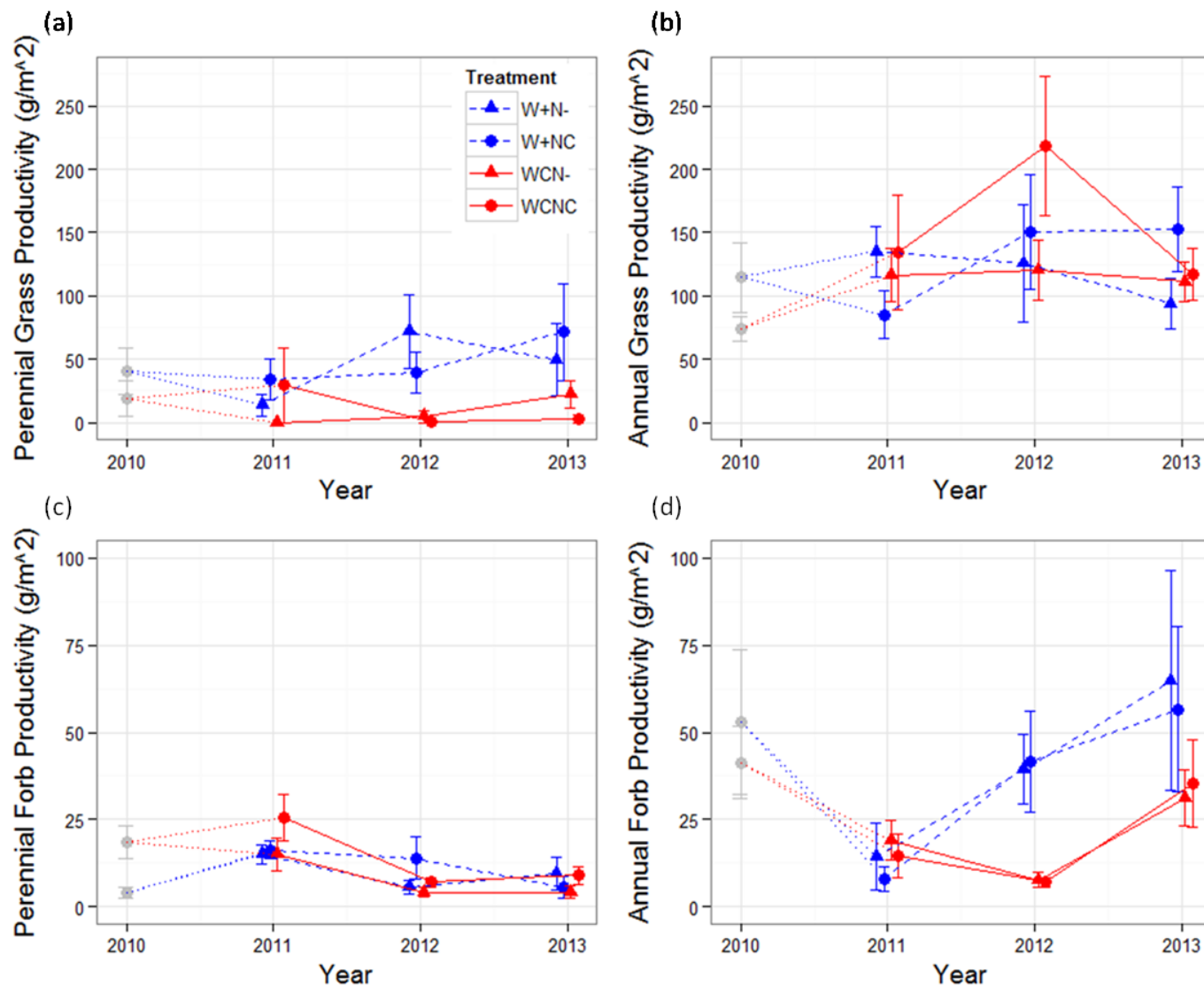


Figure 5.5: Effects of water-addition and nitrogen-fixer-removal on productivity of (a) Perennial Grasses; (b) Annual Grasses; (c) Perennial Forbs; (d) Annual Forbs. Data represent mean dry mass in g/m² $\pm$ 1 standard error for each treatment application across three successive years. W+N- denotes water-addition/nitrogen-fixer-removal; W+NC denotes water-addition/nitrogen-fixer-control; WCN- denotes water-control/nitrogen-fixer-removal; and WCNC denotes water-control/nitrogen-fixer-control. In grey are 2010 baseline data from plots featured in Suttle *et al.* (2007), prior to implementation of the nitrogen-fixer treatments. Dotted lines link baseline data to experimental data. Note the difference in scale for grass and forb species.

5.4.2 Plant diversity

Total plant diversity

Prior to implementation of the NITROX experiment, water-addition plots showed lower plant diversity than water-control plots ($P=0.013$). This difference was not apparent early in the NITROX experiment. There were no significant treatment effects on plant diversity in Year 1 (2011) or Year 2 (2012), although there was a marginally significant effect of nitrogen treatment on plant diversity in Year 1 ($P=0.067$). Nitrogen-fixer-removal plots showed higher plant diversity than nitrogen-fixer-control plots across both watering treatments in this year ($P=0.069$).

By Year 3, there was a highly significant effect of nitrogen-fixer-removal ($P=0.005$) and an even more highly significant water-addition by nitrogen-fixer-removal interaction ($P<0.001$). Least-square mean comparisons revealed that these results reflect a highly significant increase in plant diversity in nitrogen-fixer-removal plots compared to nitrogen-fixer-control plots under water-addition ($P<0.001$), but no such effect under water-control ($P=0.296$). They also revealed that water-addition plots had lower plant diversity than water control plots under nitrogen-fixer-control conditions, as prior to the start of the experiment ($P=0.020$). This effect was not apparent under nitrogen-fixer-removal conditions ($P=0.782$).

Figure 5.6 illustrates the effect of each treatment on plant diversity through time. Table 5.3 details the main effects of each treatment and their interaction on plant diversity through time.

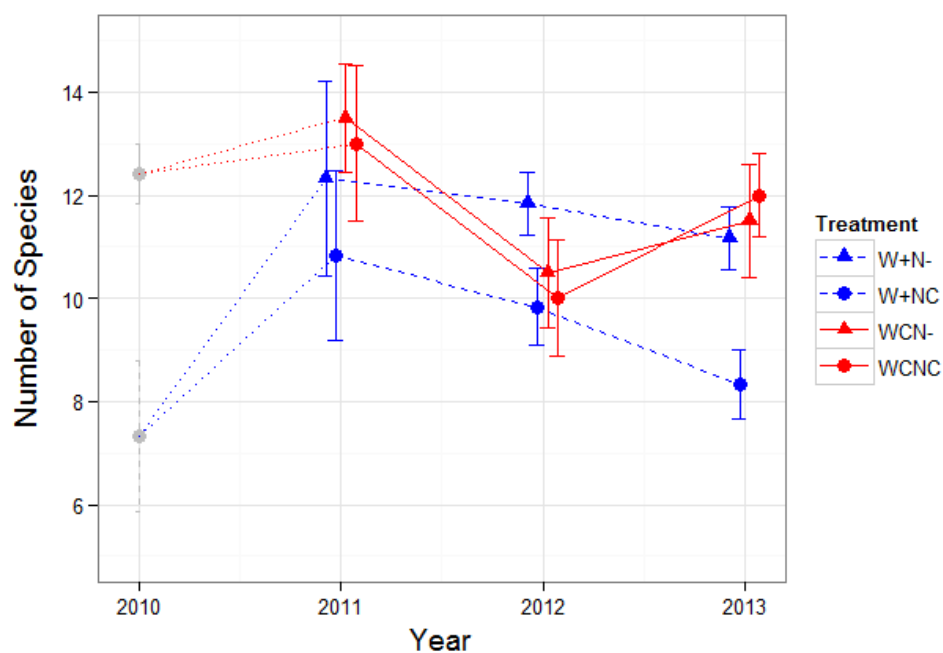


Figure 5.6: Effect of water-addition and nitrogen-fixer-removal on plant species diversity. Data represent mean number of species ± 1 standard error for each treatment combination over three successive years. W+N- denotes water-addition/nitrogen-fixer-removal; W+NC denotes water-addition/nitrogen-fixer-control; WCN- denotes water-control/nitrogen-fixer-removal; and WCNC denotes water-control/nitrogen-fixer-control. In grey are baseline data for these plots in 2010, prior to imposition of the nitrogen-fixer treatments. Dotted lines link baseline data to experimental data.

Table 5.3: Effect of watering treatment, nitrogen treatment and their interaction on species diversity, calculated using conditional F-tests with Kenward-Roger approximated degrees of freedom on linear mixed effects models. Dropped denotes a non-significant interaction term that was removed from the full model.

Year	Model Term	F-value	df	P-value
2010	Watering	14.434	1, 5	0.013 *
2011	Watering	1.306	1, 5	0.305
	Nitrogen	4.125	1, 11	0.067 +
	Watering x Nitrogen	<i>dropped</i>	<i>dropped</i>	<i>dropped</i>
2012	Watering	0.643	1, 5	0.459
	Nitrogen	2.951	1, 11	0.114
	Watering x Nitrogen	<i>dropped</i>	<i>dropped</i>	<i>dropped</i>
2013	Watering	3.265	1, 5	0.131
	Nitrogen	13.243	1, 10	0.005 **
	Watering x Nitrogen	27.027	1, 10	<0.001 ***

0.05 $\leq$ P $\leq$ 0.10, * 0.01 $\leq$ P $\leq$ 0.05, ** 0.001 $\leq$ P $\leq$ 0.01, *** 0.0001 $\leq$ P $\leq$ 0.001

Functional group-specific plant diversity

In 2010, prior to implementation of the NITROX experiment, water-addition plots had significantly lower numbers of forb species than water-control plots ($P=0.030$). This effect was not apparent early in the NITROX experiment: there were no significant treatment effects on forb diversity in Year 1 or Year 2. By Year 3, there was a significant effect of the interaction between watering treatment and nitrogen-fixer treatment ($P=0.016$). Least-square mean comparisons revealed the same trends for this year as seen for the total plant diversity results in Year 3: nitrogen-fixer-removal plots had higher numbers of forb species than nitrogen-fixer-control plots under water-addition conditions ($P=0.016$), but there was no such difference under water-control conditions ($P=0.257$). Again similar to the total plant diversity results, these comparisons also revealed that water-addition plots had lower forb diversity than water-control plots under nitrogen-fixer-control conditions, as prior to the start of the experiment ($P=0.020$). This effect was not apparent under nitrogen-fixer-removal conditions ($P=0.671$).

2010 saw no effect of watering treatment on grass diversity. This state persisted into Year 1 of the NITROX experiment, when there were no treatment effects on grass diversity. Year 2 saw a marginally significant effect of the interaction between the two treatments ($P=0.093$). Least-square mean comparisons revealed that nitrogen-fixer-removal plots showed higher grass diversity than nitrogen-fixer-control plots under water-addition ($P=0.029$), but not under water-control ($P=0.943$). Further, water-addition plots showed higher grass diversity than water-control plots under nitrogen-fixer-removal conditions ($P=0.036$), but not under nitrogen-fixer-control conditions ($P=0.947$). The grass diversity model for Year 2 had a poor residual distribution however, and thus these results should be treated with caution. By Year 3, this interaction effect was not significant, although there was a marginally significant main effect of nitrogen-fixer treatment on grass diversity ($P=0.072$): Nitrogen-fixer-removal plots showed higher grass diversity than nitrogen-fixer-control plots, across both watering treatments ($P=0.083$).

Figure 5.7 illustrates the effect of each treatment on forb and grass diversity through time. Table 5.4 details the main effects of each treatment and their interaction on forb and grass diversity through time.

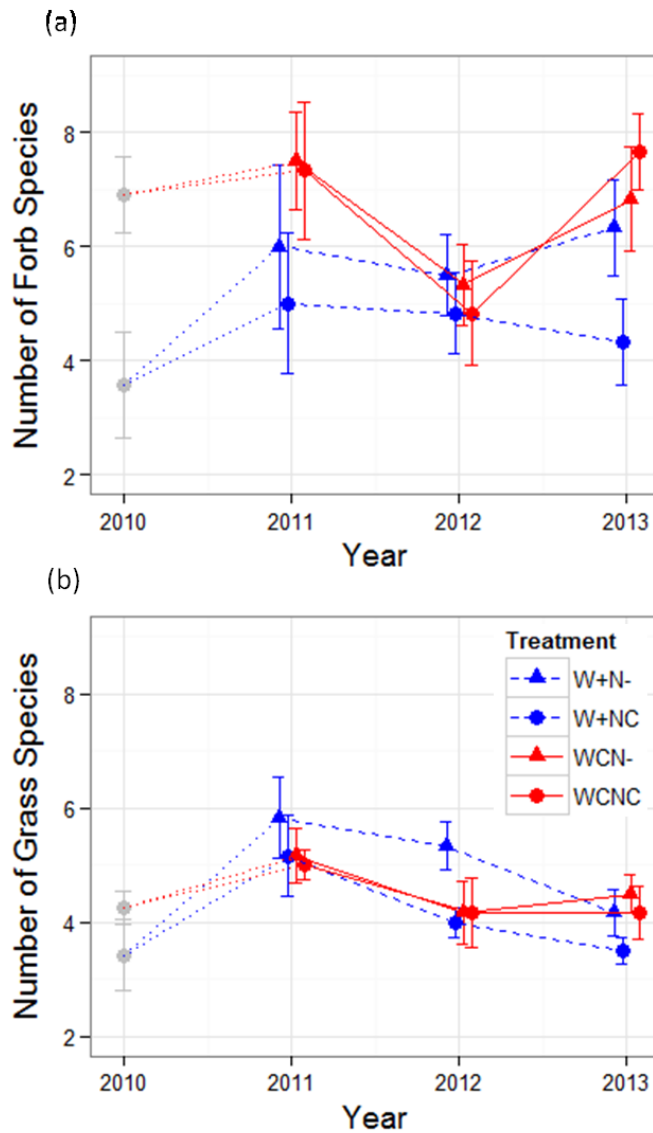


Figure 5.7: Effect of water-addition and nitrogen-fixer-removal on (a) Forb diversity (excluding nitrogen-fixers); (b) Grass diversity. Data represent mean number of Forb or Grass species $\pm$ 1 standard error for each treatment combination over three successive years. W+N- denotes water-addition/nitrogen-fixer-removal; W+NC denotes water-addition/nitrogen-fixer-control; WCN- denotes water-control/nitrogen-fixer-removal; and WCNC denotes water-control/nitrogen-fixer-control. In grey are baseline data for these plots in 2010, prior to imposition of the nitrogen-fixer treatments. Dotted lines link baseline data to experimental data.

Table 5.4: Effect of watering treatment, nitrogen treatment and their interaction on forb (excluding nitrogen-fixers) and grass species diversity, calculated using Kenward-Roger approximated sum to zero contrasts on linear mixed effects models. Dropped denotes a non-significant interaction term that was removed from the full model.

Year	Functional Group	Model Term	F-value	df	P-value
2010	Grasses	Watering	2.841	1, 5	0.153
	Non-fixing Forbs	Watering	9.050	1, 5	0.030 *
2011	Grasses	Watering	0.139	1, 5	0.725
		Nitrogen	1.876	1, 11	0.198
		Watering x Nitrogen	<i>dropped</i>	<i>dropped</i>	<i>dropped</i>
	Forbs	Watering	3.086	1, 5	0.139
		Nitrogen	2.147	1, 11	0.171
		Watering x Nitrogen	<i>dropped</i>	<i>dropped</i>	<i>dropped</i>

Continued...

Year	Functional Group	Model Term	F-value	df	P-value
2012	Grasses	Watering	2.306	1, 5	0.189
		Nitrogen	3.068	1, 10	0.110
		Watering x Nitrogen	3.442	1, 10	0.093 †
	Forbs	Watering	0.011	1, 5	0.919
		Nitrogen	1.553	1, 11	0.239
		Watering x Nitrogen	<i>dropped</i>	<i>dropped</i>	<i>dropped</i>
2013	Grasses	Watering	1.360	1, 5	0.296
		Nitrogen	3.973	1, 11	0.072 †
		Watering x Nitrogen	<i>dropped</i>	<i>dropped</i>	<i>dropped</i>
	Non-fixing Forbs	Watering	3.531	1, 5	0.119
		Nitrogen	1.416	1, 10	0.262
		Watering x Nitrogen	8.353	1, 10	0.016 *

0.05 ≤ P ≤ 0.10, * 0.01 ≤ P ≤ 0.05, ** 0.001 ≤ P ≤ 0.01, *** 0.0001 ≤ P ≤ 0.001

5.4.3 Species composition

Prior to implementation of the NITROX experiment, watering treatment was a clear determinant of patterns of plot species composition in 2010 ($P=0.065$). Water-addition plots also showed greater plot to plot compositional variation than water-control plots in this year. The compositional separation by watering treatment remained clear across all three years of the nitrogen-fixer-removal experiment (2011: $P<0.001$; 2012: $P=0.066$; 2013: $P=0.002$). Nitrogen treatment had no effect on patterns of plot species composition in any year. Figure 5.8 details each 2D NMDS ordination solution graphically. Table 5.5 details the main effects of watering treatment, nitrogen-fixer treatment and their interaction on plot compositional similarity.

Table 5.5: Effect of watering treatment, nitrogen treatment and their interaction on patterns in plot species composition, calculated using PerMANOVA with Bray-Curtis distance measures, and stratified by block. Dropped denotes a non-significant interaction term that was removed from the full model.

