Biotic interactions in a changing world: the role of feeding interactions in the response of multitrophic communities to rising temperature and nitrogen deposition

A thesis submitted in partial fulfillment of the
requirements for the Degree
of
Doctor of Philosophy
in the University of Canterbury
by

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2012

We can't solve problems by using the same kind of thinking we used when we created them

A. Einstein

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Abstract

Global warming and increasing atmospheric nitrogen deposition are ranked as second and third most important global drivers of biodiversity loss. Widespread species losses have deep implications for the functioning of ecosystems, the delivery of essential ecosystem services and their resilience to future environmental perturbations.

There is growing recognition that interactions between species play a crucial role in determining the response of ecosystems to global environmental changes. Moreover, evidence of synergistic effects between global change drivers has prompted numerous calls to integrate multiple drivers in ecological research. Nevertheless, empirical studies assessing the impacts of temperature and nitrogen on communities at multiple trophic levels are largely absent. This thesis explores the effects of temperature and nitrogen on a tri-trophic system comprising plants, herbivores and natural enemies. The first chapter shows impacts of the drivers on the composition and phenology of an herbivore community. The second chapter highlights changes in biomass under the treatments at three trophic levels. The third chapter explores, for the first time, the impacts of temperature and nitrogen on quantitative food webs. Finally, the last data chapter uses body size as an important species trait to gain insights on the mechanisms causing shifts in food web structure.

The key findings of this thesis were i) trophic interactions largely mediated the effects of both global change drivers ii) In particular, strong bottom-up effects determined the system response, with herbivores responding positively and consistently more so than plants and parasitoids in particular. However, iii) this contrasting response was not explained by a phenological mismatch. iv) Food-web structure responded to the changes in composition of herbivores and parasitoids, but shifts in interaction structure did not affect the resilience of the food. However, temperature and nitrogen impacted host-parasitoid food-web structure by altering the response of parasitoid species to host density and size structuring, which is likely to bear consequences on host-parasitoid co-evolution and future food-web architecture and stability. Finally, v) we found frequent, non-additive interactions between the

global change drivers. We conclude that co-occurring temperature and nitrogen are likely to alter food-web structure and overall ecosystem balance, with increasing herbivore dominance likely to have important implications for ecosystem functioning and food-web persistence.

Acknowledgements

I would like to acknowledge the University of Canterbury (Doctoral Scholarship 2008-2011) and the *Miss* E L *Hellaby* Indigenous Grassland Research Trust (Fellowship 2008-2009) for their financial support. The Marsden Fund and the The Miss E L Hellaby Indigenous Grassland Research Trust provided generous research funding. The University of Canterbury funded two assistants through the Summer Scholarships Program.

A number of people contributed in making this thesis possible, whilst some had a shot at trying to make it impossible[1]. Luckily, the first prevailed, and I'm very grateful to them all.

My supervisor, Jason Tylianakis had the challenging and potentially scarring experience of supervising me as his first PhD candidate. He excelled at it and made it look just easy. His calm and ever positive attitude, endless support and availability did wonders in the making of this thesis, and make me wonder what I would have achieved without it. It has been a real privilege to work with such a dedicated, driven, yet extremely generous supervisor and person.

My associate supervisor, Owen T Lewis, has been a geographically distant but reassuring presence throughout the project. He provided solid help and comments on a number of drafts.

I'm indebted to John S. Dugdale (Landcare Research), who identified my Lepidoptera and Tachinidae samples and helped develop a larval key for alpine grassland Lepitoptera. I'm equally indebted to Jo Berry (MAF Biosecurity), who dedicated a lot of her free time identifying Hymenoptera for me. Mike and Cliff Cox kindly allowed me to work and stay on their land. I shall miss the little green hut.

At UC, Jenny Ladley was great at troubleshooting, and generally supported and looked after me and my research with a tireless "can do" attitude. David Conder equally put a lot of mileage into the early days of my project, without once loosing his patience and Zen. Rebecca Jackson, Michael Bartlett and Kirsty Trotter have

[1] http://www.stuff.co.nz/the-press/news/3743507/Happiness-is-the-return-of-a-stolen-computer-with-data-intact

been precious assistants, pulled some long days in the field and some more in the lab, without a complaint. Etienne Laliberte tricked me into multivariate statistic (it's easy!), and was very helpful in many ways. Andrew Barnes shared the joys and pains of SEM.

A big highlight of this PhD was the opportunity to meet some truly good folks. Alvin, Anna, Andrew, Ceci, Kristy, James, Josh, Matt, Rocio, Sarah, Simon, the Harris family, the Lab group et al. were a pleasure to meet and share many good moments around campus and even more so when not around campus.

My family never entirely grasped what I'm I doing, let alone why I'm doing it, or why do I need to do it at the other side of the World. Nevertheless, they sustained me in every way they could.

A last, but very special thank you my partner, Liz; I'm very grateful for everything and every way you've helped me, especially in the last stretch. I look forward to doing the same for you and, although I'll never make such good PA, I hope I will be as good as you've been in helping putting things in perspective, and a smile on my face.

Authorship declaration

Chapter 2: Plant-mediated and non-additive effects of two global change drivers on an insect herbivore community

Published in Ecology

Authors: de Sassi, C., Lewis, O.T., Tylianakis, J.M.

CDS co-designed the experiments with JMT, obtained external funding, collected and analyzed the data, and wrote the manuscript. JMT critically reviewed all previous drafts and supervised this project in all its phases. OTL commented on earlier drafts.

Chapter 3: Climate change disproportionately increases herbivore biomass

Under revision for publication in PLOS One

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CDS co-designed the experiments with JMT, collected and analyzed the data, and wrote the manuscript. JMT critically reviewed all previous drafts and supervised this project in all its phases.

Chapter 4: Bottom-up effects mediate response of food-web structure and resilience to climate change and nitrogen deposition

in preparation

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CDS co-designed the field experiments with JMT, collected the data, carried out the main analyses and wrote the manuscript. ET carried out the network resilience simulations. TAR helped with the experimental set up and fieldwork. TAR and OTL commented on the concept of the manuscript, and will review future drafts prior to publication. JMT critically reviewed all previous drafts and supervised this project in all its phases. This manuscript represents the main objective of a Marsden Grant application by JMT (Principal Investigator) and Owen Lewis (Associate Investigator).

Chapter 5: Warming and nitrogen affect size structuring of a host-parasitoid food web

Accepted for publication as part of a special feature in the Phil. Trans. Roc. Soc. B

Authors: de Sassi, C., Staniczenko, P.P.A, Tylianakis, J.M.

CDS co-designed the experiment with JMT, conducted multivariate and univariate analyses, collected the data and wrote the manuscript. PPAS carried out the Bayesian analyses, provided help with the interpretation of the results and commented on the earlier drafts. JMT has critically reviewed all previous drafts and provided support wherever needed.

CHAPTER I

Introduction

1.1 A perspective of climate change in ecological research

It is only in recent times that the term "climate change" is debated by the public and not only specialist scientists. Although some sceptics still "don't believe man-made global warming is settled in science enough" (Rick Perry, US elections 2012 Republican candidate, cited in Hymas; 2011) recent decades of research have generated the prevailing understanding and sense of ownership of this global problem. The correlation between human activities and climate change was first, and unsurprisingly, suggested by meteorologists. A search in Reuters "Web of Knowledge" database reports studies on regional climatic changes as far back as the early 1900s, with at least 11 studies between 1900 and 1920 (and there could conceivably be more, as many early studies cannot be traced on such databases). It is, however, not until the 1950s that scientists began consistently inferring human influence on the world's climate, from a geophysical perspective (Plass 1956).

Despite the well-known importance of abiotic factors such as climate for determining species distributions and the niche (Rosenthal 1953, Bary 1964, Kolosova 1975, Thorp and Hoss 1975) ecologists were slower than meteorologists to embrace climate-change research. For example, an editorial dated 1965 by Paul S. Martin, entitled "The Current Consensus on Climatic Change" (Martin 1965) refers to the growing agreement among atmospheric scientists that human activities are affecting the global climate. With regards to ecology, the author contends that any "… ecologist with a taste for theory will find full measure in the meteorologists' attempts to unravel the cause(s) for climatic change. According to one school the warm-up of the last 60 years might be attributed to man, through industrialization and increased slash-burn agriculture, both acting to raise the CO_2 content of the atmosphere and thus to increase the atmospheric greenhouse effect". He further implies that atmospheric climate change publications "*commend themselves to an*

often neglected corner of the ecologists' bookshelf". Indeed, although ecological climate change research has increased exponentially over the last few decades (Figure 1.1), Martin's somewhat visionary statement had to wait nearly 40 years before titles such as "Ecological responses to recent climate change" (Walther et al. 2002) and "A globally coherent fingerprint of climate change impacts across natural systems" (Parmesan and Yohe 2003) were published and rapidly established themselves among the most cited papers in ecological research. Nowadays, understanding, predicting and mitigating the effects on the biosphere of global environmental change (GEC), with climate as a key driver, is now widely recognized as one, if not the, major challenge in ecology (Walther et al. 2005).

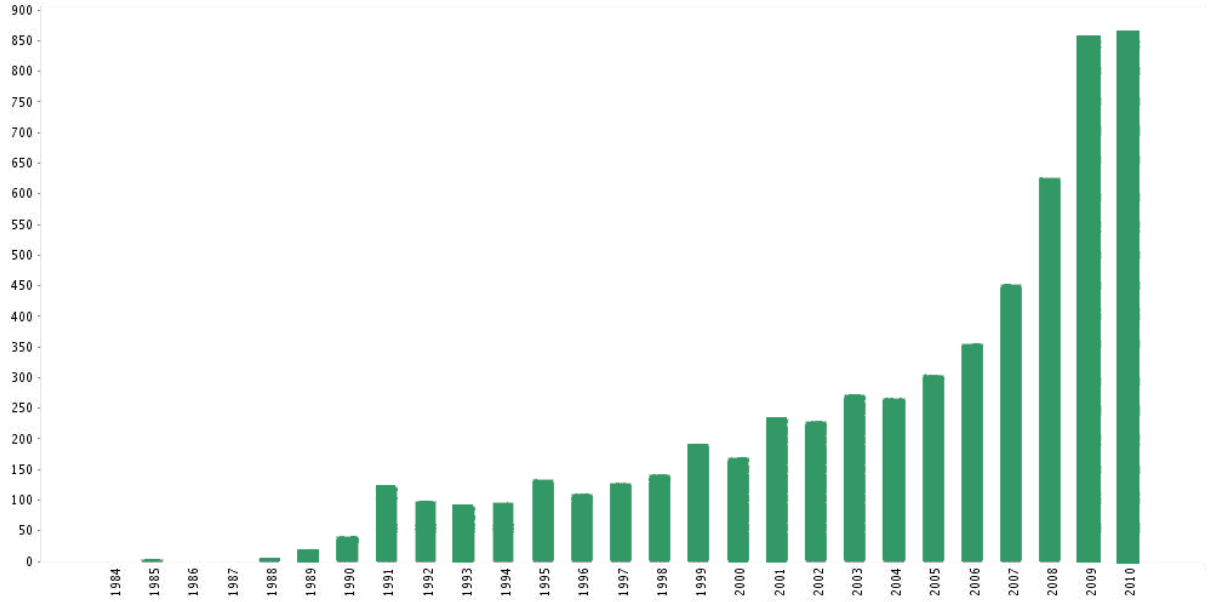

Figure 1.1 Publication report (source: ISI Web of Knowledge) in the category "Environmental Sciences and Ecology" matching with "global warming" as keyword, for the period 1960 to 2010. The same search using "climate change" as keyword yielded ca. 48,176 studies between 1960 and 2010; 41,871 of these were conducted between 2000 and 2010.