Year	Model Term	Sum of squares	df	F-value	p-value
2010	Watering	0.555	1	2.858	0.065 †
2011	Watering	1.000	1	5.999	<0.001 ***
	Nitrogen	0.119	1	0.711	0.444
	Watering x Nitrogen	<i>dropped</i>	<i>dropped</i>	<i>dropped</i>	<i>dropped</i>
2012	Watering	0.317	1	1.343	0.066 †
	Nitrogen	0.070	1	0.297	0.851
	Watering x Nitrogen	<i>dropped</i>	<i>dropped</i>	<i>dropped</i>	<i>dropped</i>
2013	Watering	0.972	1	4.676	0.002 **
	Nitrogen	0.039	1	0.189	0.940
	Watering x Nitrogen	<i>dropped</i>	<i>dropped</i>	<i>dropped</i>	<i>dropped</i>

0.05 ≤ P ≤ 0.10, * 0.01 ≤ P ≤ 0.05, ** 0.001 ≤ P ≤ 0.01, *** 0.0001 ≤ P ≤ 0.001

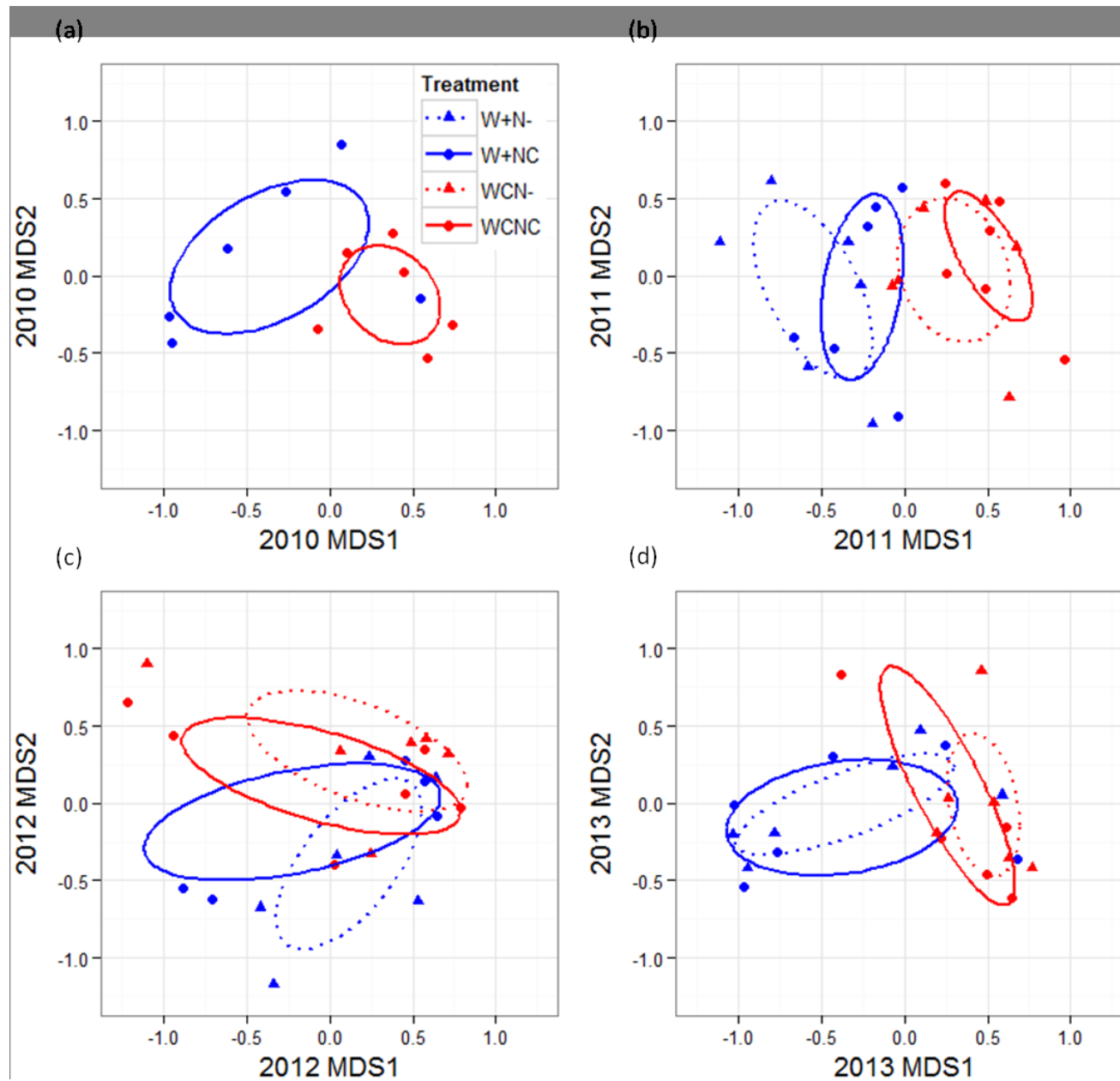


Figure 5.8: 2D Non-metric Multidimensional Scaling (NMDS) ordinations showing plot compositional similarity for each treatment application in each year: (a) 2010 (baseline data); (b) 2011; (c) 2012; (d) 2013. Distances are based on Bray Curtis measures. Ellipses represent the $\pm 1.96SE$ (95% confidence) for each treatment. For 2010, W+ denotes water-addition; and WC denotes water-control. For 2011-2013, W+N- denotes water-addition/nitrogen-fixer-removal; W+NC denotes water-addition/nitrogen-fixer-control; WCN- denotes water-control/nitrogen-fixer-removal; and WCNC denotes water-control/nitrogen-fixer-control.

5.5 Discussion

Predictions of local-scale responses of ecological communities under the changing climate are subject to great numbers of uncertainties, with many influences coming to bear upon the responses of individual species. Biotic interactions may be particularly complicating influences upon our ability to predict at these scales, as they can generate a multitude of indirect effects via unforeseen complexities in the ways that individuals respond to local climate change (Walther 2010). One way to improve predictability at these local scales would be to identify key indirect effects that most strongly govern system dynamics. As such, our understanding of these dynamics could be defined in more tractable terms. This chapter tests this idea, attempting to resolve the indirect effects observed in an experimental community to a single major driver. It further asks whether, by targeting this driver, we can reduce the role of indirect relative to direct effects in a community under climate change. The results presented have to be viewed against a backdrop of large interannual variation. Strong non-directional year to year differences in production, diversity and composition are the ambient state in most California grasslands, determined in particular by the timing and amount of annual precipitation each year (Pitt and Heady 1978, Stromberg and Griffin 1996, Hobbs *et al.* 2007). In interpreting these results then, focus needs to be directed at differences relative to controls, rather than differences from one year to the next. It is differences that emerge and persist between treatments that hold instructive value for how climate change may affect the grassland system generally, and of the importance of the proposed key driver of complex indirect effects in this system specifically. The effects of nitrogen-fixer removal on water-addition plots should be of particular focus, as it is the indirect effects prevalent in these water-addition plots that are of interest here. These dynamics make three years an abbreviated timeframe over which to be interpreting experimental effects, but even absent the opportunity for strong inference, some interesting patterns have emerged.

ANPP results from the first two years of the NITROX experiment show no clear effects of nitrogen-fixer-removal on water-addition plots. By the third year of the NITROX experiment however, the removal of nitrogen-fixers in water-addition plots had reduced ANPP significantly below that seen these plots where nitrogen-fixers were not removed, with no concomitant change under water-control conditions. Functional group production differences in Year 3 provide more information about the effect of removing nitrogen-fixers on water-addition plot productivity. It becomes clear that the ANPP difference between water-addition

plots where nitrogen-fixers were removed and those of such plots under nitrogen-fixer-control conditions is primarily attributable to exotic annual grass responses. Removal of nitrogen-fixers in water-addition plots has reduced exotic annual grass production to levels significantly below those seen in the equivalent nitrogen-fixer-control plots. There is no comparable effect under water-control conditions. Other functional groups show less of a clear contribution to the ANPP result in Year 3.

The ANPP declines under nitrogen-fixer-removal in water-addition plots are accompanied by an increase in plant diversity. Year 3 plant diversity is significantly higher in these plots than in the equivalent nitrogen-fixer-control plots, with no concomitant change under water-control conditions. Plant diversity remains lower in water-addition plots as compared to water-control under nitrogen-fixer-control conditions. Looking to functional group breakdowns in diversity changes, we can glean more information about the specifics of the effect of nitrogen-fixer-removal on plot diversity under the extended rainy season conditions in water-addition plots. The removal of nitrogen-fixers in water-addition plots appeared to affect forb diversity predominantly. Year 3 forb diversity in such plots was significantly higher than in water-addition plots under nitrogen-fixer-control conditions, showing a recovery towards levels of diversity seen in water-control plots. This effect may be caused by the observed declines in exotic annual grass production in these plots. Indeed the thatch of exotic annual grasses is well known to suppress germination and regrowth of leafy forbs in California grassland systems (Bentley *et al.* 1958; Heady 1958; Hobbs *et al.* 1988; Hueneker *et al.* 1990). As such, the observed decline in exotic annual grass production and consequent grass thatch as a result of nitrogen-fixer removal in water-addition plots could have driven the increase in forb diversity observed in these plots.

The removal of nitrogen-fixers appears to have had limited effect on the plant species composition of both water-addition and water-control plots. NMDS and PerMANOVA results indicate that plot species composition remains strongly determined by watering treatment throughout the experiment. Given the length of time the watering manipulation has been applied for, and the dominance of water as a driver of change in this system, this might be expected after only three years of nitrogen manipulation. The ANPP and plant diversity results discussed here indicate that, in the long run, nitrogen-fixer-removal may have detectable effects on the multivariate species composition of water-addition plots as well, if production continues to drop and formerly excluded species are able to take advantage of the reduction in competitive dominance to re-establish populations.

Together, the results from the first 3 years of the NITROX experiment indicate that removal of nitrogen-fixers from water-addition plots has resulted in production declines in exotic annual grass species and an increase in the number of forb species in the system. These results should be interpreted further in the context of the previous work employed in this system. Suttle *et al.* (2007) and Chapter 4 highlighted the pervasiveness of indirect effects in determining community dynamics of an experimental system subject to a climate manipulation. In line with expectations from Davis *et al.* (1998) and Petchey *et al.* (1999), they demonstrated that consideration of the direct effects of climate alone cannot explain the responses observed in this system. Specifically, component species autecology would dictate that California native plants, with their flexible flowering phenology, would benefit more from an extended rainy season (as imposed in water-addition plots) than the more phenologically constrained exotic annual grass species in the system (Laude 1953; Pitt and Heady 1978; Jackson and Roy 1986). In actuality, the direct responses that species showed to the extended rainy season appeared to have been overturned by indirect effects generated by the interactions between them, resulting in species diversity declines and community reorganisation. Chapter 4 made clear that these indirectly driven species responses are highly idiosyncratic, and do not break down according to shared traits. It did however indicate that one specific trait might be proximally driving these indirect changes, enacted via the direct response of a single species to the extended rainy season. The initial results from the NITROX experiment suggest that by removal of this key driving trait from the experimental system, the indirect effects that ensued in its wake may be terminated, and some of the negative consequences of the imposed climate change for production and diversity may start to reverse. As such, while the responses of species in this system do not breakdown according to traits, there is now solid evidence that one trait has a strongly deterministic influence on community reorganisation in response to a simulated change in climate. Furthermore, there is also evidence that by targeting this key driving trait, the role of indirect relative to direct effects in this community can be reduced.

Despite these initial results, we must be cautious in drawing concrete conclusions. As discussed earlier, strong year to year differences in production, richness, and composition are the ambient state in most California grasslands. These grasslands also show a lot of natural patchiness (Harrison 1997; Huntsinger *et al.* 2007). As such these community properties are not only variable year to year, but also within a year between even the most proximal of locales. While good experimental design can preclude the impact of some of this natural

variation on results, patchiness is so extensive in these systems that it was observed even within the individual plots in this experiment, let alone within the blocks that were set up to counter it. Furthermore, even if the results documented so far are indeed primarily attributable to the experimental manipulation, it is not yet clear whether the initial unwinding of indirect effects in this system will continue as the experiment progresses, nor how these manipulated plots will eventually be characterised. Will plots experience stable patterns of production and diversity that might be expected from direct responses of species to increased water availability, or will other pathways of indirect effects emerge in this system instead, creating further, unforeseen complexities (Burns *et al.* 2003)? Only letting the experiment run its course will allow us to understand the true nature of the impacts of this manipulation, and more specifically the relevance of nitrogen-fixation as a driver of change and the reversibility of the indirect effects that have ensued in response to it. Given the broadly documented influence of indirect effects on community responses (e.g. Klein *et al.* 2004; Berg *et al.* 2006; Munson *et al.* 2008; Barton and Schmitz 2009; Menge *et al.* 2009; Janowski *et al.* 2010; Liu *et al.* 2011), if we *can* assert more definitively the importance of a key trait driver of change in this system and in others, it would hold great promise for improved understanding of community responses to climate change, and further for the management of these responses.

Overall, by controlled removal of nitrogen-fixers from this experimental community, the NITROX experiment has tested the role of climatic effects on nitrogen-fixation in driving system dynamics. Results indicate that this one trait plays a pivotal role in how communities have reorganized under simulated climate change. Furthermore, the removal of this trait from the system has gone some way to reversing the trajectory of indirect change observed by Suttle *et al.* (2007) and Chapter 4. This particular experimental system requires a longer runtime before we can be sure of the validity of our initial results. However, it is encouraging to see that not only can our understanding of community responses to climate change be made more tractable by thinking in terms of key drivers, we may also be able to improve our adaptive management prospects by targeting them.

Chapter Six

Conclusion

The impact of climate change on ecological systems is globally demonstrable (Parmesan & Yohe 2003; Root *et al.* 2003; Chen *et al.* 2011). Understanding and predicting ecological responses to the changing climate is a huge focus of research in ecology. Any approach at predicting these responses is challenged by numerous uncertainties, although there has been good progress by researchers in contending with some of these uncertainties (e.g. Pearson and Dawson 2003). In addition to refining current approaches to prediction, it is important to seek out new sources of predictability, such that we can turn as many tools as possible towards understanding how ecological systems are responding (Dawson *et al.* 2011). This thesis looks to traits as units for prediction, assessing their utility in both predictive and adaptive contexts.

Two important ways in which species can respond to the changing climate are by shifting their phenology and by shifting their distribution. There are presently 24 studies that have provided evidence that traits are significant predictors of these two responses. They span a broad variety of taxa, including birds (e.g. Rubolini *et al.* 2010), plants (e.g. Molnar *et al.* 2012), insects (e.g. Diamond *et al.* 2011), mammals (e.g. Angert *et al.* 2011) and fish (e.g. Perry *et al.* 2005). Chapter One reviewed the basis for such trait-based approaches to prediction. It provided justification for this approach in suggesting that trait-based predictors may be transferable between focal species and sites, and may provide relatively immediate knowledge gains. It also highlighted the beneficially probabilistic nature of trait-based predictions of ecological responses to climate change.

Chapter Two set out to implement a reliable modelling approach in a proof-of-concept trait analysis on a dataset of contemporary plant phenological changes under warming (Fitter and Fitter 2002). In testing for trait-based links in the strength and direction of species responses, it demonstrated that biogeographic range and origin may be useful predictors of species responses to future climate change. The low explanatory power of the established relationships however, suggested that traits may be of most practical relevance when used in conjunction with other predictive techniques.

Chapter Three moved to address the critical issue of transferability, which was proposed as one of the key values of a trait-based approach to prediction. If traits are acting as functional respondents to climate change, in that species responses can be predicted based on the traits they possess, then insights should carry over from species to species and site to site. As such, traits may be generally applicable, site-relevant tools. There are clear parallels in the trait-based predictions of other studies (e.g. Vegvari *et al.* 2010 and Hurlbert and Liang 2012; Wolkovich *et al.* 2013 and Chapter 2). Some of these trait studies even carry out cross-site analyses, and have demonstrated consistency in trait-based predictions between sites (Thackeray *et al.* 2010; Wolkovich *et al.* 2013). However they do not formally test whether a trait-model from one area can effectively predict responses from another. Chapter Three addressed this by developing trait-based models for long-term datasets of first-arrival dates for migratory birds in two neighbouring US states, and then assessing the cross-applicability between these models. Body mass was found to show good cross-transferability between states under phylogenetic control. Despite this, the analyses in this study were confronted with problems in estimating the phylogenetic signal in relationships between traits and response, which makes it difficult to draw concrete inferences on the cross-transferability of traits in this study. Further work is required using the kinds of approaches developed in this chapter to assess whether traits do represent transferable predictors. In the longer term, transferability should also be assessed at varied scales. In so doing, it might be possible to establish a lower boundary of scale to the transferability of trait predictions, perhaps set by local level idiosyncratic community context. Such knowledge would allow some of the limitations of a trait-based approach to be established, making clear what we cannot know as much as what we can. Making known biological unknowns is an important component of response prediction under global change.

Chapters One, Two and Three reviewed and contended with a number of logistical and methodological obstacles to the use of trait-based predictors, and attempted to establish some best practices for statistical rigour and model reliability. They asserted the importance of controlling for phylogenetic non-independence in trait-based models, and also of using model selection approaches to avoid the caveats of stepwise regression modelling. Several of the trait studies already published do not test and control for phylogenetic non-independence in their data (e.g. Butler 2003; Altermatt 2010; Buskirk *et al.* 2012; Hurlbert and Liang 2012). I myself struggled with accurate assessment of the phylogenetic signal in my dataset in Chapter Three. Part of the difficulty in doing so is in the availability of reliable phylogenetic

trees. Despite some fantastic advances in the field of Phylogenetics in recent years (e.g. Jetz *et al.* 2012), the availability of reliable phylogenies is somewhat restricted, and those that are available are confronted with biases due to the chosen method of reconstruction (Barracough and Nee 2001), molecular dating (Bennett and Owens 2002), the fundamental definition of species (Agapow *et al.* 2004), the choice of taxonomic scale (Harvey and Pagel 1991) and the reliance on correlative approaches rather than experimental ones (Purvis and Agapow 2002). In running a reliable comparative model selection analysis, there are also issues, often of sample size. These sorts of analyses gain huge value from very large datasets that span many species and are collated over long time scales. Such data not only increase the reliability of any relationships that are established, but they also allow for thorough comparative analyses of several traits at once, and of interactions between these traits. This is particularly important if we are to gain a more mechanistic understanding of the relationships between traits and species responses to climate change. It is difficult when performing these sorts of comparative analyses not to turn to explanatory variables that are continuous in nature, as such variables use fewer degrees of freedom and allow for easier assessment of interactions. This can result in biases towards certain traits over others, simply because of the structure of the data. It can also result in traits that are more explicitly categorical in nature being coerced into continuous form by researchers, perhaps somewhat artificially. By using datasets spanning many species however, I would hope that the temptation to use continuous explanatory variables simply by virtue of their structure would be diminished.

The use of accurate phylogenies and of large datasets of species responses relies upon a greater sharing of data between researchers. For datasets that are readily available, access is often reliant on knowing where to find it. As such, we need to make these data more explicitly available and organised. If we increase the amount and quality of data-sharing in Ecology, the scope and reliability of work implemented across the entire spectrum, from graduate student to experienced researcher, would greatly increase. I will be publishing the raw data I collated for each of my thesis chapters online. With time, I hope that more researchers will show trust and willing in sharing their data, and thus enhance the success of our field as a whole.

Having demonstrated the functionality and transferability of the trait-based approach in Chapters One through Three, I moved to explore the bounds of its utility. If traits predict changes in species phenologies, can they in turn predict changes in species abundances under climate change as well? Abundance is an important currency in conservation, and thus if

traits can be applied to predicting abundance changes as well as changes in phenology and geographic distribution, they would have greater practical application. There is some indication in the literature that abundance changes do break down according to specific traits (Li *et al.* 2009; Genner *et al.* 2010; Terry *et al.* 2011). In Chapter Four, I examined this possibility further. Using a detailed record of community-wide changes in species abundance under ten years of experimental climate change, I attempted to establish relationships between traits and abundance changes. I also reevaluated the prevailing hypothesis that had guided the interpretation of ecological changes in this system by previous researchers (Suttle and colleagues). Contrary to expectation, the results from Chapter Four indicated no clear breakdown in species abundance responses according to shared traits. Indeed, abundance responses at the local scale of this study appeared to be highly idiosyncratic, with no trait-clustering apparent. In light of this, the results of Chapter Four also provided further evidence for what previous researchers had observed in this system (Suttle *et al.* 2007): the direct responses that species showed to the imposed climate manipulation in the first few years of this experiment were overturned by the action of indirect effects, resulting in reductions in plant diversity and substantial community reorganisation.

Retrospectively, the lack of a clear relationship between traits and abundance responses in this system makes more sense. In representing fundamental aspects of species biology and in being the units by which, theoretically, communities are organised (e.g. Kraft and Ackerly 2010), traits may have value in explaining the direct responses of species to climate. Indirect effects however, can result in unforeseen complexities in how species respond and may have large influences on community composition (Doak *et al.* 2008; Walther 2010). This may preclude our ability to use trait-based predictors in systems where the prevalence of indirect effects is high, as in the experimental community analysed in Chapter Four.

Chapter Four resolved the mechanism of change in Suttle and colleagues' experimental system to a single, proximal driver – the nitrogen-fixer, *Lotus micranthus*. This species appeared to have initiated the indirect effects that had unfolded via the action of a single trait – nitrogen-fixation. Thus while species responses in this system were shown not to cluster by traits, one trait did appear to have an important effect on changes in this community, albeit mediated by a single species. This provided me with the opportunity in Chapter Five to test the role of climatic effects on nitrogen-fixation in driving system dynamics, via a controlled removal of this trait from experimental communities. It also allowed me to assess if by

removing a key driver of indirect effects from a community, the role of these indirect effects relative to direct effects can be reduced.

The NITROX experiment designed in Chapter Five is embedded within the original watering manipulation of Suttle and colleagues (Suttle *et al.* 2007; Suttle and Thomsen 2007) and implements orthogonal treatment combinations of water-addition and nitrogen-fixer-removal. Designed to run for five years, the results through the first three years of the experiment are presented in this thesis. Results at three years indicate that a single, trait-based driver does play a pivotal role in how communities have reorganized under this simulated climate change. Furthermore, they suggest that by removing this driver from the system, at least partial reversal of long-term changes in production and diversity have started to become apparent. While I must allow for potential strengthening or weakening of these inferences in the final years of the experiment, these results provide a tantalising indication for management prospects of systems such as this under climate change in the future. Furthermore, the idea of viewing the indirect responses of communities to climate change through the lens of key drivers might help us to manage other systems where such indirect effects have been shown to complicate community responses (e.g. Klein *et al.* 2004; Liu *et al.* 2011). Finally, if the community on which NITROX acts does return to a state of predominantly direct effects, I might have more hope of developing the trait-based predictions of abundance changes that eluded me in Chapter Four.

An interesting parallel to the NITROX experiment is currently being implemented in the same study meadow, by one of the original researchers on the Angelo Reserve Rainfall Addition Experiment – Meredith Thomsen. Here, the same setup as the original Angelo experiment has been implemented in smaller, replicate plots. Nitrogen-fixers are being removed in these plots in an attempt to see what patterns emerge in light of water-addition in absence of this indirect driver. In future work, I hope to compare the results from preventing the emergence of indirect effects in a system to those from reversing their trajectory. If the removal of key drivers can both prevent and reverse indirect effects, then a key driver focus may be useful in both an adaptive and a mitigative management context.

Overall this thesis has shed new light on the role of species traits in ecological responses to climate change. In Summer 2013, a qualitative assessment of the traits that might determine species' vulnerability to climate change was assembled by experts in the field of extinction risk (Foden *et al.* 2013). The work presented in this thesis should provide an excellent

quantitative complement to these kinds of assessments, as it highlights both opportunities and limitations for using traits to organise our thinking over prediction and adaptation.

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Appendix One

Appendix 1.1: Trait Figures

For all figures, Responsiveness represents the extent of change in first flowering date of the plant species in this analysis from 1954-2000.

All boxplots are notched. If notches do not overlap between two levels, this provides strong evidence that the medians for those levels differ (Chambers *et al.* 1983).

Figure A1.1.1: Null distribution of the phenological responsiveness of the plant species in this study.

Figure A1.1.2: Scatterplot of the relationship between Plant Height and phenological responsiveness.

Figure A1.1.3: Scatterplot of the relationship between Plant Height and phenological responsiveness. Plant Height logged to improve distribution with response variable.

Figure A1.1.4: Notched box-plot of the relationship between a plant species' Native Status and its phenological responsiveness. Native Status is a factor with two levels: Native and Non-native. Black dots represent outliers.

Figure A1.1.5: Notched box-plot of the relationship between a plant species' Perennation Strategy and its phenological responsiveness. Perennation Strategy is a factor with three levels: Annual, Biennial and Perennial. Black dots represent outliers.

Figure A1.1.6: Notched box-plot of the relationship between a plant species' Woodiness and its phenological responsiveness. Woodiness is a factor with two levels: Woody and Non-Woody. Black dots represent outliers.

Figure A1.1.7: Notched box-plot of the relationship between a plant species' Biome and its phenological responsiveness. Biome is a factor with four levels: Southerly, Northerly, Temperate and Broad. Table 2.3 provides more detail on these categories. Black dots represent outliers.

Figure A1.1.8: Scatterplot of the relationship between a plant species' Total Range in Britain (km²) and its phenological responsiveness. The estimates include the area of the Isle of Man, but exclude Ireland and the Channel Islands.

Figure A1.1.9: Notched box-plot of the relationship between a plant species' Flowering Time and its phenological responsiveness. Flowering Time is a factor representing whether the first flowering of a plant species occurs in months that showed the greatest temperature increase during the study period (1954-2002), or not. Such species are classified as Peak and Sub-Peak respectively. Months showing the greatest temperature increases include January, February, March, July and August. Black dots represent outliers.

Figure A1.1.10: Notched box-plot of the relationship between whether a plant species has its northern range limit in Britain or not, and its phenological responsiveness. Northern Range Limit is a factor with two levels: Yes (i.e. northern range limit is in Britain) and No (i.e. northern range limit is not in Britain.). Black dots represent outliers.

Figure A1.1.11: Notched box-plot of the relationship between a plant species' Pollen Vector and its phenological responsiveness. Pollen vector is a factor with four levels: Insect, Wind, Selfed, and Non-Angiosperms. Black dots represent outliers.

Figure A1.1.12: Scatterplot of the relationship between a plant species' UK Habitat Breadth and its phenological responsiveness.