1.2 How does climate change affect us?

Global ecosystems are undergoing rapid change and nearly two thirds of the services provided by nature to humankind, such as provision of food and water, disease management, climate regulation, and aesthetic enjoyment, are found to be in

decline worldwide (Chapin et al. 2000, M.E.A 2005, Diaz et al. 2006, Fargione et al. 2008). Human activities since industrialization have changed ecosystems more rapidly and extensively than during any comparable period of time in human history, largely to meet rapidly-growing demands for food, fresh water, timber, fiber, and fuel (M.E.A 2005). This has resulted in a substantial, and largely irreversible, loss of biodiversity (Pimm and Raven 2000). These changes to ecosystems have contributed to substantial net gains in short-term human well-being and economic development (Raudsepp-Hearne et al. 2010), but such gains have been achieved at growing costs in the form of the degradation of many ecosystem services (M.E.A 2005). Evidence of GEC as a consequence of anthropogenic activities is now widely accepted (Sala et al. 2000), and the degradation of ecosystems could worsen significantly during the first half of this century (IPCC 2002, M.E.A 2005). Ecologists are therefore challenged with understanding the mechanisms through which GEC influences species, communities, ecosystem functioning, and consequently, the delivery of services on which we all depend (Chapin et al. 2000).

A major research theme within ecology has suggested that the rates and stability of ecosystem functions such as productivity strongly depend on biodiversity (McCann 2000, Loreau et al. 2001, Hooper et al. 2005). Consequently, the effects of GEC on biodiversity have become an ongoing central topic in ecological research. Biodiversity is rapidly declining world wide (Leakey and Lewin 1996, Pimm and Raven 2000), and numerous studies have demonstrated empirically that GEC has played a major role in this decline (Thomas et al. 2004, Botkin et al. 2007). Sala and colleagues (2000) ranked the top five GEC drivers in terms of their threat to biodiversity as: land use change (loss and fragmentation of natural habitats), climatic changes, deposition of anthropogenically-fixed nitrogen, biotic invasion and increasing atmospheric CO_2. All of these drivers are predicted to become more important as human exploitation of the environment increases over short time scales, and this is likely to accelerate the ongoing loss of biodiversity throughout the world (Thomas et al. 2004, Dobson et al. 2006).

1.3 The multiple axes of real-world complexity

1.3.1 Biodiversity and the importance of interactions among species

Decades of biodiversity research, led to growing evidence that species diversity *per se* is crucial, but not the sole parameter determining and maintaining the stability of natural ecosystems. In particular, ecosystem stability is strongly interconnected with the multitude of biotic interactions among species (McCann 2000, Bascompte et al. 2006, Suttle et al. 2007, Okuyama and Holland 2008). Biodiversity can both affect and respond to the strength of biotic interactions, whereas biodiversity loss can be at the same time a cause and consequence of changes in ecosystem processes (Hooper et al. 2005).

The role of biotic interactions in ecosystem functioning, and their response to GEC, have traditionally received less attention than biodiversity (McCann 2007), with much of it only in recent years (Tylianakis et al. 2008). This historical lack of research focus probably stems from difficulties in quantifying changes to interactions compared with species abundances or richness. Yet, global environmental changes are expected to affect interactions among all organisms, through direct effects on phenology (Root et al. 2003, Encinas-Viso et al. 2012), physiology (Chown and Gaston 2008), distribution range (Parmesan et al. 1999, Thuiller et al. 2008), behaviour (Stokes et al. 2004), the physical environment in which interactions take place (Laliberte and Tylianakis 2010), and individual fitness (Memmott et al. 2007), or indirectly through cascading responses of trophic structure (Barton 2011).

Different trophic levels have been shown to react differently to climate change (Voigt et al. 2003) to the extent that predator-prey and plant-herbivore interactions have been disrupted by warming (Parmesan 2006). Studies on multitrophic food chains have shown that changes in plant resource quality, mediated via herbivores, can cascade up to the predator level (Fox et al. 1990, de Sassi et al. 2006, Bukovinszky 2008, van Nouhuys and Laine 2008). Predator performance can also be affected directly by GEC drivers, and this may have severe consequences for their ability to control herbivore populations (Stireman et al. 2005, Wilmers and Getz 2005). In turn, plant primary production has important implications for food production and carbon sequestration (Luo 2006, Reich et al. 2006b), but a net benefit

to herbivores may signal future increases in pest outbreaks, which could arise through changes to the system stability (Haddad 1990).

Integrating ecological interactions into global change research is therefore recognized as a necessary challenge for ecology, both for understanding how ecosystems may respond to climate change, and also generally to incorporate greater real-world complexity into our understanding of species and communities (Harrington et al. 1999, Tylianakis et al. 2008, Gilman et al. 2010).

1.3.2 Biotic complexity: from species to networks

Natural systems are an integration of entities and processes that we, as ecologists, typically attempt to separate into distinct partitions that allow description and understanding. However, real-world complexity limits our ability to extrapolate conclusions from such discrete "building blocks" to larger units such as communities and ecosystems (Tylianakis et al. 2008). While research examining the effects of different GEC drivers on single species (Arft et al. 1999), plant-plant interactions (Brooker 2006b), plant-herbivore interactions (Throop and Lerdau 2004, Stiling and Cornelissen 2007) and predator prey dynamics (Parmesan 2006) has made consistent progress in the last few years, impacts on biological communities and more complex networks of feeding interactions are poorly understood (McCann 2007, Tylianakis et al. 2008, Ings et al. 2009). In the face of a time-pressured challenge such as predicting the effects of climatic changes on key ecosystems, our power of generalisation becomes paramount.

A promising way to achieve this is by scaling up our understanding from species-specific case studies to multispecies networks and processes (Bascompte 2009). With the development of new theories and tools, ecological research is now moving in this direction, producing research at increasing levels of biotic organization, from communities to multitrophic interaction patterns and even quantifying changes to entire systems.

All species interact with other species in antagonistic (e.g., predator–prey) or mutualistic (e.g., plant-pollinator) networks often referred as 'food webs'. These networks describe the feeding relationships between all species in the web. However,

for interactions between species to occur, the species must not only be present, but also co-occur in space and time, and have the physiological ability to interact. Therefore, food webs are more than the sum of their component species (Montoya et al. 2006, Bascompte and Jordano 2007, Bascompte 2009), and recent studies have shown that the structure of the food webs can respond to global environmental changes beyond changes in species diversity and or abundance (Tylianakis et al. 2007). In addition to improving realism, the incorporation of higher levels of biotic complexity may improve our understanding of ecosystem stability. The potential for a relationship between diversity and stability was suggested more than half a century ago (MacArthur 1955, Elton 1958). However, the general view that species diversity promotes stability was challenged by May (1973) who, modeling randomly-assembled food webs, found that diversity tends to destabilize community dynamics. Years later, further research showed that real interaction networks were more stable than randomly-constructed webs (Yodzis 1981), indicating that elusive mechanisms drive stability beyond the number of species or ecological interactions. More recently, it was proposed that interaction strength is crucial to stability. In particular, the interplay of strong and weak interactions could be a main mechanism conferring stability to ecosystems (McCann et al. 1998), as weakly-interacting species stabilize community dynamics by dampening strong, potentially destabilizing consumer–resource interactions (McCann 2000). This implies that decreasing biodiversity will be accompanied by increases in average interaction strengths within ecosystems, and a concomitant decrease in ecosystem stability (McCann 2000, McCann 2007). A recent explosion of food-web studies, augmented by the incorporation of network modelling techniques, confirms how complex networks of biotic interactions such as predation play an important role in the maintenance of biodiversity and ecosystem stability (Bascompte et al. 2006, Rooney et al. 2006, Karlsson et al. 2007, Montoya and Raffaelli 2010, Stouffer and Bascompte 2011).

The potential of interaction networks for mediating ecosystem responses to GEC, and the stability of the ecosystem services on which human well-being depends, remains largely unexplored. However, a small but fast-growing body of literature is providing the first insights into these questions. Tylianakis et al. (2007) showed that land-use change has significant effects on food-web structure, even

when biodiversity was not significantly altered. Exploring the effects of invasion on plant-pollinator mutualistic networks, Aizen et al. (2008) found that connectivity among native species declined in highly-invaded webs. These examples highlight potential consequences for ecosystem functioning and stability that trace back to the ways in which species interact with another, rather than being dependent on biodiversity. However, another study found that effects of land-use change on species composition were not reflected in the quantitative interaction structure of local food webs: landscape context (fragmentation and isolation) had no detectable effects on food-web topology (Kaartinen and Roslin 2011). Collectively, these studies demonstrate that global environmental changes can affect biotic interactions along with or independent of their effect on biodiversity.

Network theory provides a conceptual framework to assess the consequences of perturbations at the community level, and this may serve as a first step toward a more predictive ecology in the face of global environmental change (Bascompte 2009). Furthermore, the incorporation of network structure into conservation monitoring and strategy is a challenging yet promising too. The response of different network attributes to biotic and abiotic changes must be understood before a compelling and generalized argument for the conservation of network structure can be made (Tylianakis et al. 2010).

1.3.3 Synergistic global environmental change drivers

There is increasing recognition that the effects of different GEC drivers can interact with one another (Didham et al. 2007, Tylianakis 2008), such that their combined effect may be greater (Shaw et al. 2002) or less (Cleland et al. 2006) than that of each driver in isolation. The current literature highlights the complexity of ecosystem responses to multiple drivers, with results ranging from additive effects (Reich et al. 2001, He 2002, Zavaleta et al. 2003) to true interactive effects, where the response to one driver depends on the other driver, such that the two drivers counteract (Hattenschwiler and Schafellner 1999, Henry et al. 2005) or augment (Long et al. 2006, Reich et al. 2006a, Aronson et al. 2007, Williams 2007) each other's effect. The mounting evidence for complex, non-additive interactions

(Darling and Cote 2008) suggests that predictions of future ecosystem processes based solely on changes in a single driver are likely to be misleading (Norby et al. 2007, Tylianakis et al. 2008, Ockinger et al. 2010). Therefore, research into the effects of GEC requires factorial experiments, involving more than one driver, to achieve an understanding of how interactions between drivers might mitigate or exacerbate the net effects of global environmental change on biotic communities in the future (Shaw et al. 2002). To date, the practical constraints of manipulating multiple drivers have meant that only a fairly small number of studies have utilized this approach, and the magnitude and direction of interactive effects found are often non-linear, system- and context-dependent (Norby et al. 2004, Brooker 2006). However, the majority of these studies focused on the effect of two or more interactive drivers on plant communities only, without considering higher trophic-level interactions. Considering only one trophic level (e.g., producers) is not satisfactory in predicting a net ecosystem response, and this integrative point of view is supported by the few studies that have investigated plant-herbivore interactions under single and combined GEC drivers in fully-factorial experiments (Richardson et al. 2002, Cleland et al. 2006, Zvereva and Kozlov 2006). Here, even more than in single-trophic-level studies, the response of the system to interacting drivers was frequently different to that of each driver in isolation.