Figure A1.1.1

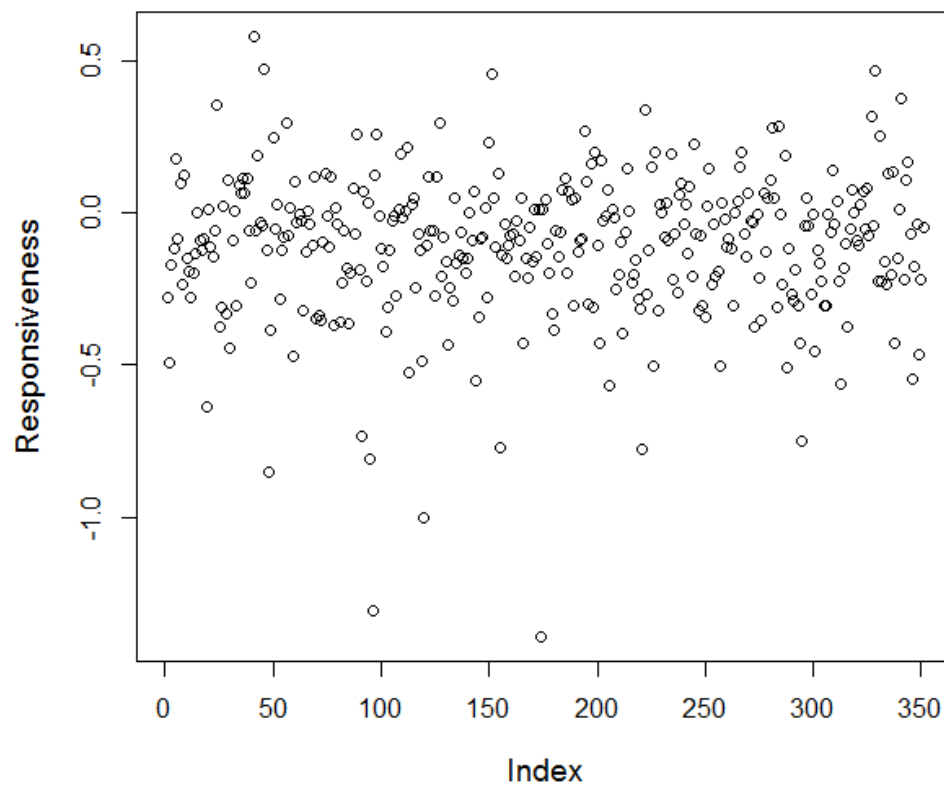


Figure A1.1.2

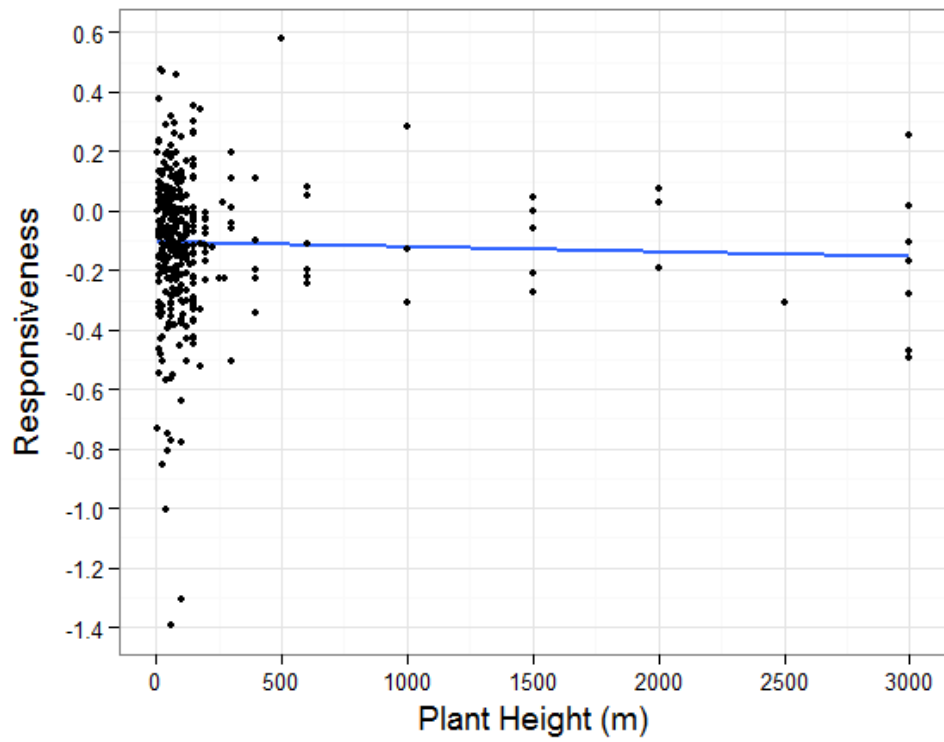


Figure A1.1.3

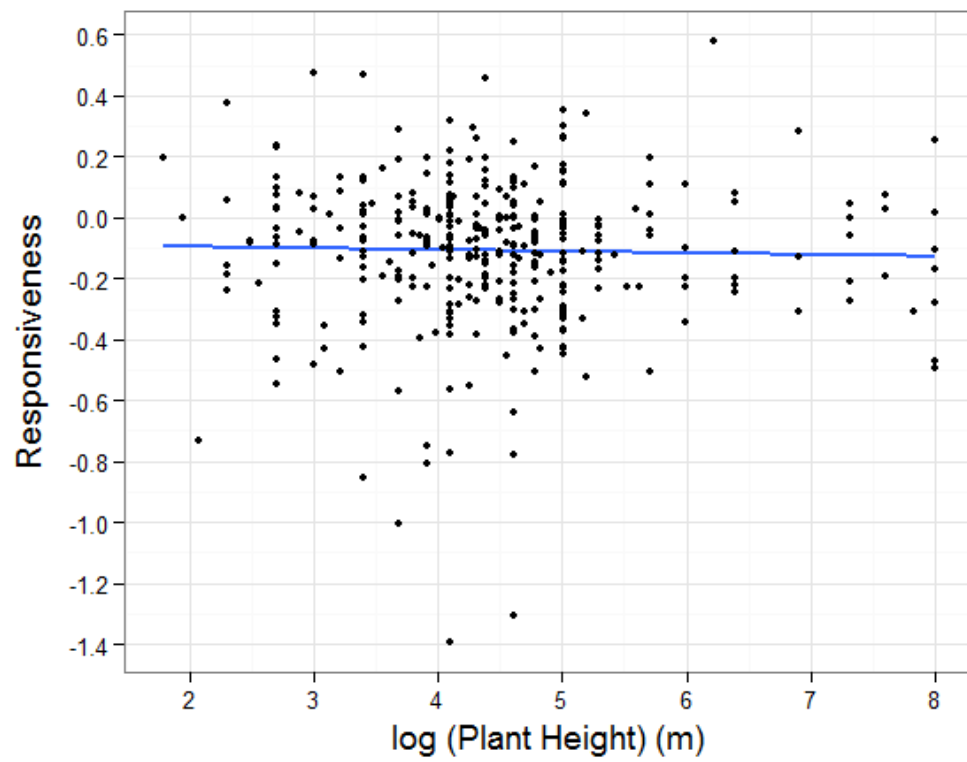


Figure A1.1.4

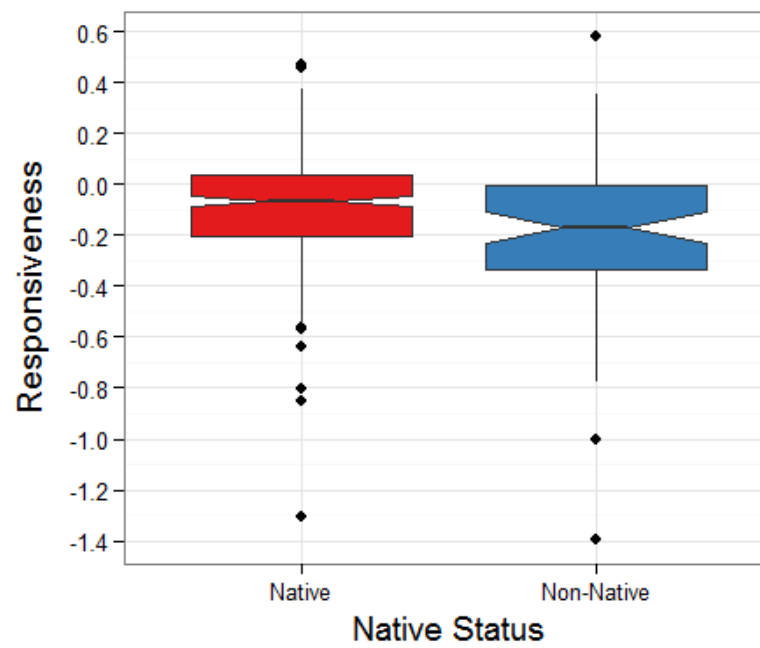


Figure A1.1.5

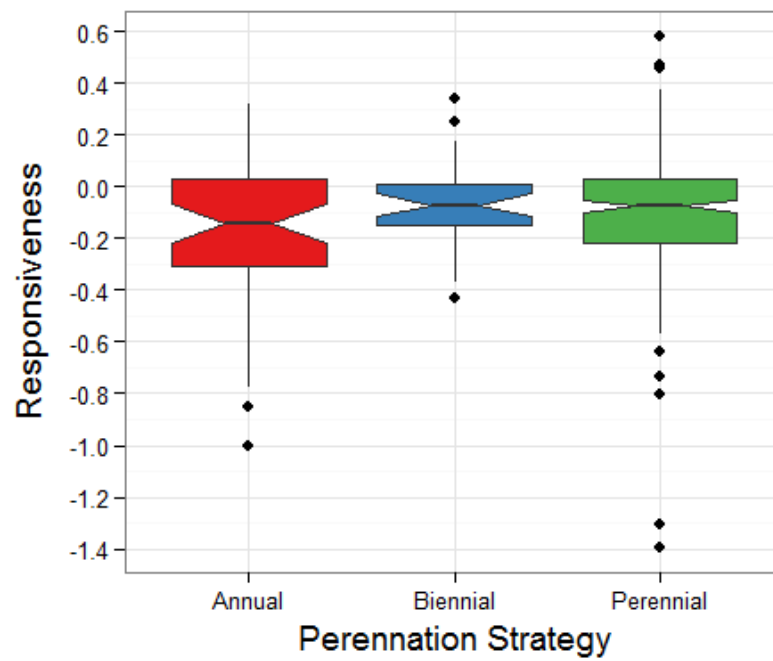


Figure A1.1.6

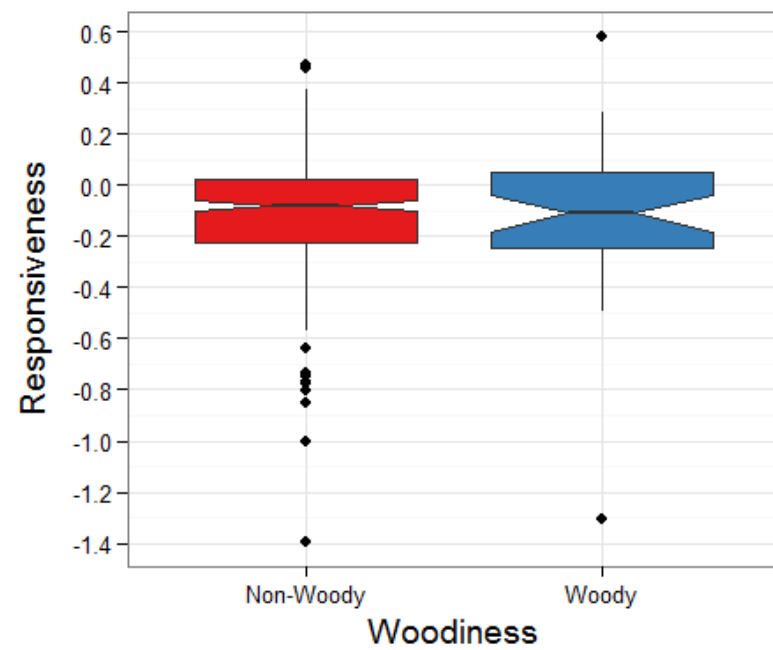


Figure A1.1.7

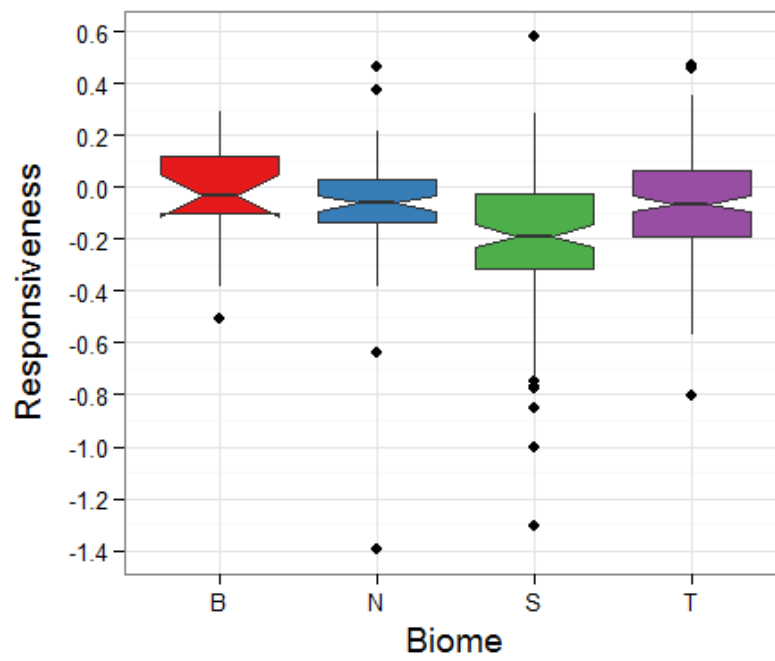


Figure A1.1.8

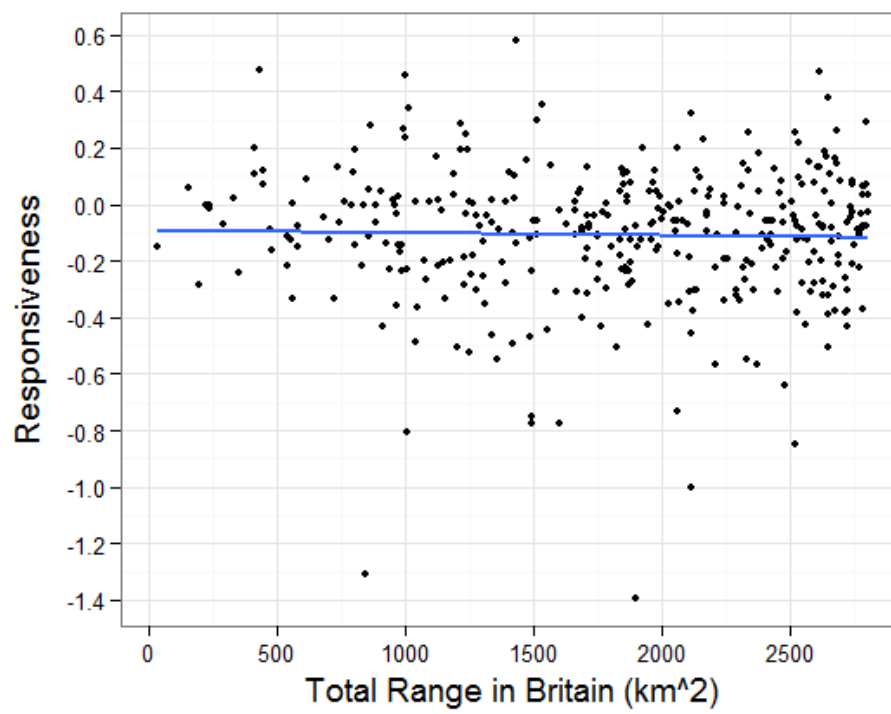


Figure A1.1.9

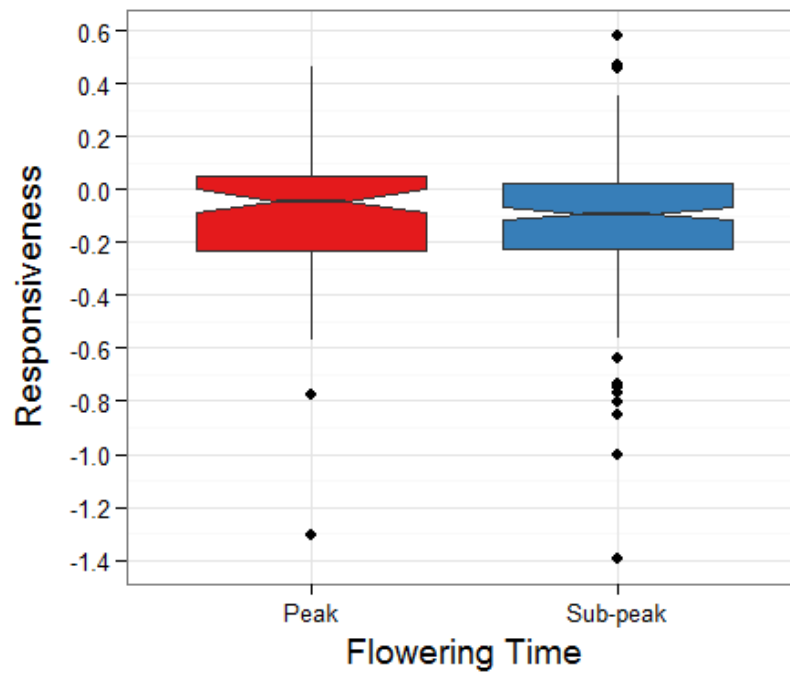


Figure A1.1.10

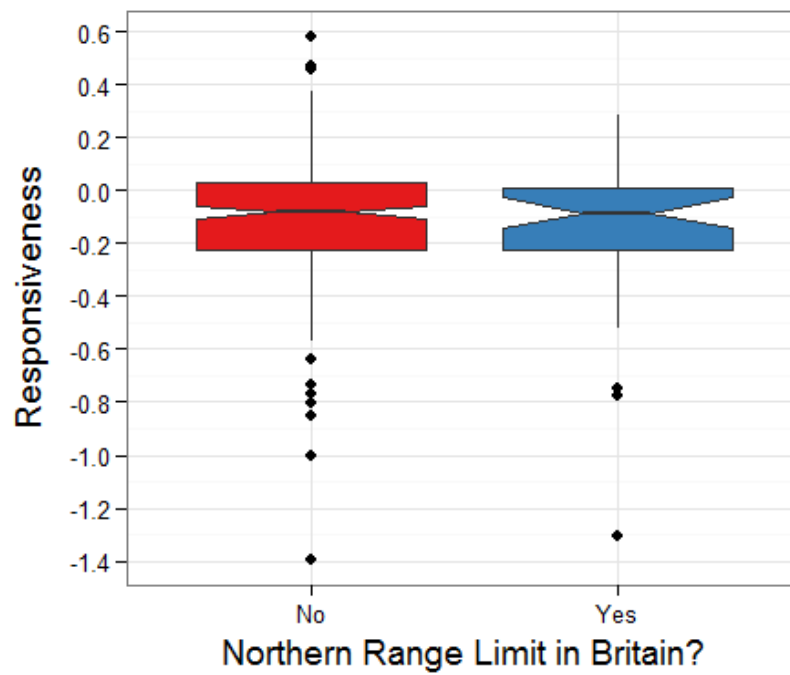


Figure A1.1.11

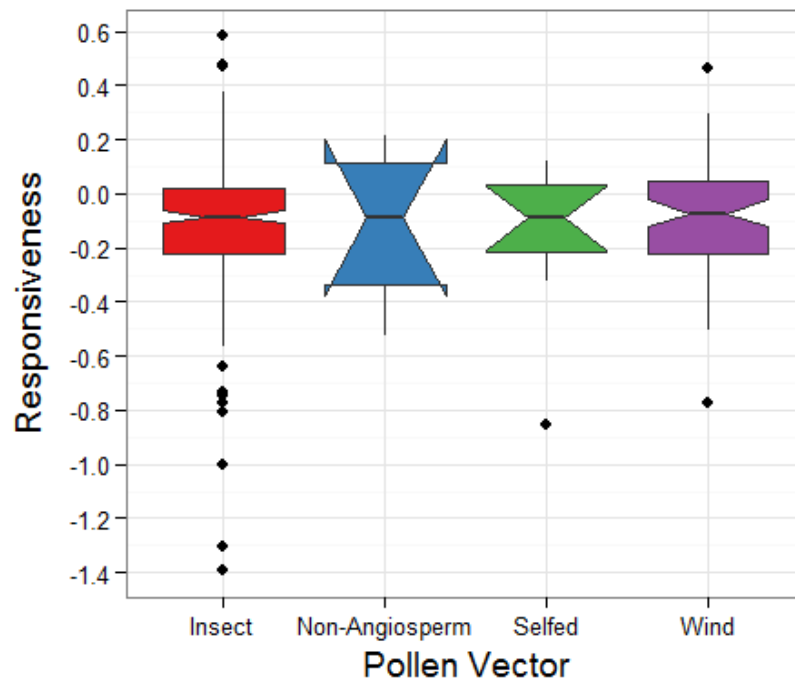
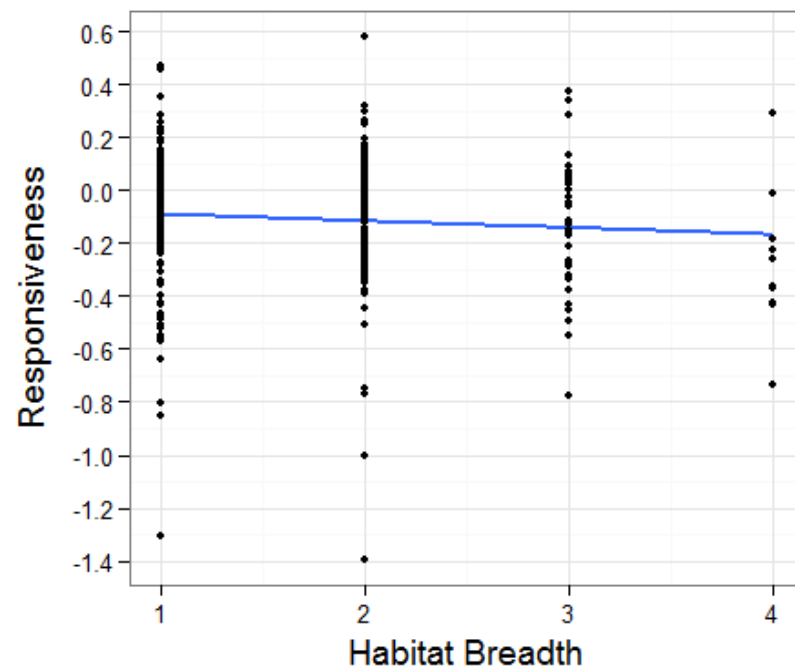


Figure A1.1.12



Appendix 1.2: Lambda maximum likelihood profile plots

Figures A1.2.1-A1.2.12 present the likelihood profiles for the estimation of a maximum likelihood (ML) lambda value for the relationship between Responsiveness and each trait to be included in model selection analyses, in turn. These estimates are bounded by 0 and 1. ML lambda estimate represented by solid red line. Dotted red lines either side of the ML lambda estimate represent 95% confidence intervals around this value. A missing upper or lower bound confidence interval indicates that that interval was at the upper or lower bound of lambda, respectively. Responsiveness represents the extent of change in first flowering date of the species in this analysis from 1954-2000. The specific models are:

Figure A1.2.1: Responsiveness ~ log (Plant Height) (m)

Figure A1.2.2: Responsiveness ~ Native Status

Figure A1.2.3: Responsiveness ~ Perennation Strategy

Figure A1.2.4: Responsiveness ~ Woodiness

Figure A1.2.5: Responsiveness ~ Biome

Figure A1.2.6: Responsiveness ~ Flowering Time

Figure A1.2.7: Responsiveness ~ Total Range in Britain (km²)

Figure A1.2.8: Responsiveness ~ Pollen Vector

Figure A1.2.9: Responsiveness ~ Habitat Breadth

Figure A1.2.1

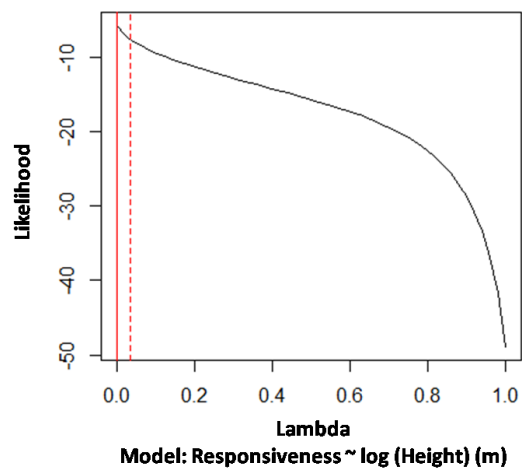


Figure A1.2.2

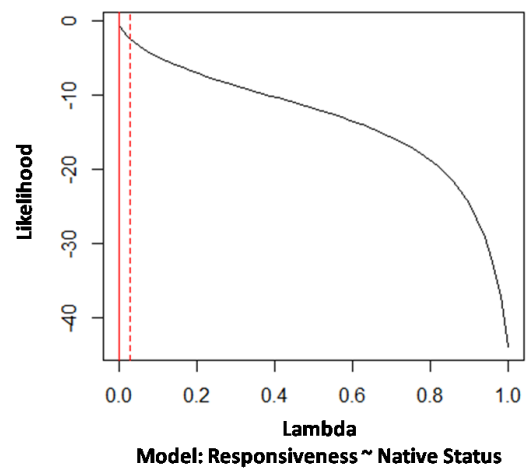


Figure A1.2.3

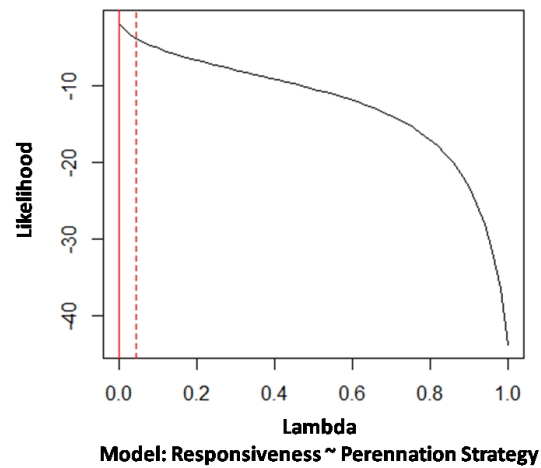


Figure A1.2.4

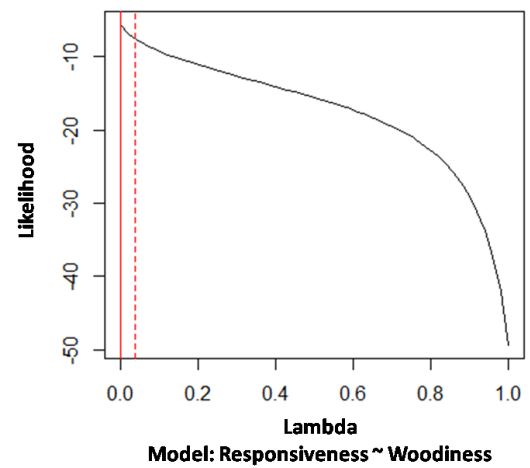


Figure A1.2.5

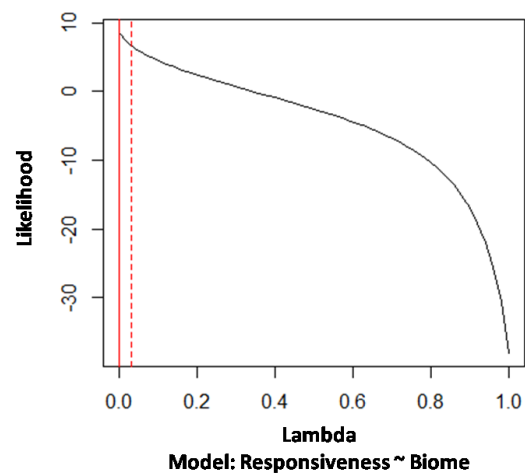


Figure A1.2.6

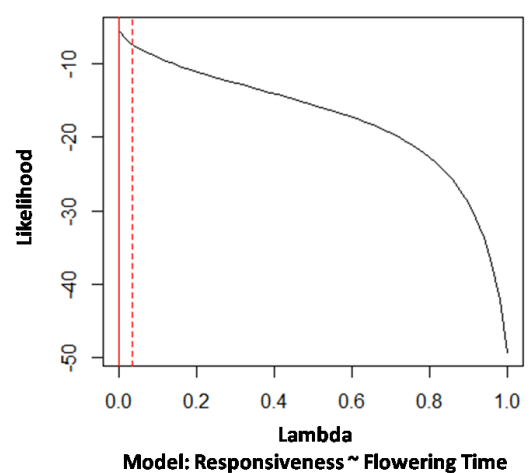


Figure A1.2.7

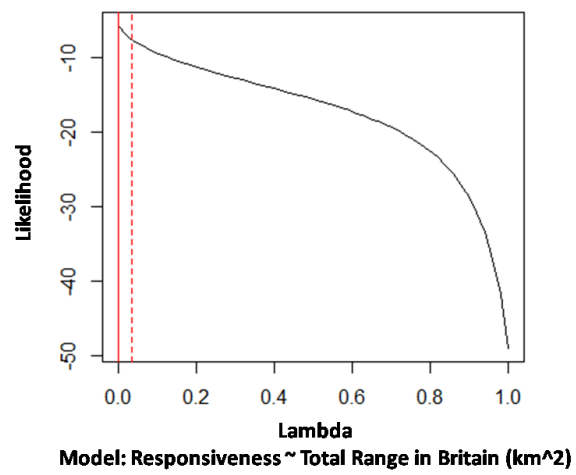


Figure A1.2.8

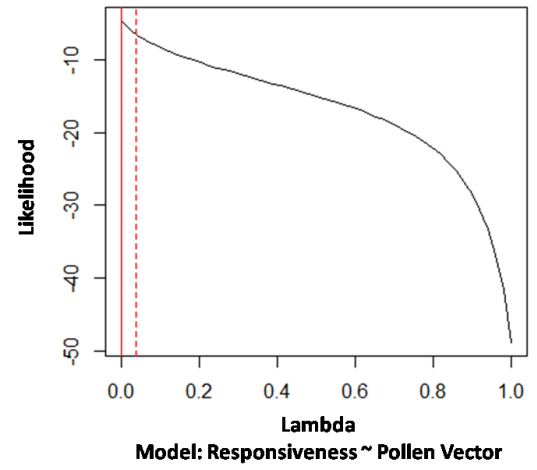
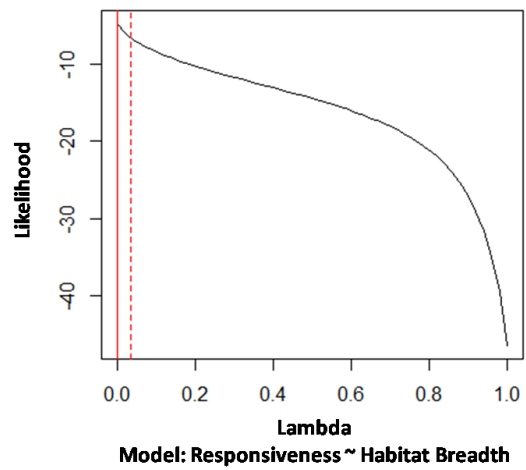


Figure A1.2.9



Appendix 1.3: Least squares means comparisons

Table 1.3.1: Details the least squares means comparisons between levels of the two significant categorical variables in the model selection analysis: Biome and Native Status, for each of the candidate models in this analysis. Southerly refers to species of a Southerly biome, Other refers to species of all other biomes in this analysis (See Table 2.3). Native refers to species Native to the UK, while Non-Native refers to species introduced into the UK.

Model Rank	Trait	Comparison	Estimate	S.E.	df	t-ratio	P-value
1	Biome	Other- Southerly	0.067	0.025	347	2.720	0.007 **
1	Native Status	Native - Non-Native	0.062	0.029	347	2.125	0.034 *
2	Biome	Other- Southerly	0.072	0.025	346	2.873	0.004 **
2	Native Status	Native - Non-Native	0.067	0.029	346	2.260	0.024 *
3	Biome	Other- Southerly	0.067	0.025	346	2.681	0.008 **
3	Native Status	Native - Non-Native	0.057	0.030	346	1.898	0.059 †
4	Biome	Other- Southerly	0.072	0.025	345	2.886	0.004 **
4	Native Status	Native - Non-Native	0.070	0.030	345	2.357	0.019 *
5	Biome	Other- Southerly	0.067	0.025	346	2.694	0.007 **
5	Native Status	Native - Non-Native	0.065	0.029	346	2.193	0.029 *
6	Biome	Other- Southerly	0.071	0.025	345	2.838	0.005 **
6	Native Status	Native - Non-Native	0.068	0.030	345	2.314	0.021 *
7	Biome	Other- Southerly	0.067	0.025	346	2.700	0.007 **
7	Native Status	Native - Non-Native	0.064	0.029	346	2.164	0.031 *
8	Biome	Other- Southerly	0.070	0.025	347	2.803	0.005 **
8	Native Status	Native - Non-Native	0.068	0.029	347	2.330	0.020 *
9	Biome	Other- Southerly	0.065	0.025	348	2.614	0.009 **
9	Native Status	Native - Non-Native	0.071	0.029	348	2.435	0.015 *
10	Biome	Other- Southerly	0.070	0.025	345	2.806	0.005 **
10	Native Status	Native - Non-Native	0.062	0.030	345	2.047	0.041 *

Continued...

Model Rank	Trait	Comparison	Estimate	S.E.	df	t-ratio	P-value
11	Biome	Other- Southerly	0.075	0.025	346	2.959	0.003 **
11	Native Status	Native - Non-Native	0.072	0.029	346	2.468	0.014 *
12	Biome	Other- Southerly	0.066	0.025	345	2.658	0.008 **
12	Native Status	Native - Non-Native	0.059	0.030	345	1.969	0.050 *

0.05≤*P*≤0.10, * 0.01≤*P*≤0.05, ** 0.001≤*P*≤0.01, *** 0.0001≤*P*≤0.001

Appendix Two

Appendix 2.1: Trait Figures

For all figures, Responsiveness represents the extent of change in first arrival date of the bird species in this analysis from 1971-2005, for each respective state. MN = Minnesota, SD = South Dakota.

Figure A2.1.1: Null distribution of the phenological responsiveness of the bird species in this study, in Minnesota.

Figure A2.1.2: Null distribution of the phenological responsiveness of the bird species in this study, in South Dakota.

Figure A2.1.3: Scatterplot of the relationship between bird Mass and phenological responsiveness in Minnesota.

Figure A2.1.4: Scatterplot of the relationship between bird Mass and phenological responsiveness in South Dakota.

Figure A2.1.5: Scatterplot of the relationship between bird mass and phenological responsiveness in Minnesota. Mass is logged to improve distribution with response variable.

Figure A2.1.6: Scatterplot of the relationship between bird Mass and phenological responsiveness in South Dakota. Mass is logged to improve distribution with response variable.

Figure A2.1.7: Scatterplot of the relationship between Maximum Clutch Size and phenological responsiveness in Minnesota.

Figure A2.1.8: Scatterplot of the relationship between Maximum Clutch Size and phenological responsiveness in South Dakota.

Figure A2.1.9: Scatterplot of the relationship between Maximum Clutch Size and phenological responsiveness in Minnesota. Maximum Clutch Size is logged to improve distribution with response variable.

Figure A2.1.10: Scatterplot of the relationship between Maximum Clutch Size and phenological responsiveness in South Dakota. Maximum Clutch Size is logged to improve distribution with response variable.

Figure A2.1.11: Scatterplot of the relationship between Maximum Longevity and phenological responsiveness in Minnesota.

Figure A2.1.12: Scatterplot of the relationship between Maximum Longevity and phenological responsiveness in South Dakota.