1.4 Thesis objectives and outline

This thesis is structured in six chapters, starting with a general introduction (Chapter 1), followed by four data chapters, which have each been written as manuscripts for submission to international peer-reviewed journals. Finally, the findings from each chapter will be synthesized in an overall discussion with conclusions (Chapter 6). The general aim of this thesis is to address gaps in our current understanding of how the interactive effects of global warming and nitrogen deposition drive changes at different trophic levels, and how these translate into overall changes in communities, specifically networks of feeding interactions.

I chose these two drivers because they are two of the most important drivers of global change (ranked second and third by Sala et al.; 2000) and their effects on

multitrophic communities remain unknown. Temperature and nitrogen are also fundamental determinants of plant growth, so these drivers are likely to cause widespread changes in basal resource availability. I use semi-natural tussock grasslands as a model system, due to their importance for agricultural food production, ubiquitous worldwide distribution (Hooper et al. 2005), rapid growth responses relative to other native systems (e.g. forests), and their sensitivity to these two drivers (Bloor et al. 2010). Parasitoids represent an extremely important group of organisms, both for their role as natural enemies in biocontrol (Cardinale et al. 2003, Schmidt et al. 2003) and their extremely high diversity (for instance, an estimated 220,000 Hymenoptera species are parasitoids, and somewhere between one and two million species across all taxonomic groups are thought to be parasitoids) (Godfray 1994). Similarly, Lepidoptera also represent a very diverse group of insects of ubiquitous worldwide distribution (Powell 2009), and of major importance as insect pests in both crop production landscapes and natural habitats (Scoble 1995).

I use data from two field experiments that I established for this research. One experiment uses an altitudinal gradient in a grassland landscape to generate a gradient in temperature (Hodkinson 2005), combined with experimental nitrogen fertilization. The second experiment, the first of its kind in New Zealand, involved artificial warming using underground heating cables and nitrogen addition in a factorial design. I established the experimental plots by planting a standardized experimental grassland community. The two experiments, whilst subject to the same GEC treatments and using the same grassland study system, are considerably different in their scale and scope. The altitudinal gradient experiment (used for Chapter 2), basing a natural temperature gradient, is well suited to capture larger-scale effects under natural field conditions. The artificial warming experiment (used for Chapter 5) is intended to capture smaller-scale effects such as behavioural choices of consumers for resources of different quality. Furthermore, the standardized plant composition of the warming experiment allows better resolution on the relative influence of bottom-up plant- or herbivore-mediated effects, versus direct effects of the drivers on higher trophic levels. The combination of these two experiments (used in Chapter 3 and 4), with different spatial scales and

methodologies, will provide a degree of confidence that the observed results are not attributable to possible limitations in either of the experimental settings.

As a first step towards the overall objectives, **Chapter 2** examines the impact of warming and nitrogen on an herbivore assemblage, exploring their interactive effects through time, and also discussing the relative strength of direct vs. plant-mediated effects of the drivers. However, the response of herbivores to environmental change drivers will depend not only on their direct response and that of their plant resources, but also on their regulation by natural enemies. Consequently, including multiple trophic levels into the factorial design of experiments may reveal new feedback mechanisms and generate a more complete knowledge of ecosystem responses to GEC.

In **Chapter 3,** I therefore expand the system to consider natural enemies in addition to plants and herbivores, and examine how biomass at each of these trophic levels responds to the drivers.

In **Chapter 4**, I aim to scale up the complexity of my system further, to include feeding interactions. We know that species interactions are capable of modulating ecosystem responses to GEC (Suttle et al. 2007), and that the structure of community interactions can be critical to ecosystem stability (McCann et al. 1998, Bascompte et al. 2006, Stouffer and Bascompte 2011). It has also been shown that food webs can be structured in part through the bottom-up availability and quality of resources (Memmott and Waser 2002, Bukovinszky 2008). Yet, the combined effects of temperature and nitrogen on food-web structure remain unknown, despite the importance of these drivers for determining basal (i.e. plant) resource availability (Reich et al. 2006a). A recent study further highlights the importance of direct and indirect effects of temperature, mediated through trophic interactions and physical changes in the environment, both for population dynamics and ecosystem processes (Beveridge et al. 2010). I use path analysis to disentangle the direct and indirect channels through which the two drivers affect the food webs, partitioning out direct effects from the effects of resource availability and species diversity at each trophic level. As a direct effect of temperature, I also test the possibility that phenological/temporal asynchrony between hosts and parasitoids affects food web

structure. Finally, I examine the consequences of any changes in the networks in relation to their equilibrium stability.

In **Chapter 5**, I further investigate the impact of GEC on structural patterns in the food web. However, I attempt to elucidate more general rules by replacing taxonomical characterization of the pairwise interactions with body size as a structuring trait. Body size determines a suite of species traits that can affect the structure and dynamics of food webs, and other ecological networks, across multiple scales of organization (Woodward et al. 2005, Brose et al. 2006, Brose 2010). Measuring body size provides a relatively simple means of encapsulating and condensing a large amount of the biological information embedded within an ecological network. (Woodward et al. 2005). The patterning of predator and prey body sizes in real ecosystems affects the arrangement of interaction strengths (Brose 2010), which in turn determines food-web stability (Emmerson and Raffaelli 2004). Body size structuring of trophic communities has been documented in marine (O'Gorman et al. 2010), freshwater (Jonsson et al. 2005) and terrestrial ecosystems (McLaughlin et al. 2010), showing that size-related structural properties can play an important stabilising role in the face of species loss and perturbations. However, the role of size structuring in host-parasitoid food webs is less clear (Cohen et al. 2005, Petchey et al. 2008), and its response to temperature and nitrogen is unknown. Finally, **Chapter 6** summarizes the findings from all chapters and highlights potential directions for future research.

CHAPTER II

Plant-mediated and non-additive effects of two global change drivers on an insect herbivore community

2.1 Abstract

Warmer temperatures can alter the phenology and distribution of individual species. However, differences across species may blur community-level phenological responses to climate or cause biotic homogenization by consistently favoring certain taxa. Additionally, the response of insect communities to climate will be subject to plant-mediated effects, which may or may not overshadow direct effects of rising temperatures. Finally, recent evidence for the importance of interaction effects between global change drivers suggests that phenological responses of communities to climate may be altered by other drivers. We used a natural temperature gradient (generated by elevation and topology), combined with experimental nitrogen fertilization, to investigate the effects of elevated temperature and anthropogenic nitrogen deposition on the structure and phenology of a semi-natural grassland herbivore assemblage (lepidopteran insects).

We found that both drivers, alone and in combination, severely altered how the relative abundance and composition of species changed through time. Importantly, warmer temperatures were associated with biotic homogenization, such that herbivore assemblages in the warmest plots had more similar species composition than those in intermediate or cool plots. Changes in herbivore composition and abundance were largely mediated by changes in the plant community, with increased non-native grass cover under high treatment levels being the strongest determinant of herbivore abundance. In addition to compositional changes, total herbivore biomass more than doubled under elevated nitrogen and increased more than four-fold with temperature, bearing important functional implications for herbivores as consumers and as a prey resource. The crucial role of non-native plant dominance in mediating

responses of herbivores to change, combined with the frequent non-additive (positive and negative) effects of the two drivers, and the differential responses of species, highlights that understanding complex ecosystem responses will benefit from multi-factor, multi-trophic experiments at community scales or larger.

2.2 Introduction

Environmental changes triggered by human activities are affecting all ecosystems, and understanding their consequences for ecological communities is a major challenge. Numerous studies have revealed effects of climate change on the distribution of different taxa (Parmesan et al. 1999, Walther et al. 2002, Hickling et al. 2006), often underpinned by range shifts (Wilson et al. 2005). Different rates of range expansion and/or contraction by different species, coupled with differential performance of species, can alter the organization of communities (Parmesan and Yohe 2003, Yang et al. 2011). Consequently, a subset of species (those with a wide thermal tolerance or an ability to exploit temperature-driven resource shifts) are likely to become more dominant within their native communities, and also to expand their ranges. If this subset is consistent across locations, their increasing range and competitive ability could drive biotic homogenization (increasing similarity of communities from different locations; Olden et al. 2004), with important consequences for ecosystem stability and functioning (Loreau et al. 2003, Olden et al. 2004). Recent studies have revealed the effects of warmer temperatures on temporal distributions of species, though species within the same community may show variable phenological responses to climate change (Primack et al. 2009, Nufio et al. 2010).

Different phenological responses to climate change across functional groups and trophic levels may disrupt crucial biotic interactions, and thereby percolate widely through ecological communities (Harrington et al. 1999, Tylianakis et al. 2008, Both et al. 2009). In the case of insect herbivores, it is well documented that changes in plant quality and composition can significantly alter herbivore life history, performance and host-plant choice (Awmack and Leather 2002, Morrison and Hay 2011). However, consumer-resource synchrony (e.g., caterpillar peak

abundance date and budburst date, Both et al. 2009) has a major impact on the population densities of herbivores such as leaf-feeding Lepidoptera, and years with high plant-herbivore synchrony may result in outbreaks of herbivorous insects (van Asch and Visser 2007). On the other hand, asynchrony of insect activity with plant resources can determine the magnitude of impact of herbivores on their host plant populations (Russell and Louda 2004), and alter insect population dynamics (Wallisdevries and Van Swaay 2006) to cause shifts in dominance of species and higher taxonomic groups (Richardson et al. 2002, Tylianakis et al. 2008). Therefore, the response of consumers to global change drivers is a complex combination of their direct response and the indirect bottom-up effect of drivers on resources, such that the net outcome can be difficult to estimate with single-trophic-level studies. Thus, there is a need to address biotic responses to global change drivers such as climate within a community context and at multiple trophic levels.

In addition to this growing emphasis on the need for community-scale data, there has been increasing concern that the effect of individual global change drivers may not reflect their synergistic effects in the real world. Recent evidence of complex interactions among co-occurring drivers (Didham et al. 2007, Tylianakis et al. 2008, Forister et al. 2010) calls for the integration of multiple drivers in global change research. For instance, nitrogen deposition, which is increasing rapidly worldwide (Vitousek et al. 1997a, M.E.A 2005), has a vast array of effects on plants, generally promoting higher biomass, affecting competition (Reich et al. 2006a), and reducing biodiversity (Stevens et al. 2004). Changes in basal plant resources are known to affect herbivore performance, which usually responds positively to elevated nitrogen (Throop and Lerdau 2004). However, such effects may need to be re-examined in the context of their interplay with temperature. For example, Wallisdevries and van Swaay (2006) showed that excess nitrogen advanced plant growth in the spring, thereby forcing herbivores to develop under colder conditions and offsetting the thermal benefit of warming via a sub-additive warming by nitrogen interaction. This and other studies (reviewed in Tylianakis et al. 2008) suggest that the ability of species to respond phenologically to warming may be altered in the context of other global change drivers acting simultaneously.