Figure A2.1.13: Scatterplot of the relationship between Maximum Longevity and phenological responsiveness in Minnesota. Maximum Longevity is logged to improve distribution with response variable.

Figure A2.1.14: Scatterplot of the relationship between Maximum Longevity and phenological responsiveness in South Dakota. Maximum Longevity is logged to improve distribution with response variable.

Figure A2.1.15: Scatterplot of the relationship between Diet Breadth and phenological responsiveness in Minnesota.

Figure A2.1.16: Scatterplot of the relationship between Diet Breadth and phenological responsiveness in South Dakota.

Figure A2.1.17: Scatterplot of the relationship between Habitat Breadth and phenological responsiveness in Minnesota.

Figure A2.1.18: Scatterplot of the relationship between Habitat Breadth and phenological responsiveness in South Dakota.

Figure A2.1.19: Scatterplot of the relationship between Habitat Breadth and phenological responsiveness in Minnesota. Habitat Breadth is logged to improve distribution with response variable.

Figure A2.1.20: Scatterplot of the relationship between Habitat Breadth and phenological responsiveness in South Dakota. Habitat Breadth is logged to improve distribution with response variable.

Figure A2.1.21: Notched box-plot of the relationship between a bird's migratory distance and its phenological responsiveness in Minnesota. Migratory Distance is a factor with two levels: short and long. If notches do not overlap between two levels, this provides strong evidence that the medians for those levels differ (Chambers *et al.* 1983). Black dots represent outliers.

Figure A2.1.22: Notched box-plot of the relationship between a bird's migratory distance and its phenological responsiveness in South Dakota. Migratory distance is a factor with two levels: short and long. If notches do not overlap between two levels, this provides strong evidence that the medians for those levels differ (Chambers *et al.* 1983). Black dots represent outliers.

Figure A2.1.1

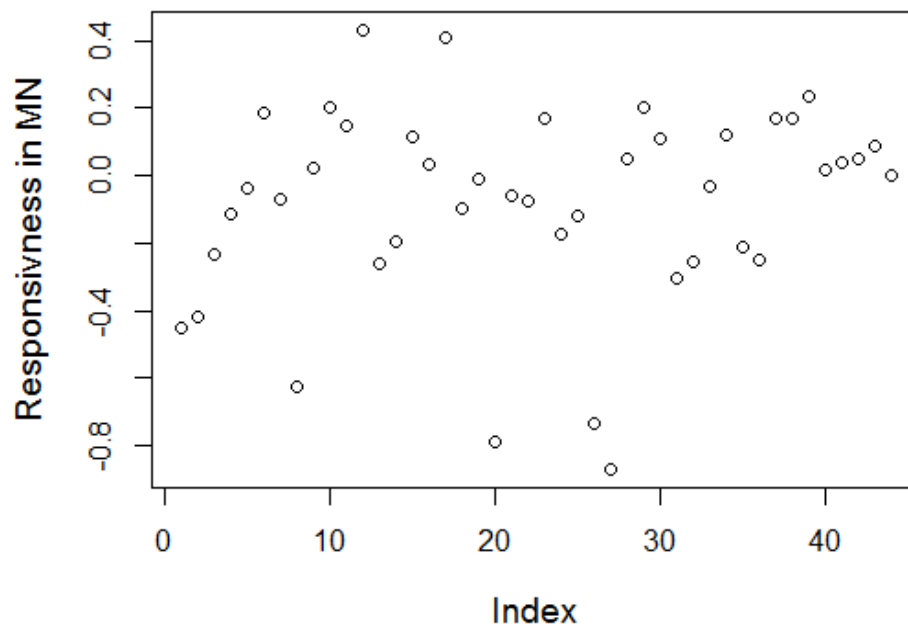


Figure A2.1.2

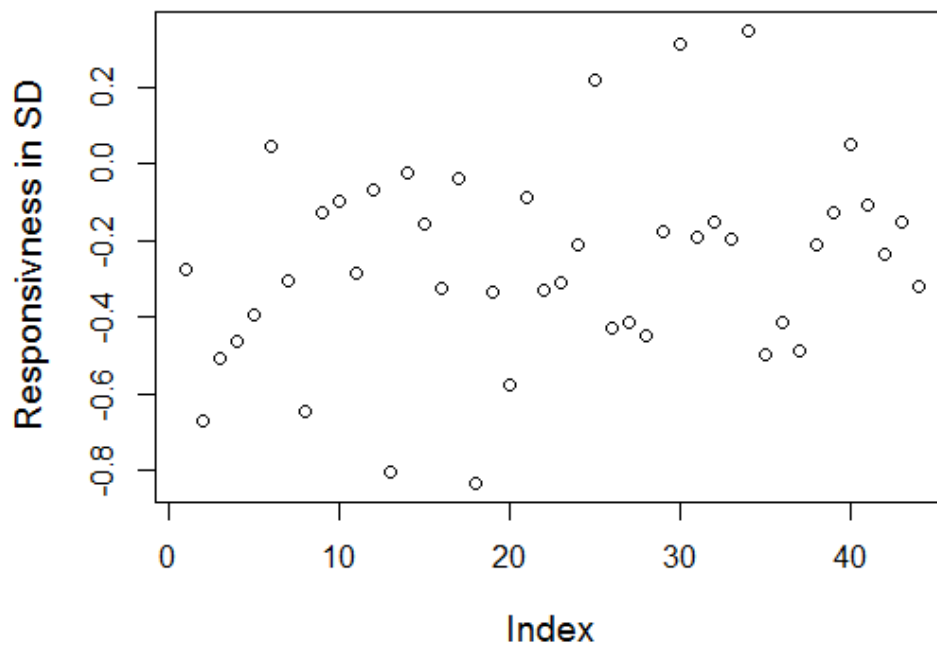


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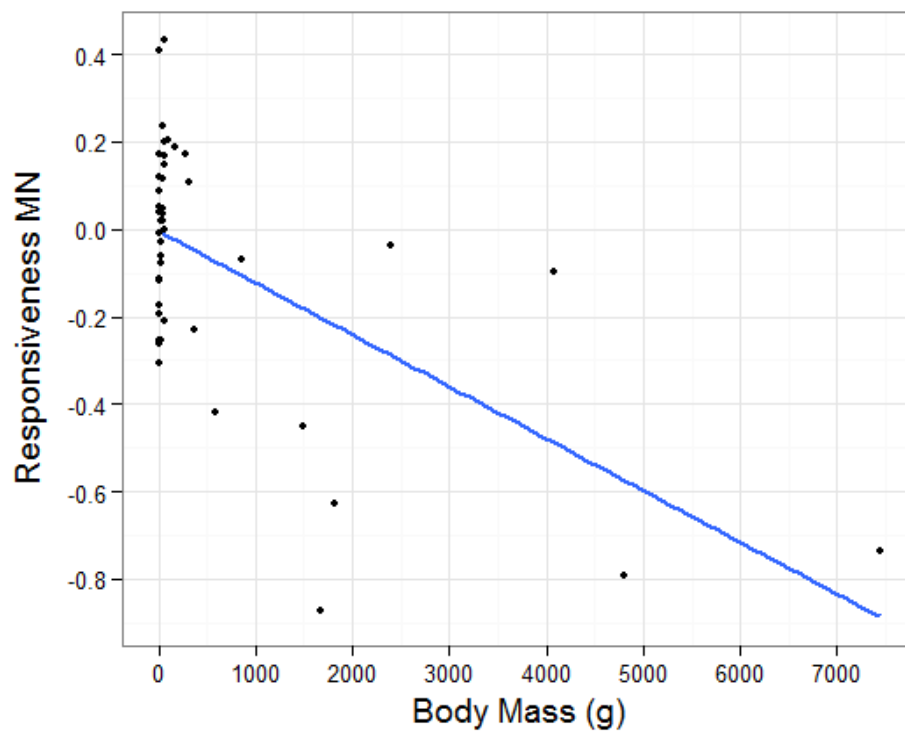


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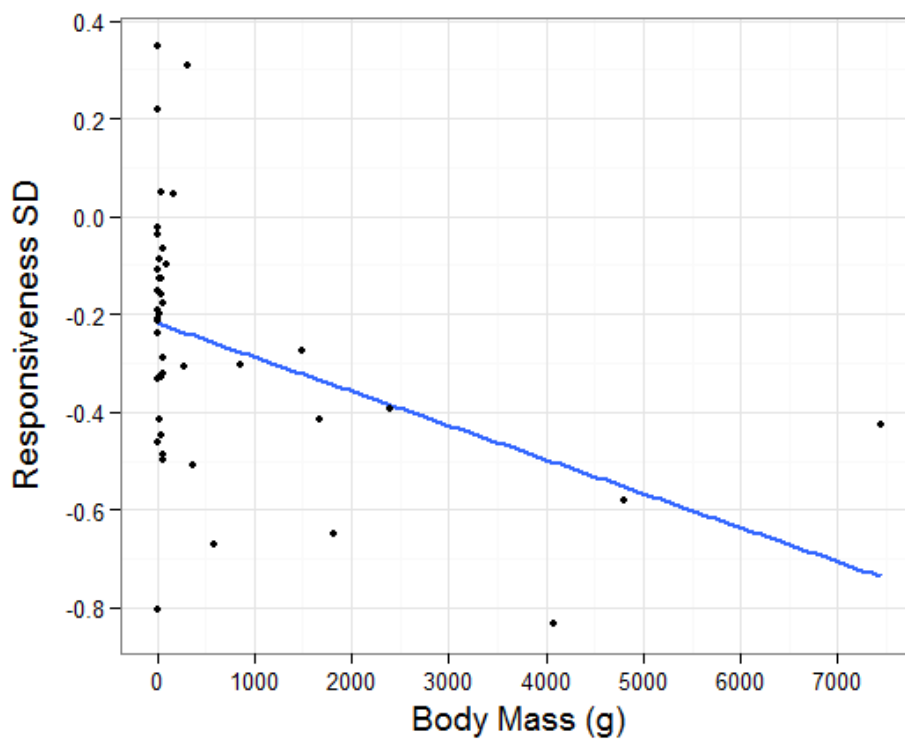


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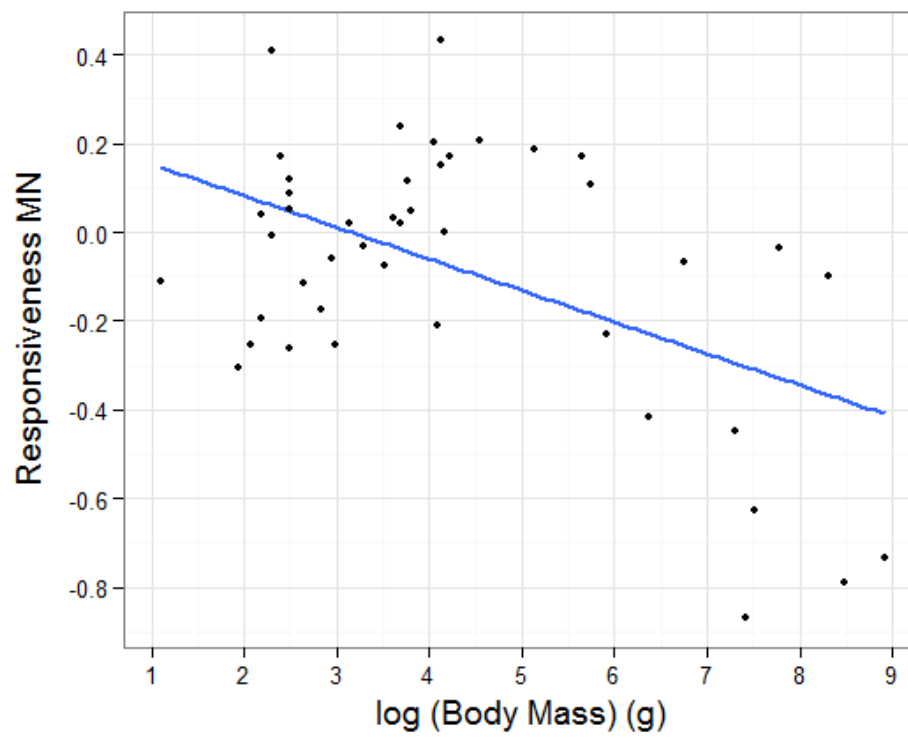


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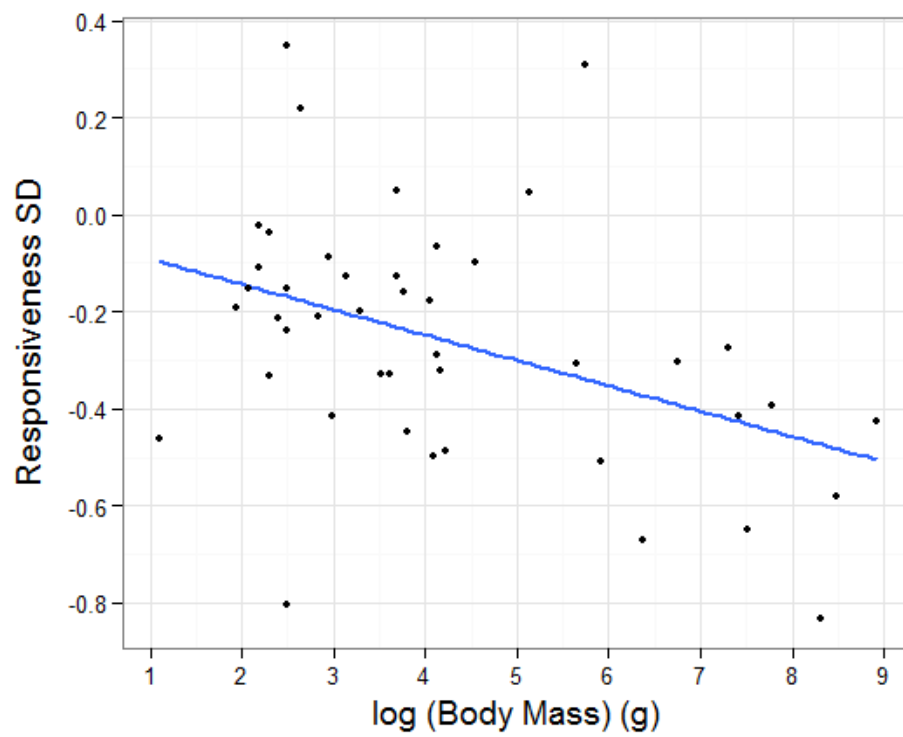


Figure A2.1.7

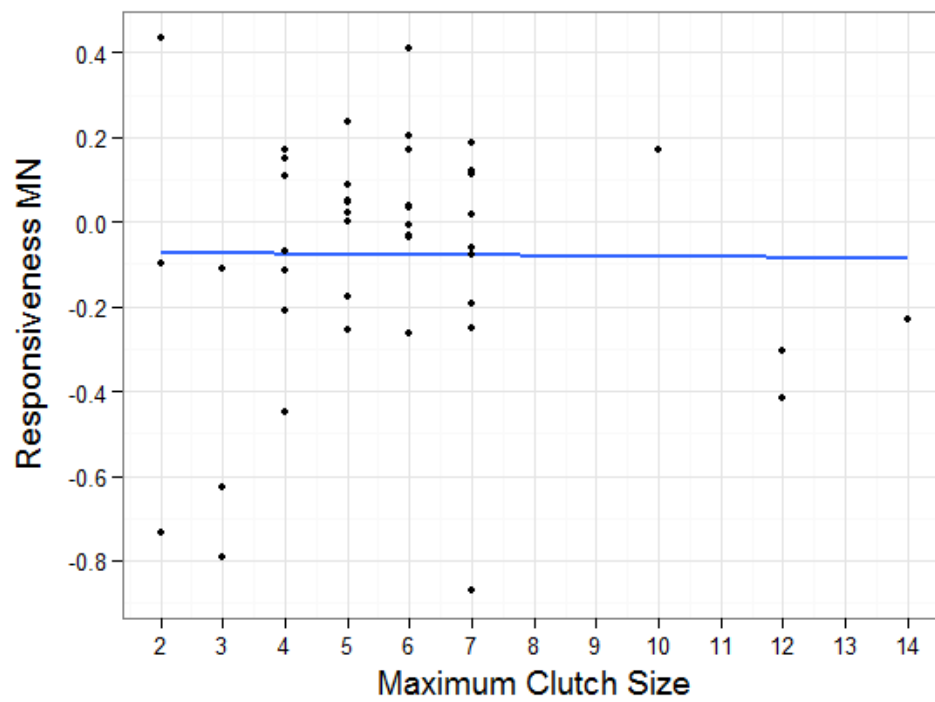


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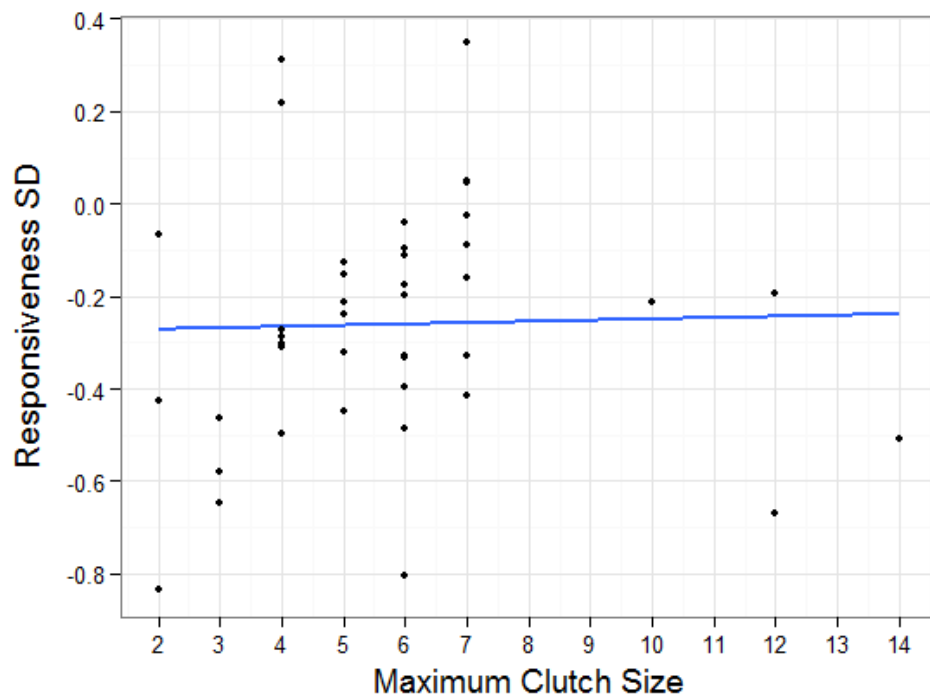


Figure A2.1.9

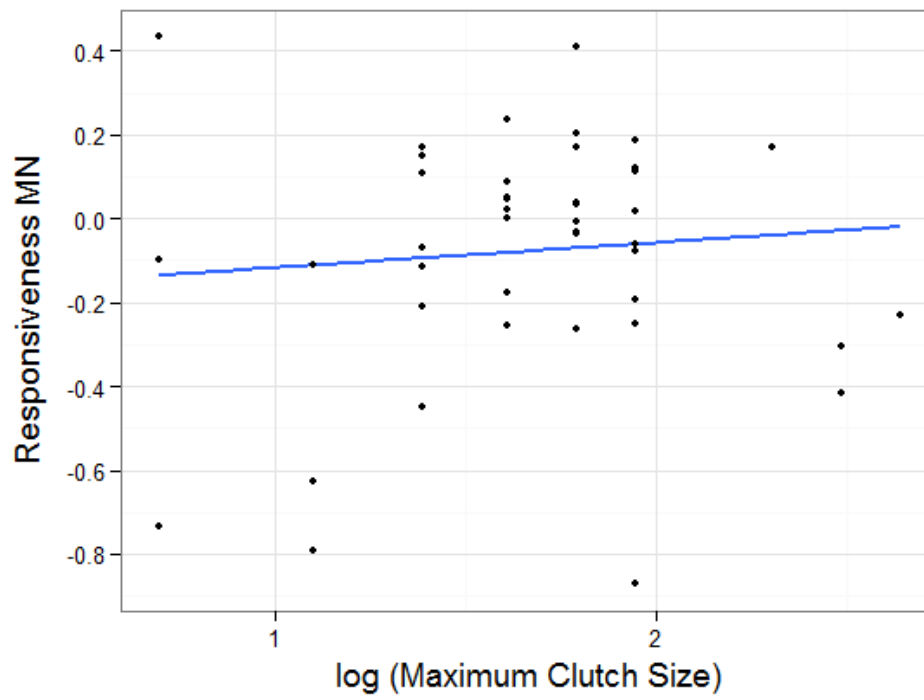


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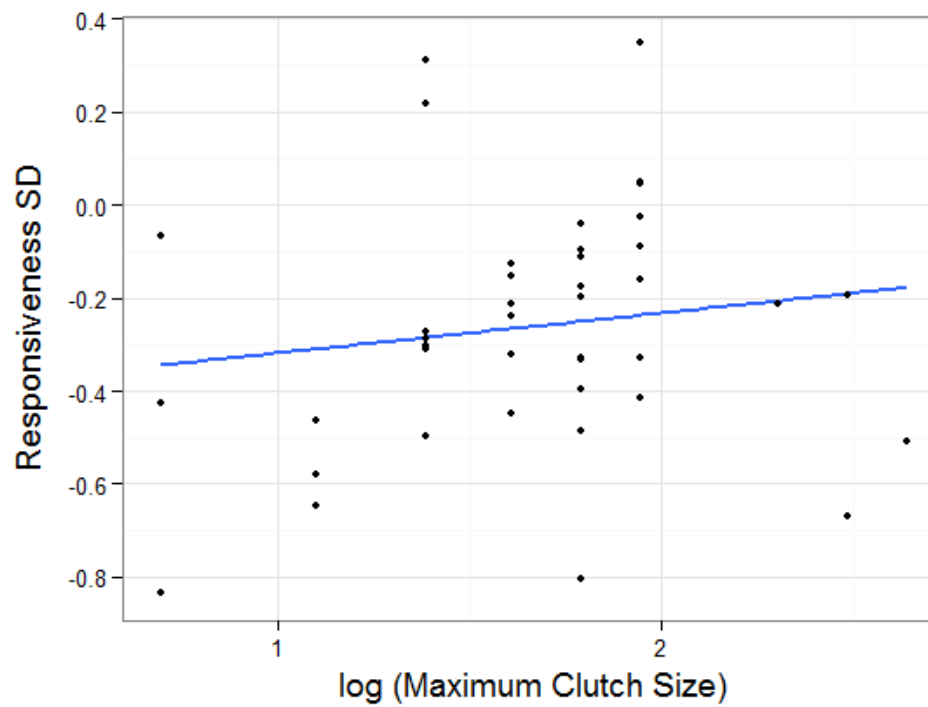


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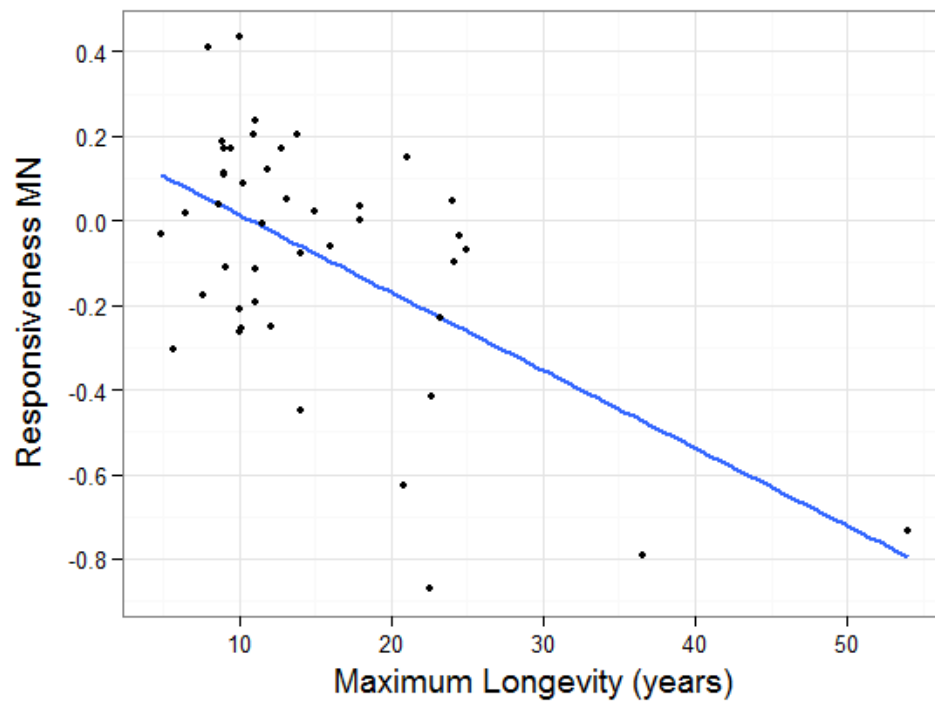


Figure A2.1.12

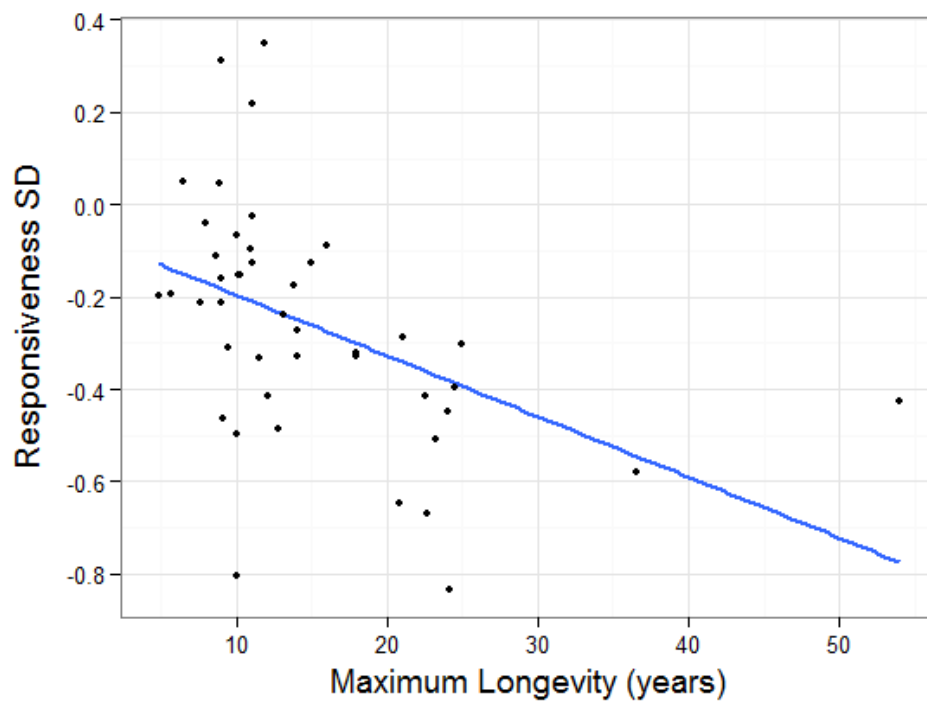


Figure A2.1.13

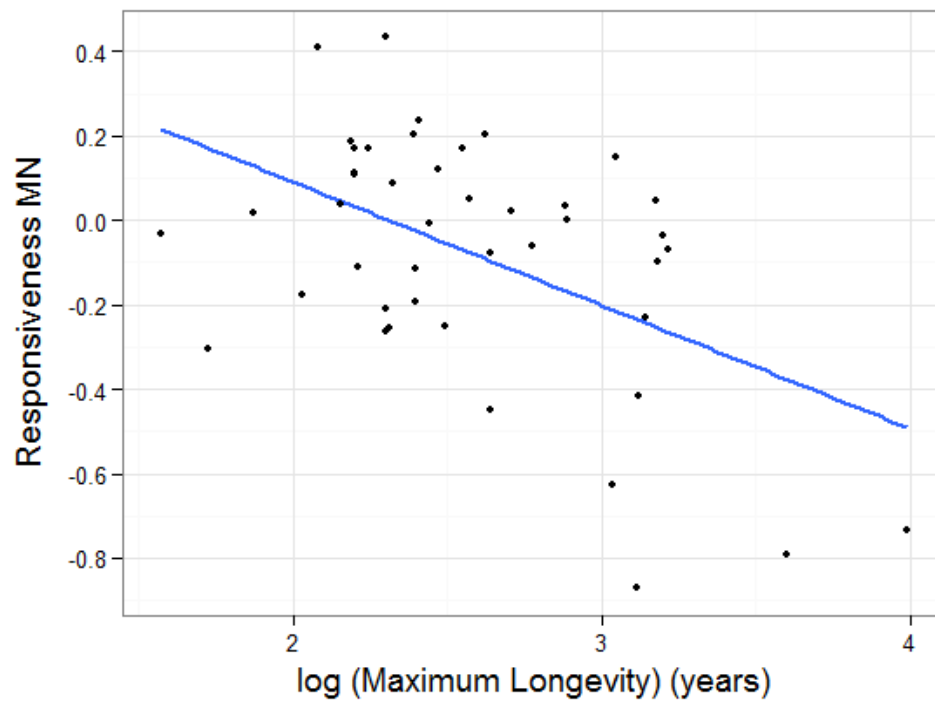


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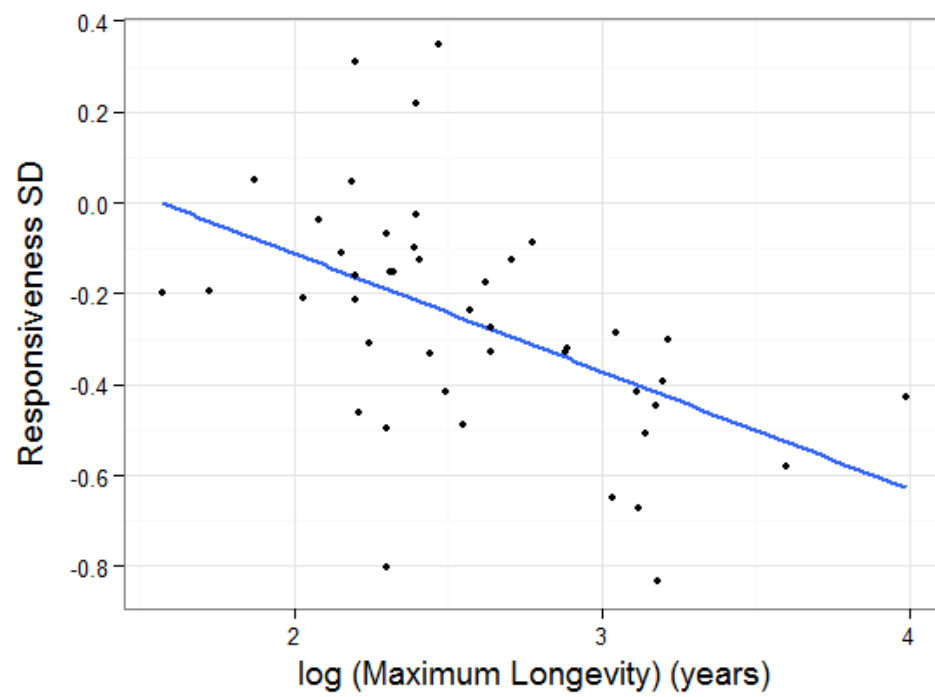


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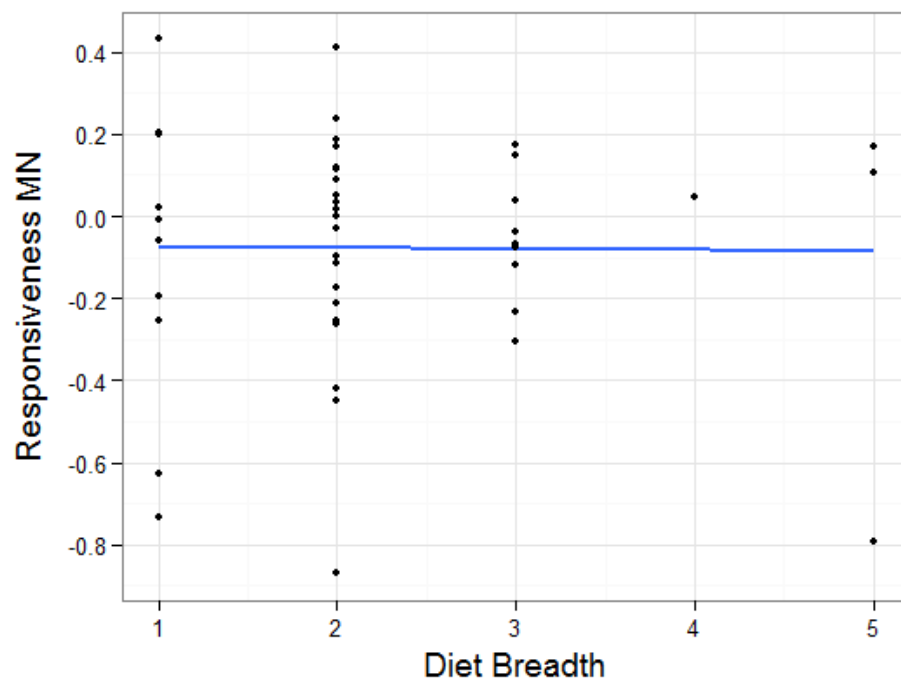


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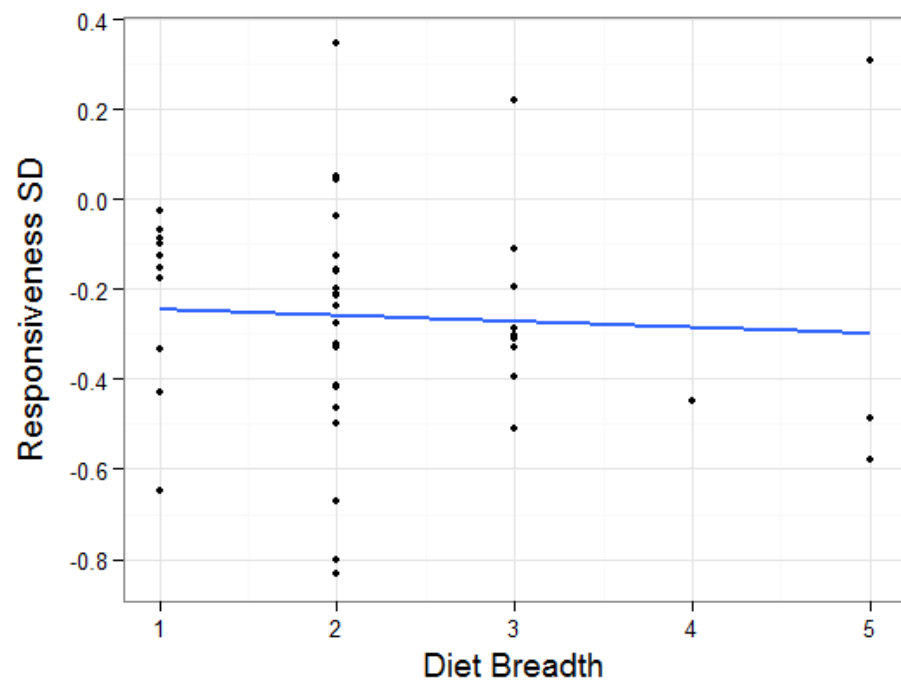


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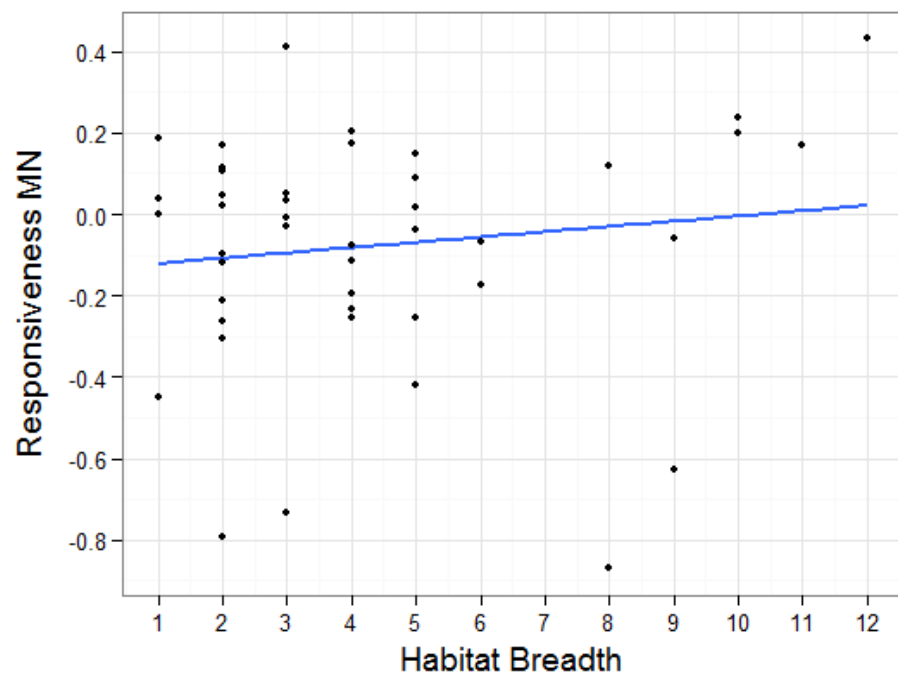


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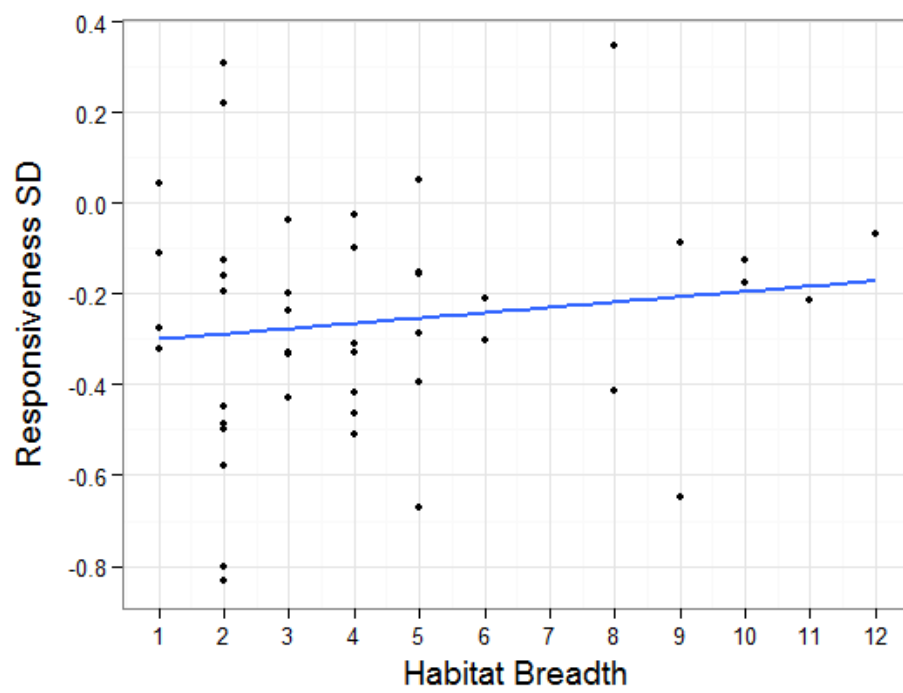