Here we examine how the phenology and structure of an insect herbivore (Lepidoptera larvae) assemblage in semi-natural grassland responds to the combined effect of temperature and simulated nitrogen deposition. We focus specifically on the following questions:

1) Do temperature variation and nitrogen affect overall plant and herbivore community composition? If so, do they alter the abundance and presence/absence of particular species consistently, such that they drive the formation of similar assemblages in different locations (i.e. biotic homogenization)?

2) Are the observed changes primarily a result of direct effects of temperature on herbivore performance, or indirect plant-mediated effects?

3) Do temperature variation and/or nitrogen deposition generate significant changes to the phenology of species and the assemblage as a whole?

4) As a measure of functional importance, are changes in community structure associated with altered total biomass of the herbivore assemblage?

5) Do the two drivers have independent effects, or complex, non-additive interactions?

2.3 Material and Methods

2.3.1 Study site

We established our experiment in tussock grasslands of the Hope River Valley, North Canterbury, New Zealand, which is located at the foothills of the Southern Alps, and ranges from 600 to 1,700 m elevation (see Study site in Appendix 1.1). Large amounts of forest were cleared by early European settlers in the mid 1800's, and later over-sown for pasture. These grasslands are now characterized by a mixture of native and non-native flora (Barratt et al. 2005), with the native component largely comprising tussock grasses that previously inhabited open areas (usually above the treeline) and the exotic component being mainly pasture plants. A large proportion of New Zealand's insect fauna is endemic (Myers et al. 2000, Barratt et al. 2005). In particular, the lepidopteran fauna shows very high levels of endemism (White 1991); all 39 species identified in this experiment were endemic, and were historically

limited to the alpine grasslands above tree line prior to forest clearing. Thus, their down-slope migration reflects each species' ability to follow the range expansion of their habitat and persist under altered conditions, rather than a historical preference of certain taxa for the climate below tree line. Of these species, 37 are generalist grass (Poaceae) feeders, and are therefore not limited in their range expansion by a specialist association with exclusively alpine plants. Similarly, host plants of the two specialist species were also found below the tree line. Our experimental plots are all situated below the natural tree line, thereby offering a comparison between newly-generated communities that differ in climate, rather than between original alpine vs. newly-created communities. Therefore, this represents an ideal system for climate-change research, although potential implications of rapid evolutionary adaptation remain unexplored

2.3.2 Experimental design

As a climatic gradient, we used an elevation gradient as a 'space for time substitution' (Pickett 1989, Hodkinson 2005). We established five vertical transects (Figure S1 in Appendix 1.1) of three plots, each at 150 m intervals of elevation, such that there was a total of 300 m difference in altitude between the lowest and the highest plot in each transect (see Site locations and details and Table S1 in Appendix 1.1). The total temperature gradient across all plots amounted to 2.83 °C (the average temperature in each plot over the entire period of data recording ranged from 3.89 to 6.72 °C). This temperature gradient falls within the range of temperature increases predicted by all IPCC scenarios within the next 100 years (IPCC 2007). The topography of the area meant that temperature did not vary consistently with elevation (i.e. some sites were slightly warmer or colder than expected for their elevation). This allowed us to test the effects of temperature alongside elevation (to account for other environmental variables that co-vary with elevation, such as oxygen availability and radiation; Hodkinson 2005). Local topography may create significant microclimatic variation, which could modify temperature over short vertical distances that override the more general altitudinal trends (Weiss et al. 1988). Therefore, we used the overall mean site temperature for the period February

to December 2009 (during which consistent data were available for all sites) as a covariate to elevation in the analysis. However, changes to mean temperatures following global warming may be strongly influenced by changes to frequency and magnitude of extreme temperature events (IPCC 2007), which remain unaccounted for in our study. Note, however, that analyses incorporated transect as a random (blocking) factor, so any environmental differences among transects would not confound treatment effects.

At each elevation, we established a 24 x 12 m sampling plot. We further subdivided each plot into two 12 x 12 m subplots, and randomly assigned one of these to a nitrogen addition treatment (addition or control with no added N). This resulted in a split-plot design, with temperature varying at the scale of plots (n = 15), blocked by transects (n = 5), and N treatments applied to subplots (n = 30) nested within plots (see Site locations and details and Table S1 in Appendix 1.1). The N-fertilisation treatment comprised a total application of 50 Kg ha-1 yr-1, (see Nitrogen treatment application in Appendix 1.1), which falls within the current range of globally-observed rates of atmospheric deposition (M.E.A. 2005).

Sampling of insects began in October 2008, and continued at monthly intervals until December 2009. Sampling was interrupted over the winter period June-August 2009, when snow cover made the sites inaccessible. In April 2009, adverse weather also prevented access to some sites due to river flooding. We completed a total of 11 sampling rounds successfully. To minimize disturbance and depletion of caterpillars in the experimental area, we subdivided each 12 x 12 m subplot into 4 strips of 3 x 12 m each, and sequentially sampled one strip only during each sampling round. This ensured a time window of at least 4 months before re-sampling of the same section. This timeframe is substantially longer than the average larval life stage of Lepidoptera, and therefore prevented bias in the abundance of any sample caused by depletion from previous sampling rounds. Plant searches for larvae involved thorough teasing apart of denser vegetation to locate any hidden larvae.

Morphospecies were validated as true species through identification of reared adults or larval characteristics, so that 6143 caterpillars were identified successfully. The adult identities were confirmed by lepidopteran taxonomist J.S. Dugdale, who

also provided support in developing diagnostic features for larval identification (see Experimental sampling and rearing in Appendix 1.1).

2.3.3 Vegetation survey

In December 2009, we carried out a vegetation survey of each 12 x 12m subplot, using the percent cover (PC) method (described by Mueller-Dombois and Ellenberg 2003), which provides an accurate estimation of plant cover and species composition. For each subplot separately, percent cover data were transformed to relative abundances by dividing the percent cover of each species by the sum of percent cover values for all species present. As tussocks were the primary food plant for Lepidoptera larvae, we determined tussock biomass by estimating their average size and abundance in each plot (see Vegetation survey in Appendix 1.1).

2.3.4 Data analysis

We performed all analyses on plant and herbivore community composition and phenology using permutational distance multivariate ANOVA, carried out with the PRIMER V6 software and the PERMANOVA package (Clarke and Gorley 2006, Anderson et al. 2008). We conducted two sets of analyses using two different dissimilarity measures, one accounting for species composition and abundance (Modified Gower base 10) and one focusing on species presence/absence (Jaccard dissimilarity, see Dissimilarity measures in Appendix 1.2). For both plant and herbivore analyses, we included nitrogen (control vs. elevated) and plant composition (see Plant composition in the herbivore community composition analyses in Appendix 1.2) as fixed effects. We included temperature as a covariate to elevation (low, mid, or high within each transect) using Type I, sequential sums of squares, to test if there were any elevation effects (e.g., due to solar radiation) beyond those explained by temperature. We ran all models by entering temperature first followed by elevation. However, we also ran all the models with an inverted order of predictors and found no significant effect of elevation, which indicates that any temperature effects were not confounded by other factors correlated with

elevation. Nevertheless, we retained elevation as a fixed factor in models, to be conservative when attributing variance to the temperature covariate.

For the analyses on herbivore phenology, we did not include plant variables as predictors, because we did not collect measures of plant phenology (such as onset of spring growth) or growth rates, and the effect of a static measure of plant composition on herbivore phenology would be uninformative (the same applies for the univariate analyses below).

We tested the effect of the drivers on community phenology by including time (sampling round) in the model, with an interaction term between the drivers and time (i.e. to test whether changes in community composition through time were dependent on the level of temperature and/or nitrogen). Transect, plot and subplot were treated as nested random factors. The error structure followed a split-plot design, with transects acting as the error term for testing effects of temperature (with elevation as a cofactor, see above), plots acting as the error term for testing the nitrogen effect, and finally subplots acting as the error for the repeated sampling through time.

We tested for biotic homogenization of both plant and herbivore composition using a permutational distance-based test for homogeneity of multivariate dispersions, based on a modified Gower dissimilarity to account for both relative abundance and presence of species (Anderson et al. 2006). This test compares community similarity within different levels of a factor, in our case, among replicates of temperature and nitrogen treatments (see Test for biotic homogenization in Appendix 1.2). Increasing similarity of replicates of a given treatment would therefore indicate that the treatment selects consistently for the same community composition.

To account for our split-plot design, we used generalized linear mixed effects models for all remaining univariate analyses (Bolker et al. 2009), which were conducted using the lme4 package (Bates and Maechler 2010) in R version 2.10.1 (R Development Core Team 2009). These included plots nested in transects as random effects, and also subplots nested in plots where repeated measures through time were being tested. To ascertain the main determinants of change in plant community composition, we tested the effect of the drivers and elevation on the proportion cover

of exotic grasses (which are known to be food plants for caterpillars), nitrogen leaf content, plant richness (native, exotic and total) and tussock biomass.

To test for changes in herbivore phenology, we analysed larval abundance, biomass and individual larval bodyweight through time, with elevation, nitrogen treatment and time as fixed factors and temperature as a covariate to elevation. For analysis of individual larval bodyweight, we also included species identity as a random factor, crossed with the nested random factors (transect, plot, and subplot), to test how bodyweight changed within each species in response to the drivers.

Overall herbivore species richness, total (summed) larval biomass and herbivore abundance were tested with elevation, temperature and nitrogen as predictors, to test the net effects of the drivers. In addition, to compare the direct vs. indirect effects of the drivers, we then included plant composition, proportion of exotic grasses and tussock biomass as covariates to the drivers to find the best-fitting model.

In these models, we used Poisson errors for abundance and species richness data and Gaussian errors for biomass, individual bodyweight, and proportion cover data. Proportion cover was arcsine square root transformed to meet the assumptions of normality and homoscedasticity. We included all interactions between temperature, nitrogen and time (where applicable) in the initial (maximal) model. Final simplified models were then fitted using restricted maximum likelihood (REML), as recommended by Bolker et al. (2009), and tested for overdispersion. Elevation was not significant in any model (tested alongside temperature), and provided an inferior fit when models with temperature were directly compared with models that included elevation instead. Therefore, we removed elevation from the final models (see Mixed effects models and Table S3 in Appendix 1.2).

2.4 Results

We found a suite of direct and plant-mediated effects of the drivers on the herbivore assemblage and evidence of non-additive, interactive effects of the drivers on phenology (Figure 2.1).