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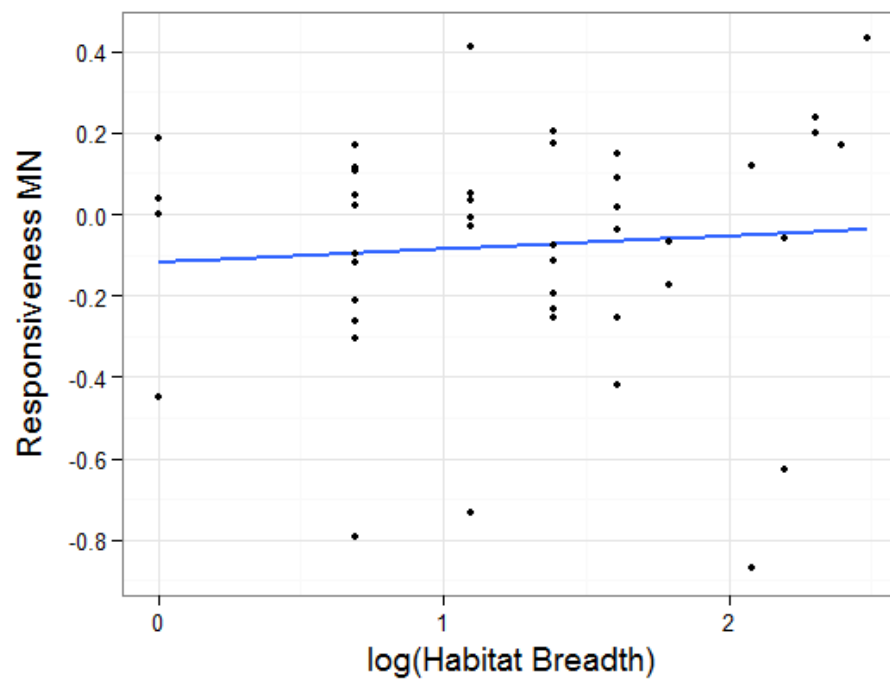


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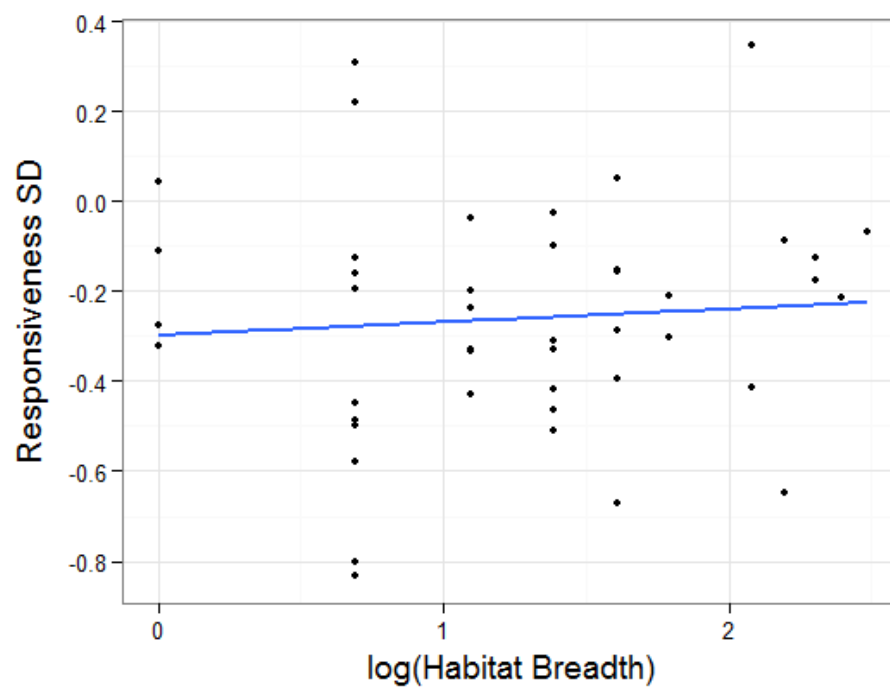


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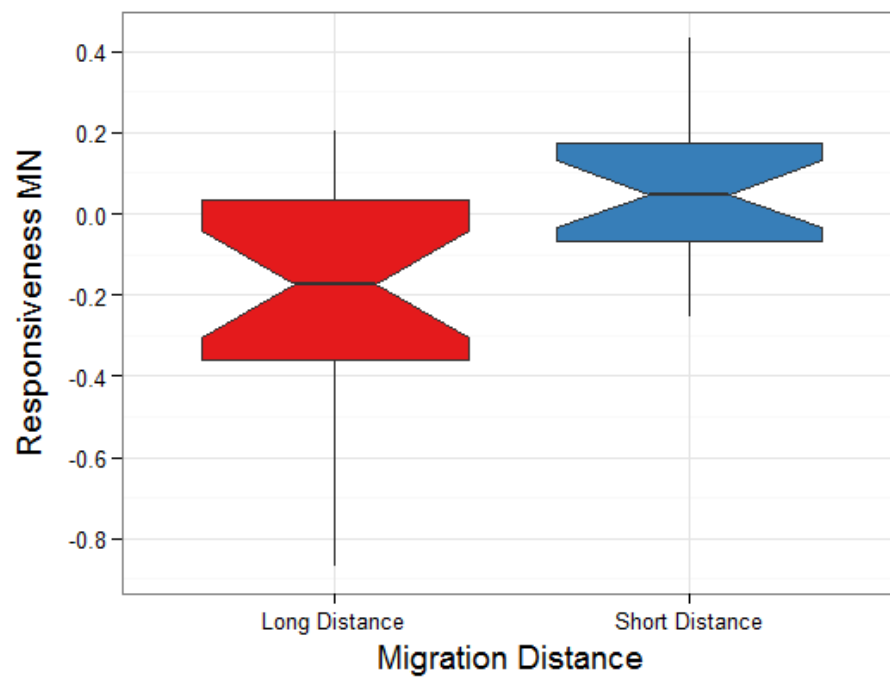
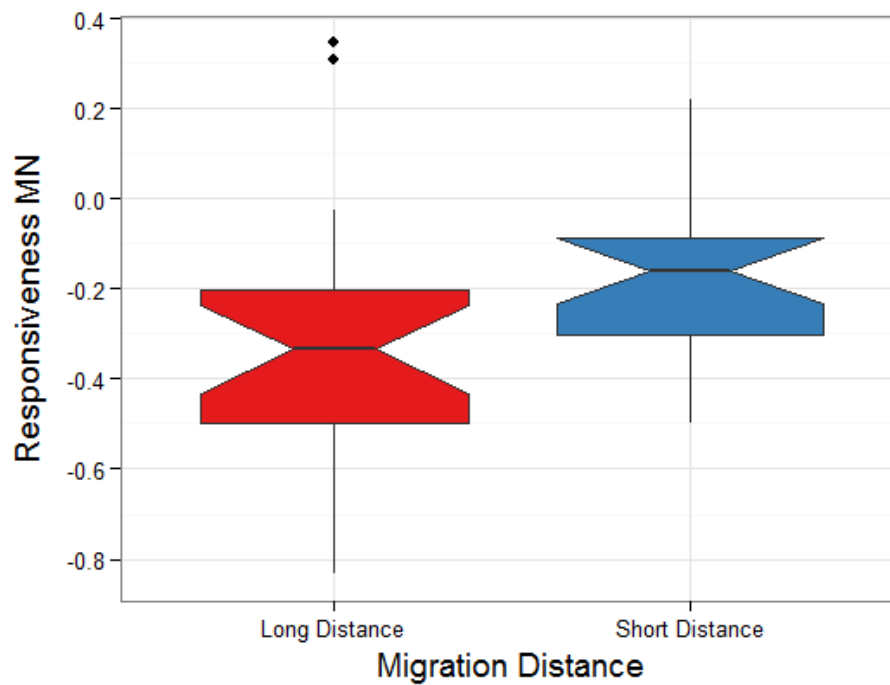


Figure A2.1.22



Appendix 2.2: Lambda maximum likelihood profile plots

Figures A2.2.1-A2.2.20 present the likelihood profiles for the estimation of a maximum likelihood (ML) lambda value for each relationship between Responsiveness and each trait, in each state. These estimates are bounded by 0 and 1. ML lambda estimate represented by solid red line. Dotted red lines either side of ML lambda estimate represent 95% confidence intervals around the ML lambda estimate. A missing upper or lower bound confidence interval indicates that interval was at the upper or lower bound of lambda, respectively. Responsiveness represents the extent of change in first arrival date of the species in this analysis from 1971-2005, for each state. MN = Minnesota, SD = South Dakota.

The specific models are:

Figure A2.2.1: Responsiveness $\sim$ log (Mass) (MN)

Figure A2.2.2: Responsiveness $\sim$ log (Mass) (SD)

Figure A2.2.3: Responsiveness $\sim$ log (Maximum Clutch Size) (MN)

Figure A2.2.4: Responsiveness $\sim$ log (Maximum Clutch Size) (SD)

Figure A2.2.5: Responsiveness $\sim$ log (Maximum Longevity) (MN)

Figure A2.2.6: Responsiveness $\sim$ log (Maximum Longevity) (SD)

Figure A2.2.7: Responsiveness $\sim$ Diet Breadth (MN)

Figure A2.2.8: Responsiveness $\sim$ Diet Breadth (SD)

Figure A2.2.9: Responsiveness $\sim$ log (Habitat Breadth) (MN)

Figure A2.2.10: Responsiveness $\sim$ log (Habitat Breadth) (SD)

Figure A2.2.11: Responsiveness $\sim$ Migration Distance (MN)

Figure A2.2.12: Responsiveness $\sim$ Migration Distance (SD)

Figure A2.2.1

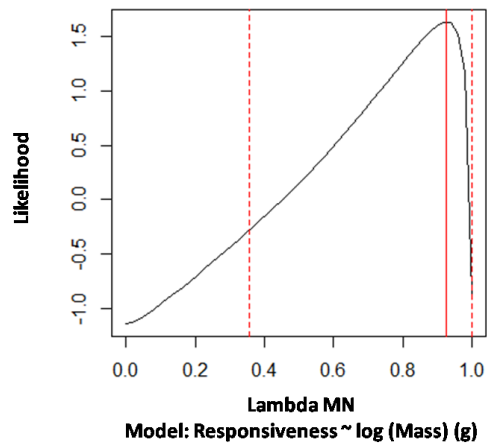


Figure A2.2.2

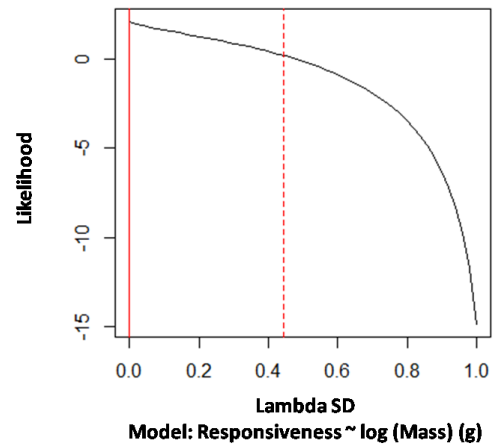


Figure A2.2.3

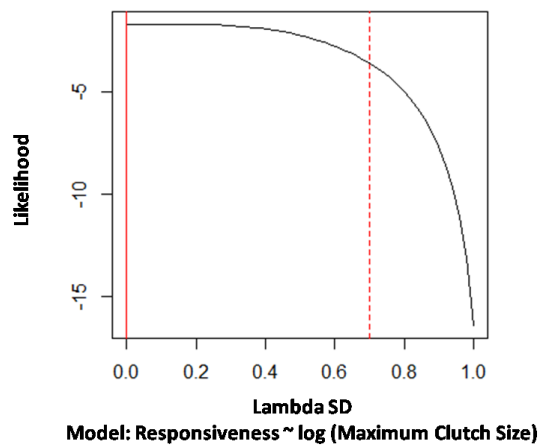


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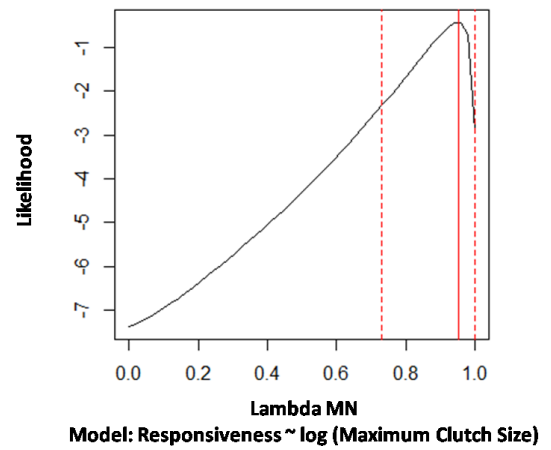


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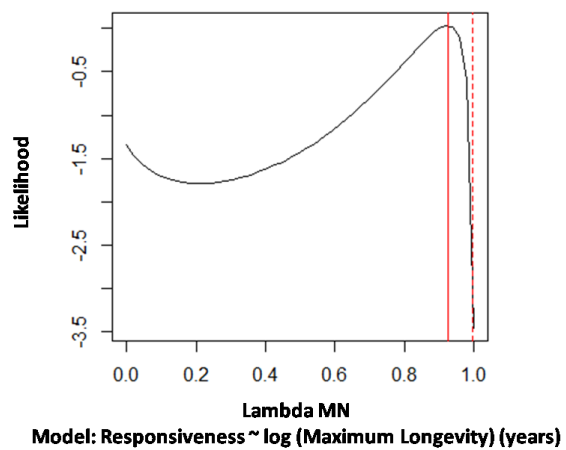


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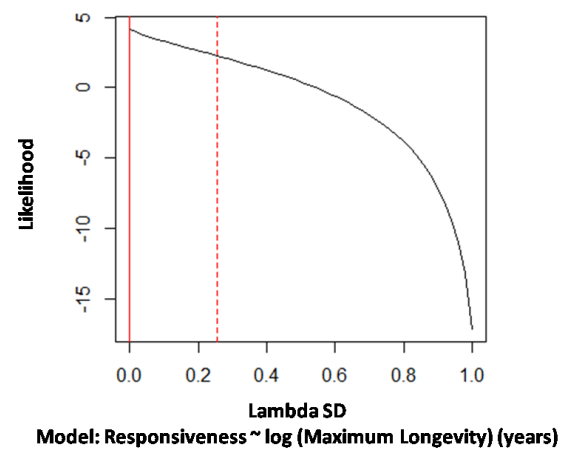


Figure A2.2.7



Figure A2.2.8

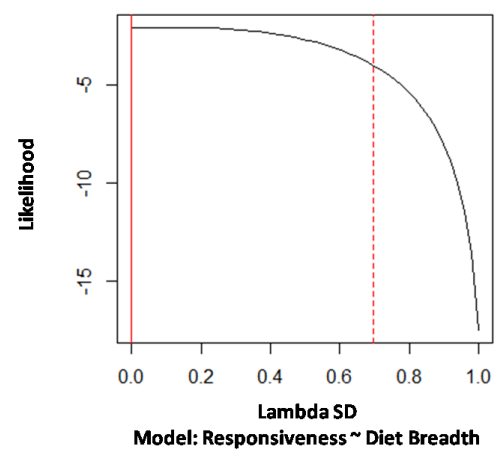


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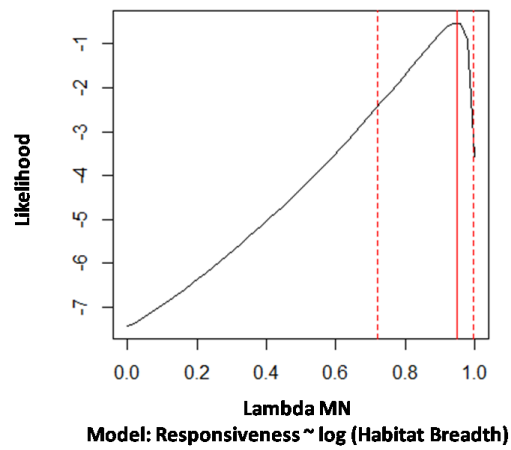


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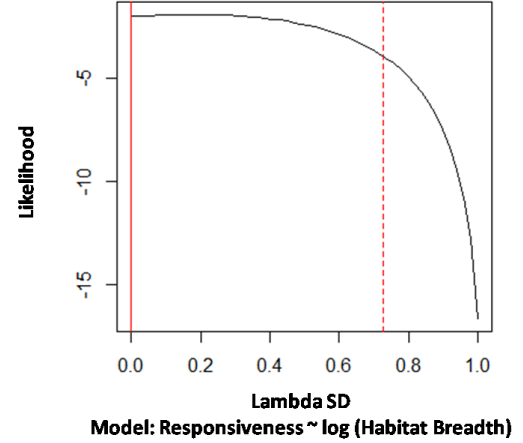


Figure A2.2.11

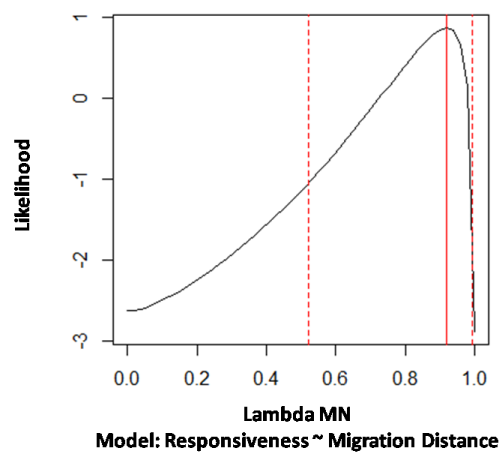
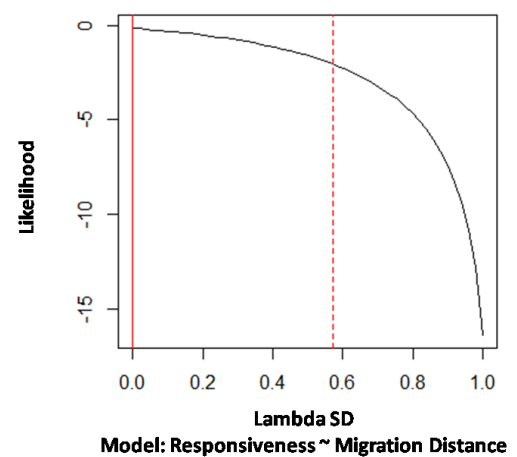


Figure A2.2.12



Appendix 2.3: Comparisons of changes in bird first arrival dates between states

Are the responses of individual species different between states?

Before investigating how consistent traits are in predicting FAD change between states, it is instructive to first examine the geographical consistency in the phenological responses of the individual study species between them. I combined the raw response data for Minnesota and South Dakota. Coding Area as a two level factor (Minnesota/South Dakota), I ran three regressions for each species:

- 4) $FAD \sim Year * Area$ (i.e. fitting two separate slopes for Area)
- 5) $FAD \sim Year + Area$ (i.e. fitting one common slope for Area)
- 6) $FAD \sim Year$

The fit of these models was compared using Akaike Information Criterion (AIC). A difference of >2 between the AIC values of two compared models suggests a difference in fit, with the model with the smaller AIC value fitting better. An improved fit by model 2 over model 3 indicates that the intercept of a species' response varies between states (i.e. there is a magnitude difference in the FAD trends with time between states). An improved fit of model 1 over model 2 indicates that the slope of a species' response varies between states (i.e. the rate of change in FAD with time varies between states). If there is no improved fit in either case, the simpler model should be chosen.

Table A2.3.1 details the AIC values of each model for each species, and the differences between these values for Model 1 and Model 2 (Model 1 AIC minus Model 2 AIC), and for Model 2 and Model 3 (Model 1 AIC minus Model 2 AIC). An AIC difference of <-2 in both comparisons indicates that the first (more complex) model in both fits better.