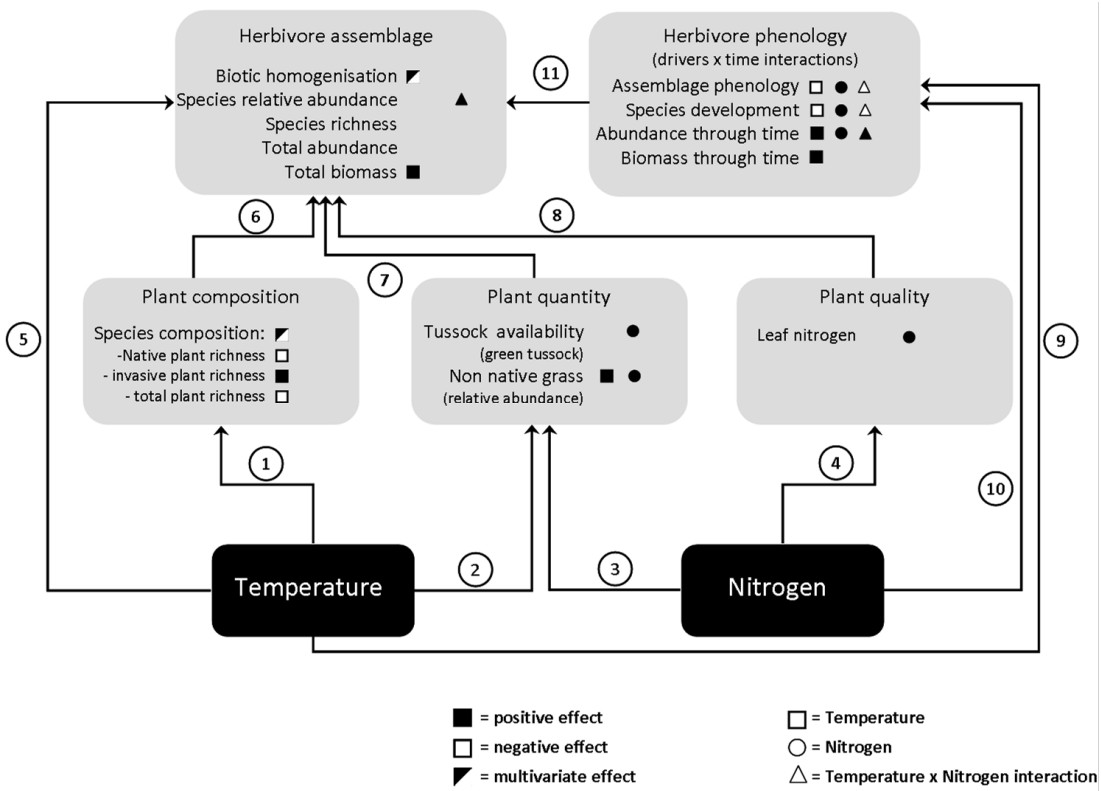

Figure 2.1: Flow diagram of the study system, showing the pathways through which temperature and nitrogen deposition could affect the herbivore community. Arrows indicate direct effects on different community variables (shaded areas). Effects of each driver (temperature, nitrogen and their interaction) are represented with symbols (see key), with no symbol indicating no significant effects. In the"Herbivore phenology" compartment, symbols represent a driver x time interaction . Temperature affected plant composition (Pathway 1), which involved a reduction in native and total plant diversity, despite increasing non-native plant diversity. Both drivers had a positive effect on the relative abundance of non-native grasses (Pathways 2 and 3). Nitrogen also affected the proportion of living (green) tussock leaf over dead standing grass stems (Pathway 3), and altered plant quality by increasing the leaf nitrogen content (Pathway 4). Temperature directly affected herbivore community structure, reducing spatial variability in composition (biotic homogenization, Pathway 5). Changes in plant composition (Pathway 6), quantity (Pathway 7) and quality (Pathway 8) altered the relative abundance and composition of herbivore species. Both drivers also affected the phenology of the herbivore assemblage (defined as the turnover of species through time, Pathways 9 and 10), whereby different species responded differently in their abundance, development and biomass through time. Here, the drivers showed a sub-additive effect on assemblage phenology, and phenological shifts had a strong impact on overall composition of the assemblage at any given point in time (Pathway 11). The phenological response could be partly mediated by plant traits, but this potential pathway remains untested.

2.4.1 Plant community response to the environmental change drivers:

The multivariate analysis showed a strong effect of temperature and a more subtle effect of nitrogen on the plant community. Temperature affected both species composition and relative abundance ($F_{1,13}$ = 3.40, P = 0.002) within the plant community. Temperature was correlated with a reduction of native species richness (Z = -5.11, P < 0.001) and an increase in exotic species (Z = 2.21, P = 0.030), which resulted in an overall decrease in plant species richness (Z = -2.91, P = 0.004). This result was supported by a strong positive effect of temperature on the relative proportion cover of exotic grasses in the vegetation (t = 4.86, P_{MCMC} < 0.001). We found no homogenization of the plant community (test for homogeneity of multivariate dispersion: Temperature: $F_{2,12}$ = 0.07, P = 0.925; Nitrogen: $F_{1,28}$ = 0.35, P = 0.586), indicating that shifts in composition were not uniform across sites.

Nitrogen fertilization did not significantly affect overall plant composition or species richness, but rather favored an increase in exotic grasses, which had a higher proportion cover in the fertilized plots than in the controls (N: 30.34% ±3.21, Control:23.67 ±3.9, t = 3.34, P_{MCMC}=0.02). Additionally, nitrogen increased the proportion of green leaf relative to dead-standing brown leaf (t = 5.12, P_{MCMC} < 0.001), thereby increasing the biomass of live tussock available as a food source for herbivores. The nitrogen content of tussock leaves was significantly higher in the nitrogen-addition plots (on average 20.7% (± 4.2 SE) higher, P < 0.001), confirming that the fertilization treatment affected plant nitrogen content, and could therefore potentially affect herbivores.

2.4.2 Herbivore assemblage response to global change drivers:

We found effects of both temperature and nitrogen addition on herbivore community structure. In particular, both drivers caused a shift in community composition, altering the relative abundance and presence/absence (Jaccard dissimilarity) of larvae from different species (Figure S2 and Tables S4 and S5 in Appendix 1.3). Total herbivore species richness varied under the different treatments, but differences in species richness were driven by the effect of the treatments on total

abundance (sample size), which affected richness, rather than a treatment effect on richness *per se* (sample size effect on richness: $Z = 5.11$, $P < 0.001$).

Warmer temperatures homogenised herbivore assemblages, such that they were most similar to each other in the warmest plots from the different transects ($F_{2,12} = 6.08$, $P = 0.015$), despite being further apart spatially than plots within each transect. However, dispersion did not differ significantly between sites at moderate and coldest temperatures (Figure S2 in Appendix 1.3 and Figure S3 in Appendix 1.4). Nitrogen addition and the temperature by nitrogen interaction did not significantly affect community dispersion ($P > 0.05$ in both cases).

2.4.3 Relative importance of direct vs. plant mediated effects

We found strong collinearity between the effects of the global change drivers and plant composition on herbivore community structure (see Appendix 1.3). Although this strongly suggests that the effects of temperature and nitrogen on the herbivore community may have been mediated via plant community shifts, we cannot objectively attribute this shared variance to either predictor with certainty. Nevertheless, a significant temperature by nitrogen interaction term present in all models after controlling for plant-mediated effects indicated that temperature retained a direct effect on herbivore community structure that was independent from its effect on plants, but was dependent on nitrogen availability ($F_{1,28} = 2.13$, $P = 0.033$).

Changes in total herbivore abundance were largely associated with temperature-correlated changes in plant composition, in particular an increase in cover of non-native grasses (effects of plant composition, proportion exotic grasses and tussock biomass: $|Z| > 2.10$, $P < 0.05$ in all cases), and increased plant quality (leaf nitrogen: $Z = 5.44$, $P < 0.001$) caused by nitrogen addition.

2.4.4 Phenology of herbivore assemblage and common species

We found strong evidence for phenological effects of the drivers on herbivores at the community scale. Temperature influenced herbivore community-compositional

change through time (positive temperature x time interaction; Tables S4, S5 in Appendix 1.3), such that temporal changes in community composition (i.e. community-level phenological changes) were greater at higher temperatures, producing a temporally more-variable community. Higher temperatures caused an earlier peak of larval abundance by one month, and were associated with higher overall larval abundance (Figure 2.2) and biomass (Tables S6-S9 in Appendix 1.5).

Nitrogen addition was also associated with higher larval abundances, and this effect became stronger through time (positive N x time interaction; Tables S6 and S7 in Appendix 1.5). The effect of the two drivers in combination was less than additive (negative N x temperature interaction; Tables S6 and S7 in Appendix 1.5), such that the effect of nitrogen was strongest in colder sites and weakened with increasing temperature. Finally, the effect of temperature on the change in larval abundance through time depended on nitrogen availability, indicating an interactive effect of the two drivers on phenology (significant temperature x nitrogen x time interaction, Tables S6 and S7 in Appendix 1.5 and Figure S4 in Appendix 1.6).

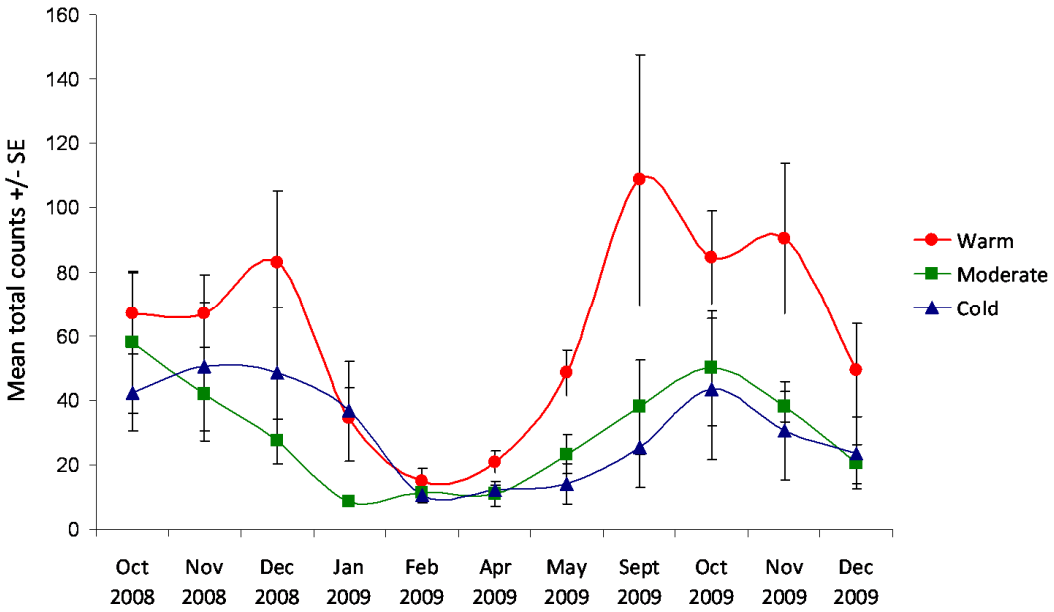

Figure 2.2: Phenological response of the community to temperature, shown as mean (+/- SE) total abundance (counts) of caterpillars through time (in months). For visual clarity, plots are grouped into three temperature categories, though analyses treated temperature as a continuous predictor.

For the three most common species (namely *Persectania aversa, Orocrambus ramosellus,* and *Orocrambus simplex*), which were present at all sites, we were able to test how abundance changed through time in response to the treatments. All three species responded positively to both drivers separately, though with varying magnitude (Appendix 1.7). Similarly, all three species showed a positive interaction between temperature and time, indicating that phenological changes in abundance depended on temperature. However, these three species showed different responses to the interaction of the drivers (temperature x nitrogen), which ranged from negative to positive. Consequently, their phenological response (i.e. change in abundance through time) to the drivers in combination also ranged from negative to non-significant or positive (Appendix 1.7). We found virtually identical results when analyzing the mean body mass of each larva across the whole assemblage as an estimate of larval development rate (see Appendix 1.8).

2.4.5 Total herbivore biomass

Changes in abundance were reflected in the total biomass of the herbivore assemblage. Total lepidopteran herbivore biomass responded positively to warmer temperatures (414% increase in total biomass compared with colder conditions; $t = 5.98$, $P_{MCMC} < 0.001$) and nitrogen-richer conditions (267% increase in total biomass compared with control plots; $t = 2.38$, $P_{MCMC} = 0.02$). After testing the direct effect of the drivers, we included plant parameters to identify plant-mediated effects. Plant composition had a significant influence on herbivore biomass ($|t| > 2.70$, $P_{MCMC} < 0.05$ for the first two PCA axes). In contrast with the results on abundance, plant composition did not overshadow the significance of temperature, but absorbed the effect of nitrogen. In particular, the availability of green tussock biomass ($t = 4.18$, $P_{MCMC} = 0.0016$) and the proportion of non-native grasses to other plants ($t = 2.81$, $P_{MCMC} = 0.02$) best explained herbivore biomass, alongside a strong direct effect of temperature ($t = 5.14$, $P_{MCMC} = 0.0001$).

2.5 Discussion

Our results showed an interactive effect of two global change drivers (temperature and nitrogen deposition) on the composition and phenology of a lepidopteran herbivore assemblage in a sub-alpine grassland. Overall, herbivore community structure was affected by both temperature and nitrogen addition, which individually altered the relative abundance and identity (presence/absence) of species. Although use of natural climatic gradients, such as elevation, has a number of caveats (Hodkinson 2005), we found no effects of elevation beyond those explained by temperature, providing a degree of confidence that the effects we present are likely to have been driven by temperature. Total herbivore species richness was not affected by nitrogen or temperature after controlling for sample size, indicating that the differences in composition reflected replacement or altered dominance within the herbivore assemblage, rather than changes in the number of species *per se.*

In our study, consistent range expansion by a subset of species led to homogenization of the assemblages at higher temperatures, showing that spatial beta diversity can be altered by climate, even when alpha diversity (richness per plot in our case) is not. It has been proposed previously that climate may partly drive interglacial periods of diversification and homogenization of plant taxa (Feurdean et al. 2010). However, biotic homogenization is normally associated with the spread of cosmopolitan invasive species (Qian and Ricklefs 2006), even though this spread may be driven by climate (Marini et al. 2009) or land-use practices (White and Kerr 2007). In contrast, our homogenized herbivore assemblages solely comprised endemic species, indicating that climate may drive significant community-scale changes even in the absence of other drivers such as invasion. We found no evidence of a similar community homogenization effect on plants, despite the presence of non-native species that could potentially become invasive under climate change. This suggests that consumer composition may be more sensitive than plants to warming. Following the ‘insurance hypothesis’ (Yachi and Loreau 1999, Loreau et al. 2003), loss of biodiversity at a regional scale (i.e. biotic homogenization) could reduce spatial complementarity, thereby making these communities less resilient to further changes or perturbation. This loss of insurance value could be particularly

significant, as warming is likely to select for species with similar functional traits, further reducing functional diversity.

We found that temperature significantly altered phenology at the community scale, advancing the time of peak abundance for individual species, increasing their peak abundance levels, and altering the identity and relative abundance of species through time. As a whole, the herbivore assemblage showed a strong response to temperature, in particular through greatly increased abundance. Species at higher latitudes and elevation could have a broader thermal tolerance and be living in climates that are currently cooler than their physiological optima, in which case they would be likely to respond strongly to rising temperature (Deutsch et al. 2008). The three numerically-dominant species differed remarkably in their response to the interactive drivers, ranging from negative (*O. simplex*), neutral (*P. aversa*)_ to positive (*O. ramosellus*) responses of their abundance. We found similar results in larval development (bodyweight through time), providing mechanistic support for the observed abundance patterns. Different responses are likely to be caused by the specific thermal physiology of species, and these differences could be exacerbated by shifts in competitive abilities within the community (Huey et al. 2009). Ultimately, elevated nitrogen affected the phenological and developmental responses of species to temperature, effectively disrupting the consistent positive interaction between temperature and time. This result indirectly suggests that the effects of temperature on phenology may be at least partially plant mediated, through changes in plant quality or phenology (Hodkinson 2005), a pathway that we were unable to test in this study. The contrasting responses of individual species to the interacting drivers confounded the results at the community level, where no unidirectional interaction between nitrogen and time was apparent. However, complex, non-additive, species-specific responses to the drivers played a central role in the observed shifts of the assemblage composition and its change through time.

In the face of rising temperatures, a major concern is how changes to the timing of biological events will affect overall ecosystem functioning and resilience (Edwards and Richardson 2004). Abundance and biomass changes through time were affected by temperature and nitrogen, as a consequence of increased dominance by a few species and earlier development of the whole community with warmer

temperatures. These results carry important implications for herbivores as both consumers and prey, as several studies have revealed decoupling of consumer-resource dynamics following climate change (Memmott et al. 2007, Tylianakis et al. 2008, Both et al. 2009). Trophic mismatch between herbivores and their natural enemies could lead to important cascading effects on herbivory (Stireman et al. 2005), and studies of such mismatch at a community level are needed.

We observed a shift in plant composition from native to non-native species with increasing temperature and nitrogen, as well as an increase of available native tussock biomass and leaf nitrogen content in our fertilization treatment. Because elevated temperature and nitrogen were associated with components of plant composition that related to increased non-native grass cover, tussock availability, and plant quality (leaf nitrogen content), their effects on herbivore biomass and abundance could not be separated. These correlations suggest that plants mediated the overall effect of the global change drivers on herbivore community structure and abundance, as the variance explained by the drivers diminished almost completely when plant quality and composition effects were included ahead of the drivers in the model.

Plant-mediated effects on herbivore communities could arise through a number of pathways. Changes in plant availability and quality are known to be exploited differently by different herbivore species (Awmack and Leather 2002), potentially leading to shifts in herbivore dominance and abundance as we observed. Beyond the simple increase in resource availability, herbivores may also benefit from access to the different nutritional content of different plants. Additionally, naïve non-native plants may lack appropriate defense mechanisms against local herbivores (Parker et al. 2006, Morrison and Hay 2011). Alternatively, altered community-wide plant phenology could extend the overall availability of plants as a food resource through time, favoring particular species that develop at the extremes of the growing season, and therefore contributing to changes in herbivore assemblage and its temporal dynamics.

With this study, we showed that warming and nitrogen directly affected the organization of herbivore communities and their phenology, and promoted the establishment of simplified, more homogenous communities even without affecting

alpha diversity. These results highlight the importance of empirical studies at the community level, rather than a species-by-species approach, since individual species can respond in idiosyncratic ways that do not reflect average community-wide responses. Furthermore, we demonstrated that plant-mediated effects can strongly contribute to overall changes in herbivore abundance, species dominance and biomass, in addition to the direct effects of the drivers. Understanding the relative importance of different effect pathways is crucial to global change research, with particular relevance to predicting herbivore outbreaks. Furthermore, the combination of two drivers (temperature and nitrogen) caused frequent, non-additive interactions that affected the response of community structure and phenology to either driver on its own. This contributes rare empirical evidence of real-world responses of natural systems to interacting global environmental changes, which has been highlighted as a necessary challenge for ecology (Didham et al. 2007; Tylianakis et al. 2008). Studies of single drivers would not have generated an adequate understanding of the community responses we observed, nor could these have been predicted from the known effects of temperature (Bale et al. 2002) and nitrogen (Throop and Lerdau 2004) in isolation on herbivore performance. Only by scaling up our understanding of changes from species to higher levels of organization, can we fully understand how current and future environmental changes are likely to affect biodiversity, ecosystem functioning and community stability.

CHAPTER III

Climate change disproportionately increases herbivore over plant or parasitoid biomass

3.1 Abstract

All living organisms are linked through trophic relationships with resources and consumers, the balance of which determines overall ecosystem stability and functioning. Ecological research has identified a multitude of mechanisms that contribute to this balance, but ecologists are now challenged with predicting responses to global environmental changes. Despite a wealth of studies highlighting likely outcomes for specific mechanisms and subsets of a system (e.g., plants, plant-herbivore or predator-prey interactions), studies comparing overall effects of changes at multiple trophic levels are rare. We used a combination of experiments in a grassland system to test how biomass at the plant, herbivore and natural enemy (parasitoid) levels responds to the interactive effects of two key global change drivers: warming and nitrogen deposition.

We found that higher temperatures and elevated nitrogen generated a multitrophic community that was increasingly dominated by herbivores. Moreover, we found synergistic effects of the drivers on biomass, which differed across trophic levels. Both absolute and relative biomass of herbivores increased disproportionately to that of plants and, in particular, parasitoids, which did not show any significant response to the treatments. Reduced parasitism rates mirrored the profound biomass changes in the system. These findings carry important implications for the response of biota to environmental changes; reduced top-down regulation is likely to coincide with an increase in herbivory, which in turn is likely to cascade to other fundamental ecosystem processes. Our findings also provide multitrophic data to support the general concern of increasing herbivore pest outbreaks in a warmer world.

3.2 Introduction

Global environmental changes affect all living organisms, with complex consequences for biodiversity, ecosystem structure and function (Chapin et al. 2000, Thomas et al. 2004). Predicting generalities in the direction of such changes represents one of the major challenges in ecology. However, the complexity of this task is exacerbated by the great variability of responses observed across biomes, space, time, and scales of biotic organization (Gilman et al. 2010). Climate has effects at all levels of organization, from population dynamics to community composition and species-specific responses (Parmesan 2006, Tylianakis et al. 2008), and it has strong impacts on ecosystems and their services (Chapin et al. 2000, Parmesan and Yohe 2003, Tscharntke and Tylianakis 2010). A wealth of studies have shown that climate warming, provided it is not too extreme, generally increases plant net primary production (Rustad et al. 2001). However, warming has also been shown to have positive effects on herbivore population size and herbivory (Bale et al. 2002), which may counteract the increased plant growth. Furthermore, the net effect of climate on herbivores will result both from direct and plant-mediated effects and from top-down control by natural enemies, and this complexity may be partly responsible for the highly-variable responses of herbivores to different environmental change drivers (Tylianakis et al. 2008).