Table A2.3.1

Species	AIC Model 1	df	AIC Model 2	df	AIC Model 3	df	AIC difference between Model 1 and Model 2	AIC difference between Model 2 and Model 3
<i>Aechmophorus occidentalis</i>	382.818	5	381.824	4	388.055	3	0.993	-6.231
<i>Anas clypeata</i>	345.838	5	345.324	4	346.616	3	0.514	-1.292
<i>Anas discors</i>	401.623	5	401.046	4	403.673	3	0.577	-2.627
<i>Archilochus colubris</i>	316.332	5	322.374	4	374.445	3	-6.043	-52.071
<i>Ardea herodias</i>	353.404	5	355.572	4	388.377	3	-2.168	-32.805
<i>Bartramia longicauda</i>	388.707	5	387.098	4	387.750	3	1.609	-0.652
<i>Buteo swainsoni</i>	400.367	5	399.454	4	397.469	3	0.913	1.985
<i>Cathartes aura</i>	398.282	5	396.288	4	418.990	3	1.994	-22.702
<i>Chaetura pelagica</i>	376.187	5	375.354	4	390.854	3	0.833	-15.500
<i>Charadrius vociferus</i>	334.866	5	337.254	4	341.119	3	-2.389	-3.865
<i>Chlidonias niger</i>	383.708	5	386.275	4	403.037	3	-2.567	-16.762
<i>Chordeiles minor</i>	378.714	5	380.418	4	406.864	3	-1.704	-26.445
<i>Dendroica coronata</i>	412.459	5	414.167	4	414.844	3	-1.708	-0.677
<i>Dendroica petechia</i>	331.600	5	331.063	4	336.090	3	0.537	-5.027
<i>Dolichonyx oryzivorus</i>	411.313	5	410.649	4	411.246	3	0.664	-0.597
<i>Dumetella carolinensis</i>	455.855	5	454.894	4	458.508	3	0.960	-3.614
<i>Empidonax minimus</i>	362.208	5	368.102	4	384.603	3	-5.893	-16.501
<i>Gavia immer</i>	405.432	5	412.719	4	413.823	3	-7.288	-1.104
<i>Geothlypis trichas</i>	314.989	5	319.909	4	321.336	3	-4.920	-1.427
<i>Grus canadensis</i>	381.315	5	380.934	4	390.993	3	0.381	-10.059
<i>Hirundo rustica</i>	379.471	5	377.502	4	393.615	3	1.969	-16.112
<i>Icterus galbula</i>	348.458	5	347.717	4	354.833	3	0.741	-7.116
<i>Larus pipixcan</i>	442.082	5	443.814	4	442.319	3	-1.733	1.496
<i>Melospiza lincolni</i>	379.409	5	377.428	4	377.354	3	1.980	0.074
<i>Passerina cyanea</i>	340.846	5	341.994	4	341.994	3	-1.148	0.000
<i>Pelecanus erythrorhynchos</i>	396.690	5	396.075	4	394.786	3	0.615	1.289

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<i>Phalacrocorax auritus</i>	384.365	5	387.247	4	398.752	3	-2.883	-11.505
<i>Phalaropus tricolor</i>	337.344	5	344.435	4	346.683	3	-7.091	-2.248
<i>Pheucticus ludovicianus</i>	354.812	5	356.709	4	376.708	3	-1.897	-19.999
<i>Progne subis</i>	394.957	5	394.200	4	403.980	3	0.757	-9.780
<i>Recurvirostra americana</i>	388.106	5	386.552	4	406.320	3	1.554	-19.768
<i>Regulus calendula</i>	399.935	5	398.137	4	408.593	3	1.798	-10.456
<i>Setophaga ruticilla</i>	310.286	5	309.945	4	320.236	3	0.341	-10.291
<i>Spiza americana</i>	382.104	5	380.877	4	379.094	3	1.227	1.783
<i>Spizella passerina</i>	406.425	5	405.492	4	408.534	3	0.933	-3.042
<i>Tachycineta bicolor</i>	390.692	5	389.963	4	464.047	3	0.729	-74.084
<i>Toxostoma rufum</i>	422.885	5	424.262	4	426.340	3	-1.377	-2.078
<i>Troglodytes aedon</i>	362.268	5	364.937	4	373.238	3	-2.669	-8.302
<i>Tyrannus tyrannus</i>	389.180	5	390.213	4	408.808	3	-1.032	-18.596
<i>Tyrannus verticalis</i>	338.946	5	337.014	4	362.560	3	1.932	-25.546
<i>Vermivora celata</i>	345.951	5	345.341	4	347.081	3	0.611	-1.741
<i>Vireo gilvus</i>	332.945	5	336.343	4	351.739	3	-3.399	-15.395
<i>Vireo olivaceus</i>	309.234	5	309.652	4	319.104	3	-0.418	-9.452
<i>Xanthocephalus xanthocephalus</i>	382.582	5	381.801	4	381.306	3	0.780	0.495

Appendix Three

Appendix 3.1: Treatment means

Table A3.1.1: Treatment means for proportions (relative abundance) of nitrogen-fixers as a functional group, *Lotus micranthus*, *Lotus wrangelianus*, *Trifolium purpureum*, *Lupinus bicolor* and Other Fixers (denoting all fixers except *L. micranthus*) in experimental plots from 2002-2010. Proportions calculated as number of pointframe hits for each group in each treatment, divided by the total number of pointframe hits for each treatment (not including bare ground, litter and moss hits). W+ denotes water-addition plots; WC denotes water-control plots.

Table A3.1.2: Treatment means for aboveground net primary production, perennial grass production, annual grass production, perennial forb production, annual forb production and nitrogen-fixer production in experimental plots from 2001-2010, in units of g/m². W+ denotes water-addition plots; WC denotes water-control plots.

Table A3.1.3: Treatment means for plant diversity in experimental plots from 2001-2010, measured as number of species. W+ denotes water-addition plots; WC denotes water-control plots.

Table A3.1.4: Treatment means for the C:N ratio of *Bromus hordeaceus* in experimental plots from 2001-2006. W+ denotes water-addition plots; WC denotes water-control plots.

Table A3.1.5: Treatment means for soil nitrogen concentrations in experimental plots from 2006-2008, in units of µg/cm². W+ denotes water-addition plots; WC denotes water-control plots.

Table A3.1.1

Year	Treatment	Proportion of Nitrogen-fixers	Proportion of <i>Lotus micranthus</i>	Proportion of <i>Lotus wrangelianus</i>	Proportion of <i>Trifolium purpureum</i>	Proportion of <i>Lupinus bicolor</i>	Proportion of Other Fixers
2002	W+	0.209	0.144	0.000	0.064	0.000	0.064
2002	WC	0.141	0.052	0.008	0.043	0.027	0.090
2003	W+	0.180	0.147	0.000	0.026	0.008	0.033
2003	WC	0.144	0.089	0.000	0.033	0.022	0.055
2004	W+	0.106	0.082	0.000	0.024	0.000	0.024
2004	WC	0.091	0.052	0.000	0.039	0.000	0.039
2005	W+	0.119	0.098	0.000	0.021	0.000	0.021
2005	WC	0.082	0.040	0.000	0.042	0.000	0.042
2006	W+	0.017	0.017	0.000	0.000	0.000	0.000
2006	WC	0.000	0.000	0.000	0.000	0.000	0.000
2007	W+	0.217	0.176	0.013	0.026	0.000	0.041
2007	WC	0.159	0.082	0.000	0.047	0.030	0.077
2008	W+	0.208	0.110	0.000	0.056	0.011	0.098
2008	WC	0.315	0.225	0.000	0.047	0.072	0.090
2009	W+	0.316	0.250	0.000	0.056	0.010	0.066
2009	WC	0.139	0.063	0.006	0.032	0.037	0.077
2010	W+	0.255	0.241	0.001	0.010	0.002	0.013
2010	WC	0.189	0.070	0.005	0.063	0.050	0.118

Table A3.1.2

Year	Treatment	ANPP	Perennial Forb Productivity (g/m ²)	Annual Forb Productivity (g/m ²)	Perennial Grass Productivity (g/m ²)	Annual Grass Productivity (g/m ²)	Nitrogen-fixer Productivity (g/m ²)
2001	W+	257.686	13.067	29.343	31.268	87.156	96.853
2001	WC	81.670	6.160	17.116	11.829	46.565	0.000
2002	W+	377.153	58.717	33.310	15.864	145.306	123.955
2002	WC	182.433	23.759	43.733	15.428	70.486	29.026
2003	W+	321.005	7.439	17.731	16.807	188.130	90.898
2003	WC	147.700	14.355	37.766	3.438	70.922	21.220
2004	W+	281.712	7.902	13.447	31.601	202.347	26.414
2004	WC	200.486	12.631	40.450	7.796	130.209	9.401
2005	W+	283.314	15.748	19.762	22.463	177.442	47.900
2005	WC	249.070	12.596	79.056	4.680	137.574	15.163
2006	W+	329.097	14.054	63.947	48.801	179.565	22.729
2006	WC	201.771	13.188	79.751	15.897	92.898	0.037
2007	W+	379.382	6.879	91.819	39.996	192.555	48.134
2007	WC	175.628	12.434	49.344	18.332	60.799	34.719
2008	W+	405.388	8.515	58.111	54.717	151.614	132.431
2008	WC	205.931	14.432	46.819	20.368	93.111	31.201
2009	W+	368.040	4.238	58.051	58.976	152.647	94.129
2009	WC	200.632	6.181	58.076	11.388	102.953	22.035
2010	W+	252.197	3.981	52.893	40.098	114.470	40.755
2010	WC	166.854	18.359	41.237	18.980	74.104	14.175

Table A3.1.3

Year	Treatment	Plant Diversity
2001	W+	18.583
2001	WC	11.667
2002	W+	17.333
2002	WC	14.667
2003	W+	14.167
2003	WC	15.833
2004	W+	7.417
2004	WC	13.500
2005	W+	9.333
2005	WC	17.500
2006	W+	9.333
2006	WC	11.500
2007	W+	10.500
2007	WC	15.833
2008	W+	11.333
2008	WC	16.083
2009	W+	11.083
2009	WC	18.083
2010	W+	8.417
2010	WC	17.083

Table A3.1.4

Year	Treatment	Bromus hordeaceus C:N ratio
2001	W+	23.148
2001	WC	24.805
2002	W+	20.997
2002	WC	24.807
2003	W+	21.591
2003	WC	23.365
2004	W+	20.115
2004	WC	25.508
2005	W+	23.703
2005	WC	25.912
2006	W+	17.887
2006	WC	21.429

Table A3.1.5

Year	Treatment	Soil Nitrogen Concentration (µg/10 cm ²)
2006	W+	14.467
2006	WC	12.333
2007	W+	15.950
2007	WC	9.700
2008	W+	16.850
2008	WC	7.933

Appendix Four

Appendix 4.1: Treatment means

Table A4.1.1: Treatment means for aboveground net primary production, perennial grass production, annual grass production, perennial forb production and annual forb production in each year (2010-2013) in units of g/m^2 . W+ denotes water-addition plots; WC denotes water-control plots; W+NX denotes water-addition/nitrogen-fixer-removal plots; W+NC denotes water-addition/nitrogen-fixer-control plots; WCNX denotes water-control/nitrogen-fixer-removal plots; WCNC denotes water-control/nitrogen-fixer-control plots.

Table A4.1.2: Treatment means for total plant species diversity, forb species diversity and grass species diversity for each year (2010-2013), measured as numbers of species. W+ denotes water-addition plots; WC denotes water-control plots; W+NX denotes water-addition/nitrogen-fixer-removal plots; W+NC denotes water-addition/nitrogen-fixer-control plots; WCNX denotes water-control/nitrogen-fixer-removal plots; WCNC denotes water-control/nitrogen-fixer-control plots.

Table A4.1.1

Treatment	Year	ANPP (g/m²)	Perennial Grass Productivity (g/m²)	Annual Grass Productivity (g/m²)	Perennial Forb Productivity (g/m²)	Annual Forb Productivity (g/m²)
W+	2010	211.442	40.098	114.470	3.981	52.893
WC	2010	152.679	18.980	74.104	18.359	41.237
W+N-	2011	178.204	14.017	134.912	14.943	14.332
W+NC	2011	143.356	34.108	85.103	16.276	7.870
WCN-	2011	150.078	0.000	116.081	14.961	19.035
WCNC	2011	203.869	29.645	134.283	25.331	14.610
W+N-	2012	242.217	71.660	125.543	5.592	39.422
W+NC	2012	245.235	38.978	150.763	13.795	41.700
WCN-	2012	136.709	4.851	120.192	4.055	7.610
WCNC	2012	232.662	0.648	218.200	6.907	6.907
W+N-	2013	217.991	49.606	94.091	9.536	64.758
W+NC	2013	286.166	71.548	152.490	5.462	56.665
WCN-	2013	168.707	22.146	111.267	4.148	31.147
WCNC	2013	164.702	2.907	117.472	8.888	35.435

Table A4.1.2

Treatment	Year	Plant Diversity (no of species)	Forb Diversity (no of species)	Grass Diversity (no of species)
W+	2010	7.333	3.583	3.417
WC	2010	12.417	6.917	4.250
W+N-	2011	12.333	6.000	5.833
W+NC	2011	10.833	5.000	5.167
WCN-	2011	13.500	7.500	5.167
WCNC	2011	13.000	7.333	5.000
W+N-	2012	11.833	5.500	5.333
W+NC	2012	9.833	4.833	4.000
WCN-	2012	10.500	5.333	4.167
WCNC	2012	10.000	4.833	4.167
W+N-	2013	11.167	6.333	4.167
W+NC	2013	8.333	4.333	3.500
WCN-	2013	11.500	6.833	4.500
WCNC	2013	12.000	7.667	4.167

Appendix 4.2: Least squares means comparisons

The effects of watering treatment, nitrogen treatment and their interaction on aboveground net primary productivity (ANPP), functional group productivity, plant diversity and functional group diversity were modelled, for each response in turn. Where there was a significant effect of either treatment, or their interaction, pairwise comparisons between least squares means of treatments were calculated using Kenward-Roger approximated degrees of freedom, for each year (2011-2013). Where only watering treatment was significant, comparisons between water-addition (W+) and water-control (WC) plots were run. Where only nitrogen treatment was significant, comparisons between nitrogen-fixer removal (N-) and nitrogen-fixer-control (NC) plots were run. Where there was a significant interaction term, four sets of comparisons were run:

- W+NC plots versus W+NX plots
- WCNC plots versus WCNX plots
- W+NC plots versus WCNC plots
- W+NX plots versus WCNX plots

This process was repeated for baseline data from 2010, where watering treatment alone was modelled for each dependent variable. Here, comparisons between W+ and WC plots were run if watering treatment had a significant main effect in the model being considered.

Table A4.2.1: Pairwise least squares means comparisons for ANPP.

Table A4.2.2: Pairwise least squares means comparisons for the productivity of four functional groups in turn: perennial grasses, annual grasses, perennial forbs and annual forbs.

Table A4.2.3: Pairwise least squares means comparisons for plant diversity.

Table A4.3.4: Pairwise least squares means comparisons for diversity of two functional groups: forbs and grasses.

Table A4.2.1: ANPP

Year	Significant effects	Comparison	Estimate	S.E.	df	t-ratio	P-value
2010	Watering	WC - W+	-58.763	21.996	5.000	-2.672	0.044 *
2011	Watering x Nitrogen	WCNC - WCNX	53.791	29.071	10.000	1.850	0.094 †
		W+NC - W+NX	-34.848	29.071	10.000	-1.199	0.258
		WCNC - W+NC	60.512	30.772	12.309	1.966	0.072 †
		WCNX - W+NX	-28.127	30.772	12.309	-0.914	0.378
2012	None	NA	NA	NA	NA	NA	NA
2013	Watering, Nitrogen, Watering x Nitrogen	WCNC - WCNX	-4.005	18.476	10.000	-0.217	0.833
		W+NC - W+NX	68.175	18.476	10.000	3.690	0.004 **
		WCNC - W+NC	-121.464	36.170	6.539	-3.358	0.013 *
		WCNX - W+NX	-49.284	36.170	6.539	-1.363	0.218

0.05 ≤ P ≤ 0.10, * 0.01 ≤ P ≤ 0.05, ** 0.001 ≤ P ≤ 0.01, *** 0.0001 ≤ P ≤ 0.001

Table A4.2.2: Productivity of different functional groups

Functional Group	Year	Significant effects	Comparison	Estimate	S.E.	df	t-ratio	P-value
Perennial grass	2010	Watering	WC - W+	-21.118	12.497	5.000	-1.690	0.152
	2011	Watering, Nitrogen	WC - W+	-1.630	0.622	5.000	-2.619	0.047 *
			NC - NX	0.794	0.436	11.000	1.822	0.096 †
	2012	Watering	WC - W+	-2.953	0.460	5.000	-6.414	0.001 **
	2013	Watering x Nitrogen	WCNC - WCNX	-1.410	0.525	10.000	-2.687	0.023 *
			W+NC - W+NX	0.208	0.525	10.000	0.396	0.701
			WCNC - W+NC	-1.950	1.001	6.637	-1.948	0.095 †
			WCNX - W+NX	-0.332	1.001	6.637	-0.331	0.751
Annual grass	2010	None	NA	NA	NA	NA	NA	NA
	2011	None	NA	NA	NA	NA	NA	NA
	2012	None	NA	NA	NA	NA	NA	NA
	2013	Nitrogen, Watering x Nitrogen	WCNC - WCNX	6.205	13.255	10.000	0.468	0.650
			W+NC - W+NX	58.400	13.255	10.000	4.406	0.001 **
			WCNC - W+NC	-35.019	28.987	6.193	-1.208	0.271
			WCNX - W+NX	17.176	28.987	6.193	0.593	0.574
Perennial forb	2010	Watering	WC - W+	14.378	4.475	5.000	3.213	0.024 *
	2011	None	NA	NA	NA	NA	NA	NA
	2012	Nitrogen	NC - NX	0.608	0.331	11.000	1.840	0.093 †
	2013	None	NA	NA	NA	NA	NA	NA
Annual forb	2010	None	NA	NA	NA	NA	NA	NA
	2011	None	NA	NA	NA	NA	NA	NA

Continued...

Functional Group	Year	Significant effects	Comparison	Estimate	S.E.	df	t-ratio	P-value
	2012	Watering	WC - W+	-1.241	0.555	5.000	-2.236	0.076 †
	2013	None	NA	NA	NA	NA	NA	NA

0.05 ≤ P ≤ 0.10, * 0.01 ≤ P ≤ 0.05, ** 0.001 ≤ P ≤ 0.01, *** 0.0001 ≤ P ≤ 0.001

Table A4.2.3: Total Plant Diversity

Year	Significant effects	Comparison	Estimate	S.E.	df	t-ratio	P-value
2010	Watering	WC - W+	5.083	1.338	5.000	3.799	0.013 *
2011	Nitrogen	NC - NX	-1.000	0.492	11.000	-2.031	0.067 †
2012	None	NA	NA	NA	NA	NA	NA
2013	Nitrogen, Watering x Nitrogen	WCNC - WCNX	0.500	0.453	10.000	1.103	0.296
		W+NC - W+NX	-2.833	0.453	10.000	-6.249	<0.001***
		WCNC - W+NC	3.667	1.152	5.854	3.182	0.020 *
		WCNX - W+NX	0.333	1.152	5.854	0.289	0.782

0.05 ≤ P ≤ 0.10, * 0.01 ≤ P ≤ 0.05, ** 0.001 ≤ P ≤ 0.01, *** 0.0001 ≤ P ≤ 0.001

Table A4.2.4: Forb Diversity and Grass Diversity

Functional Group	Year	Significant effects	Comparison	Estimate	S.E.	df	t-ratio	P-value
Forb	2010	Watering	WC - W+	3.333	1.108	5.000	3.008	0.030 *
	2011	None	NA	NA	NA	NA	NA	NA
	2012	None	NA	NA	NA	NA	NA	NA
	2013	Watering x Nitrogen	WCNC - WCNX	0.833	0.693	10.000	1.202	0.257
			W+NC - W+NX	-2.000	0.693	10.000	-2.885	0.016 *
			WCNC - W+NC	3.333	1.132	7.380	2.946	0.020 *
			WCNX - W+NX	0.500	1.132	7.380	0.442	0.671
Grass	2010	None	NA	NA	NA	NA	NA	NA
	2011	None	NA	NA	NA	NA	NA	NA
	2012	Watering x Nitrogen	WCNC - WCNX	0.008	0.111	10.000	0.073	0.943
			W+NC - W+NX	-0.283	0.111	10.000	-2.550	0.029 *
			WCNC - W+NC	0.008	0.120	11.962	0.068	0.947
			WCNX - W+NX	-0.283	0.120	11.962	-2.363	0.036 *
	2013	Nitrogen	NC - NX	-0.130	0.065	11.000	-1.993	0.072 †

0.05 ≤ P ≤ 0.10, * 0.01 ≤ P ≤ 0.05, ** 0.001 ≤ P ≤ 0.01, *** 0.0001 ≤ P ≤ 0.001