The ecosystem equilibrium arising from the combination of these effects therefore depends on the relative response of individual trophic levels. A vast body of literature has addressed the effect of climate on plant-herbivore and prey-predator systems, but it disproportionately represents studies looking at pairs of interacting species, rather than larger modules or communities at once (Tylianakis et al. 2008, Gilman et al. 2010). Despite the insights into specific mechanisms (e.g., phenological mismatches, shifts in competition, prey defense and palatability) gained from this approach, such studies do not allow generalizations to be made on the relative impact of climate or other change drivers at different trophic levels. In fact, only a handful of investigations have specifically considered overall responses at different trophic levels. For example, Voigt and colleagues focused on covariance in the response to multiple climatic factors of community composition, at different trophic levels

(Voigt et al. 2003) and functional groups (Voigt et al. 2007), and concluded that sensitivity (i.e population fluctuations) to climate increases with trophic level. Focusing on a model system including a raptor bird, four passerine species and two caterpillar species, Both et al. (2009) showed that the response of consumers was weaker than that of their resource. However, this result is contrasted by a recent study showing a climate-induced increase in synchrony between food demand and availability in a similar caterpillar-passerine system (Vatka et al. 2011). This latter result indicates that variability in species responses may not necessarily match the overarching community-wide response. Nevertheless, these results imply that climate change is likely to prompt changes in the trophic structure of communities, which could directly or indirectly affect ecosystem processes such as nutrient cycling, herbivory and predation (Petchey et al. 1999, Kishi et al. 2005).

Finally, in addition to indirect effects on species through changes at adjacent trophic levels, organismal responses to climate could be altered by co-occurring changes in the biotic and abiotic environment, such that recent literature has called for the integration of multiple drivers in global change research (Didham et al. 2007, Tylianakis et al. 2008). For example, biologically-available nitrogen deposition in non-agricultural systems has increased rapidly and become a major driver of biotic change (Vitousek et al. 1997a). As well as generally increasing net primary productivity (NPP), nitrogen has been shown to alter plant competitive interactions (Zavaleta et al. 2003, Brooker 2006b, Tylianakis et al. 2008) and drive biodiversity losses (Stevens et al. 2004, Clark and Tilman 2008); effects that can percolate to higher trophic levels (Richardson et al. 2002). Despite the logical assumption that nitrogen will, in contrast to temperature, only affect herbivores via bottom-up effects (Tylianakis et al. 2008), the interaction of the direct effect of temperature with changes in basal resource availability triggered by nitrogen, create a complex interplay that shows more context dependence than either effect in isolation (Wallisdevries and Van Swaay 2006, Thompson et al. 2008). Finally, the combined impact of warming and nitrogen on natural enemies is largely unknown, though N deposition tends to benefit predators (Tylianakis et al. 2008), while climate warming can destabilize predator-prey interactions (Rall et al. 2011). Thus, the interactive effects of temperature and N on plant growth (Reich et al. 2006a) and herbivores

(Tylianakis et al. 2008), complicated by the general absence of data on their effect on natural enemies, suggest that these drivers may have complex, non-additive effects on trophic balance.

In this study, we examine how biomass at three trophic levels (plants, lepidopteran herbivores and their parasitoids) responds to co-occurring increases in temperature and nitrogen. We use seminatural grasslands as a model system, due to their global ubiquity (Hooper et al. 2005) and importance for grazing agriculture. Furthermore, they are known to respond to N addition (Stevens et al. 2004), and more strongly to warming than to other climate drivers such as CO_2 concentration and drought (Bloor et al. 2010). We use a field experiment along an altitudinal gradient, combined with an artificial warming experiment under controlled field conditions, and measure how total biomass of plants, herbivores and parasitoids, as well as parasitism rates, respond to elevated temperature and nitrogen treatments.

3.3 Material and Methods

3.3.1 Study site: altitudinal gradient experiment

We established our experiment near Lewis Pass, North Canterbury, New Zealand (Appendix 2.1). The valley is located at the foothills of the Southern Alps, and ranges from 600 to 1,700 m elevation. The climate is cool and humid, with a mean annual rainfall of 1560 mm and a mean annual temperature of 9.1°C (Williams and Courtney 1995). The wider experimental area is characterized by montane tussock grassland, dominated by native species in the genera *Festuca*, *Poa*, *Rytidosperma*, and *Chionochloa* at higher altitudes. These species are typical of semi-arid to humid, montane and subalpine zones in New Zealand (Rose et al. 2004). The inter-tussock ground is generally dominated by stock-palatable Eurasian species (particularly *Agrostis capillaris*, *Anthoxanthum odoratum*, *Trifolium* spp.), which were over-sown after forest clearing in the late 1800s. At present, the area is farmed at very low intensity, with a stock density of less than 1 sheep per hectare, and no nitrogen fertilizer is applied.

3.3.2 Experimental design and sampling of altitudinal gradient experiment

To generate a climatic gradient, we used an elevation gradient as a 'space for time substitution' (Pickett 1989, Hodkinson 2005). We established five vertical transects of three plots, each at 150 m intervals of elevation, such that there was a total of 300 m difference in altitude between the lowest and the highest plot in each transect. Transects were at least 600 m apart (twice the vertical length of each individual transect, see Appendix 2.1, Table S11 and Figure S5). All plots had a similar incline and vegetation type, and faced north or north-west. Note, however, that analyses incorporated transect as a random (blocking) factor, so any environmental differences among transects would not confound treatment effects. To maintain similar characteristics, transects were not all positioned at exactly the same elevation, so plots ranged from 650 m at the lowest point to 1073 m a.s.l at the highest (423 m of total elevation span). This provided a total temperature gradient of 2.83 °C across all plots (the average temperature in each plot over the entire period of data recording ranged from 3.89 to 6.72 °C). This temperature gradient falls within the range of temperature increases predicted for all IPCC scenarios within the next 100 years (IPCC 2007).

The topography of the area meant that temperature did not vary consistently with elevation (i.e. some sites were warmer or colder than expected for their elevation). This allowed us to test the effects of temperature *per se*, partially uncoupled from the effects of other environmental variables that co-vary with elevation (such as oxygen availability and radiation; Hodkinson 2005). Temperature was recorded in each plot using Hobo series ProV2 data loggers, protected by a sun shield, logging temperature at 1h intervals from February to December 2009. We used the overall mean site temperature for this period as a predictor variable in the analysis.

At each elevation, we established a 24 x 12 m sampling plot. We further subdivided each plot into two 12 x 12 m subplots, and randomly assigned one of these to a nitrogen addition treatment (addition or control with no added N). This resulted in a split-plot design, with temperature varying at the scale of plots (n = 15), blocked by transects (n = 5), and N treatments applied to subplots (n = 30) nested within plots. The N fertilization treatment comprised a total application of 50 Kg ha-

1 yr-1, which falls within the current range of globally-observed rates of atmospheric deposition (M.E.A 2005). Precise N deposition rates for the study region are not known, but expansion of dairy farming across New Zealand is driving rapid increases in N fertilizer application (Austin et al. 2007), which will likely impact adjacent semi-natural grasslands. Nitrogen fertilizer was applied in the form of Calcium Ammonium Nitrate (CAN) granules (Ravensdown LTD, New Zealand). This form of fertilizer combines fast and slower release of biologically-available nitrogen, and has been used previously to simulate atmospheric deposition (Clark and Tilman 2008).
We began N addition in September 2008, by adding 40% of the total year budget (20 Kg ha-1 yr-1 , 1066 g CAN per subplot) and applying the remaining 60% in 4 pulses, evenly distributed over the next 12 months, by sprinkling the dry granules throughout the treated subplot. Fertiliser addition continued at a rate of 50 Kg ha-1yr-1 until sampling was completed in December 2009.

Although initial sampling of insects began in October 2008, here we present data only from samples where biomass was measured, which were those collected from May to December 2009, i.e., approximately a year after starting the nitrogen fertilization treatment. To minimize disturbance and depletion of caterpillars in the experimental area, we subdivided each 12 x 12 m subplot into 4 strips of 3 x 12 m each, and sequentially sampled one strip only during each sampling round. This ensured a time window of at least 4 months before re-sampling of the same section. This timeframe is substantially longer than the average larval life stage of Lepidoptera in our study area, and therefore prevented bias in the abundance of any sample caused by depletion from previous sampling rounds.

We searched all the tussocks within the 3 x 12 m strip at each sampling round. Plant searches involved thorough teasing apart of denser vegetation to locate any hidden larvae.

3.3.3 Study site: artificial warming experiment

We set up an artificial warming experiment adjacent to the University of Canterbury field station at Cass in the Waimakariri River catchment, South Island of New Zealand (Appendix 2.1, Figure S5). The Cass field station lies at 640 m a.s.l., a

mean annual rainfall of 1300 mm (1918-1965) is uniformly distributed throughout the year, and typical monthly mean air temperatures range from 1.6°C (July) to 15.7°C (February). Snow lies for some days each winter (June-September). The climate of the area is described in detail by Greenland (1977). The study area is embedded in a montane short-tussock grassland with very similar characteristics to the environment of the altitudinal gradient experiment, although the two experimental locations are over 60 km apart and are in different catchments. The intertussock area is dominated by Eurasian grasses oversown for pastoral purposes. The area surrounding the field station shows dominance cover of *Agrostis capillaris* and *Anthoxanthum odoratum* (Barratt et al. 2005).

3.3.4 Experimental design and sampling of warming experiment

The experiment comprised a 2 x 2 factorial design, with warming and nitrogen as treatments with two levels each (control vs. elevated) and five true replicates per treatment combination, totaling 20 plots. We dug a 24m by 19m experimental area in October 2008, to a depth of 20 cm to establish the twenty 3.5 x 3.5m plots (12.25 m^2), each separated by a 1m corridor. We then leveled the ground and installed custom-made electric heating cables (Argus Heating Ltd, Christchurch, New Zealand: coiled copper wire on fiberglass core and silicon coating) in half of the plots, and dummy cables in the remaining (unheated) plots. Each plot was fitted with two coils of 45m meters each, resulting in a spacing of 14 cm between cable lines. Heating power totals 940 Watts per plot or a power density of 76W/ m^2. Similar power output has been recommended (Peterjohn et al. 1993) and successfully used in previous underground heating experiments (Melillo et al. 2002). In each warmed plot, we installed three thermocouples (Type E, Chromel-Constantan, Campbell Scientific, USA) at 10 cm depth and standardized position relative to the heating cables (1 directly above the cable, 1 between two heating cables, 1 between the other two thermocouples), to capture any potential temperature differences within the plot driven by distance from the heating cables. In each control (unheated) plot, we installed 1 thermocouple at the same depth. The thermocouple in the control plot

provided a baseline measure of ambient temperature so that the warmed plots could be kept at a constant temperature above ambient.

We homogenized all the extracted soil by mixing it with a digger to remove any confounding nutrient or bio-geochemical gradient before re-installing it in the experimental area and leveling the ground to ensure constant ground depth relative to the cables We planted well-established (at least 3 month old) individuals of four species of tussock grasses, which were common to the general area and also present in the altitudinal gradient experiment (50x *Poa cita*, 50x *Festuca novae-zelandiae*, 12x *Chionochloa rigida* and 12x *Chionochloa flavecens* per plot), in a consistent composition and layout for each plot). This resulted in each plot being planted with 144 individual plants, amounting to 2880 tussocks in total. We completed the set up and planting in January 2009 (see Figure S6 in Appendix 2.1). To minimize water stress to the recently-planted tussocks in the height of the first summer, we installed an automated watering system, which ran for half an hour at dawn and after sunset until May 2009.

We first activated the warming treatment in April 2009, after the plants had established for over three months. However, adjustment and tuning of temperature control meant that the experiment was fully operational by late June 2009. We paired each warming plot with its spatially-closest control plot to keep the warmed treatments at 3°C above ambient, logging the temperature of all thermocouples every minute using two Campbell CR1000 (Campbell Scientific, USA) data loggers. The average temperature of the thermocouples in the warming plots is used against the control plot to switch the power on and off as required (see Figures S7 and S8 in Appendix 2.1 for details on the temperature control). The three degrees of warming achieved in this experiment is in line with the temperature gradient we found in the field experiment and with the predictions of global (and New Zealand) warming scenarios for the next 100 years (IPCC 2007).

The nitrogen treatment application, using the same fertilizer as the gradient experiment, began shortly after planting, by adding the equivalent of 25 Kg ha^{-1} yr^{-1} in late January 2009. Applications reached a total of 50 Kg $ha^{-1}yr^{-1}$ with three evenly-distributed applications during the rest of the year. Fertilization treatments continued in 2010 with five applications of 10 Kg $ha^{-1}yr^{-1}$, one every two months except the

winter months of July and August, when the plots were often covered in snow. The decision to use five applications arose from a tradeoff between maximizing frequency of applications (to resemble natural deposition), yet applying enough to practically allow even application across the entire treated plot.

We began sampling lepidopteran larvae in January 2010, that is, a full year after plot establishment and planting. Sampling continued at monthly intervals until June 2010 (i.e. mid winter, when snow cover made sampling impractical), and resumed at monthly intervals from September to December 2010, totaling 11 sampling rounds. To minimize disturbance and depletion of caterpillars in the experimental area, we sampled half of each plot during each round, alternating between the two halves. This ensured a time window of at least 8 weeks before re-sampling of the same section. Sampling entailed visually searching for caterpillars on tussock plants, teasing apart the dense vegetation to find any hidden larvae.

Both the artificial warming and the gradient experiment present a number of caveats in their design: using natural-gradient studies has limitations in the ability to explain the response of communities to temperature changes, as populations may already have adapted to the different conditions (Hodkinson 2005). Additionally, changes to mean temperatures following global warming may be strongly influenced by changes to frequency and magnitude of extreme temperature events (IPCC 2007), which remain unaccounted for in our study. Similarly, artificial warming experiments such as the one presented in this study can be criticized for the necessarily small scale, and limitations of any heating method used in simulating global change (Kimball 2008, Peterjohn et al. 1993). However, most experiments to date have used one of these methods. In this study, we used both a large-scale field experiment combined with a manipulative controlled field experiment, finding largely consistent results that provide a good degree of confidence that the patterns found were due to the generalities of communities response to simulated global-change drivers, rather than spurious effects of any particular experimental approach.

3.3.5 Insect rearing

For both experiments, we identified each individual larva to morphospecies. To allow collection of parasitoids, we individually reared all larvae to maturity

(emergence of the adult moth or parasitoid) in a climate-controlled room, with a constant temperature of 16 degrees, relative humidity of 60% and a light cycle of 16L:8D. The feeding protocols varied according to the species requirements. All parasitoids were identified to species level where possible, and to morphospecies for organisms lacking a recognized classification. We sought the expertise of two taxonomists to help with the identification: John S. Dugdale (Landcare Research, Nelson) developed a larval key for Lepidoptera and confirmed the identity of all the tachinid flies, and Jo Berry (MAF Biosecurity, Wellington) validated hymenopteran morphospecies and formally identified all known species. The individual rearing of every herbivore larva allowed us to estimate the rate of parasitism (proportion of larvae from which a parasitoid emerged). Larvae that died during rearing were excluded from all analyses to avoid underestimation of parasitism and overestimation of herbivore biomass that would arise from including the dead herbivore but excluding non emerged parasitoids.

3.3.6 Biomass measurements

To estimate effects of temperature and N on larval biomass, we weighed the caterpillars (Mettler Toledo analytical balance accurate to 0.0001g) directly after collection for all samples. We estimated total herbivore biomass as the sum of the larval weight of all individuals in each plot. As we could not always observe parasitoids as soon as they emerged, there is a risk that individual parasitoid weight could be biased by the time between emergence and being discovered. Furthermore, unlike herbivore mass, which was measured directly after collection, parasitoid body mass can only be measured at emergence, and could therefore be strongly determined by the age at which the host larva was brought into the laboratory for rearing, and the food provided to the growing larva. This procedure could carry a possible bias, if parasitoids emerging under any treatment were consistently larger. However, there is no other practical way to collect emerging parasitoids than rearing the caterpillars, which could generate spurious differences across treatments. Therefore, we calculated the total parasitoid biomass for a plot by multiplying the total counts of each species by the average adult weight of that species. We obtained each species

average by weighing 20 adult individuals of each species, or all individuals for species totaling less than 20 individuals.

To estimate plant biomass without disruptive sampling of the plots, we estimated the total tussock volume in each plot. To obtain the total tussock volume, we first calculated the mean tussock volume per plot by measuring a subset of randomly-selected tussocks (20 in the warming experiment, 30 in the gradient experiment). We measured basal circumference and height from the ground to the highest leaf, and then calculated the cylinder volume (Laliberte et al. 2010). After obtaining the average tussock volume for each plot, we multiplied it by the total count of tussock individuals. To convert plant volume to biomass, we measured the volume of 10 tussock plants from our glasshouse cultures following the same procedure as above. We then clipped them to ground level and dried the leaf material at 60°C for 48 hours. We used a linear regression to test how well volume approximated dry weight, and found a significant relationship ($F_{1,8}$ = 20.68, P = 0.001, R^2 = 0.72).

3.3.7 Data analysis

We carried out all analyses using R version 2.12.0 (R Development Core Team; 2010). To account for our split-plot design in the gradient experiment, we used general linear mixed effects models (Bolker et al. 2009), within the nlme package (Pinheiro et al. 2011). We used total (summed) plant, herbivore or parasitoid biomass as the response variable, with a Gaussian error distribution. We included nitrogen treatment as a fixed factor and temperature as a (fixed) variate, with plots nested in transects as random effects. Biomass of consumer trophic levels is likely to be highly correlated with the biomass of the trophic level below (its resource). Therefore, we included the biomass of plants as a covariate in the model predicting herbivore biomass, and herbivore biomass in the model for parasitoids. We initially included all possible interactions, then simplified the model by removing non-significant interaction sequentially, each time assessing changes in Akaike Information Criterion (AIC) scores before any further simplification. This allowed us to determine if the effects of the drivers on consumer biomass persisted after accounting for variation

explained by resource biomass, i.e. if there was any direct effect of the drivers beyond the bottom-up, resource-biomass-mediated effects. To highlight the differential response between trophic groups, we calculated a herbivore to plant biomass ratio and a parasitoid to herbivore biomass ratio. We used a logit transformation for these ratios to meet the assumptions of normality, then tested them each as a response variable in a mixed effects model with temperature and nitrogen as predictors and a Gaussian error distribution.

In addition to biomass changes, the activity of natural enemies may respond to the treatments (e.g., higher activity due to higher metabolic rates with increasing temperature, or altered attack rates as host quality changes under elevated N). Because such a response may not have been captured by looking solely at changes in biomass, we tested the response of overall parasitism rates to the drivers. We modeled parasitism rates using a generalized linear mixed model with a binomial error distribution, carried out in the lme4 package (Bates and Maechler 2010). The proportion of all herbivores that were parasitised was the response variable, and the drivers temperature and nitrogen were included as predictors.

To test for changes in biomass at each trophic level in the artificial warming experiment, given the full factorial design, we used general linear models (the lm function in the base package of R). We used total (summed) plant, herbivore or parasitoid biomass as the response variable, with temperature and nitrogen as fixed factors. We followed the same procedure as in the altitudinal gradient experiment by including resource biomass (biomass of plants as a variate in the model predicting herbivore biomass, and herbivore biomass in the model for parasitoids) alongside the drivers, including all interactions and subsequently simplifying the model as above.

To highlight changes in total biomass within each trophic level, we also tested the relative percentage increase in biomass (arcsine square root transformed to meet the assumptions of normality and homoscedasticity) compared with the control treatment for each trophic level. We did not carry out this analysis in the gradient experiment because the use of temperature as a variate rather than the categorical (warming vs. control) used here did not allow an equally effective comparison. We tested the biomass ratio using the same procedure as in the altitudinal gradient

experiment. To test the response of parasitism rates to the driver treatments, we used binomial errors and a logit link function in the glm function of the base package in R.

3.4 Results

3.4.1 Altitudinal gradient experiment

In the gradient experiment, we found no effect of the drivers on plant biomass (Table 3.1 A). Herbivore biomass was positively correlated with plant biomass, but a strong effect of temperature on herbivores remained even after controlling for resource biomass (Table 3.1 B). In contrast, a trend towards a positive effect of nitrogen on herbivore biomass ($t = 1.82$, $d.f = 9$, $P = 0.090$) disappeared when plant biomass was included in the model. Parasitoid biomass was positively correlated with host resource biomass (Table 3.1 C), but did not respond directly to the treatments. Interestingly, we found a negative interaction between herbivore biomass and temperature, such that the positive relationship between herbivore biomass and parasitoid biomass was significantly weaker at higher temperatures (Table 3.1 C, Figure 3.1).

We also found that increasing temperature led to an overall increase in the biomass ratio of herbivores to plants ($t = 3.66$, $d.f. = 9$, $P = 0.005$) and a tendency for a decrease in the ratio of parasitoid biomass to herbivore biomass ($t = -1.91$, $d.f. = 9$, $P = 0.084$). Concordantly, we found a negative effect of both drivers on parasitism rates, with a significant interaction such that the drivers acted sub-additively (Temperature: $Z = - 2.15$, $P = 0.031$, Nitrogen: $Z = - 2.11$, $P = 0.034$, Interaction: $Z = 1.98$, $P = 0.047$).

Table 3.1: Elevational gradient experiment: coefficient table for the combined effect of the drivers on A) plant biomass, and the effect of the drivers and resource (plant or herbivore host respectively) biomass on total biomass of B) herbivores and C) parasitoids. Asterisks indicate level of significance (. ≤ 0.1, * ≤ 0.05, ** ≤ 0.01).

	Value	Std.Error	df	t-value	P-value	
A) Plants						
(Intercept)	9830.12	10020.47	13	0.98	0.345	
Temperature	-232.27	1873.78	9	-0.12	0.904	
Nitrogen	10577.54	10907.60	13	0.97	0.350	
Temperature : nitrogen	-1408.00	2042.77	13	-0.69	0.503	
B) Herbivores						
(Intercept)	-43.10	10.53	12	-4.09	0.002	**
Plant biomass	0.0005	0.0002	12	2.37	0.036	*
Temperature	9.32	1.94	9	4.80	0.001	**
Nitrogen	-10.87	13.60	12	-0.80	0.440	
Temperature : nitrogen	2.43	2.53	12	0.96	0.355	
C) Parasitoids						
(Intercept)	0.07	0.18	11	0.38	0.713	
Herbivore biomass	0.051	0.01	11	4.41	0.001	**
Temperature	-0.003	0.03	9	-0.07	0.943	
Nitrogen	-0.29	0.20	11	-1.45	0.174	
Herbivore biomass : warming	-0.007	0.002	11	-3.72	0.003	**
Temperature : nitrogen	0.04	0.04	11	1.26	0.231	

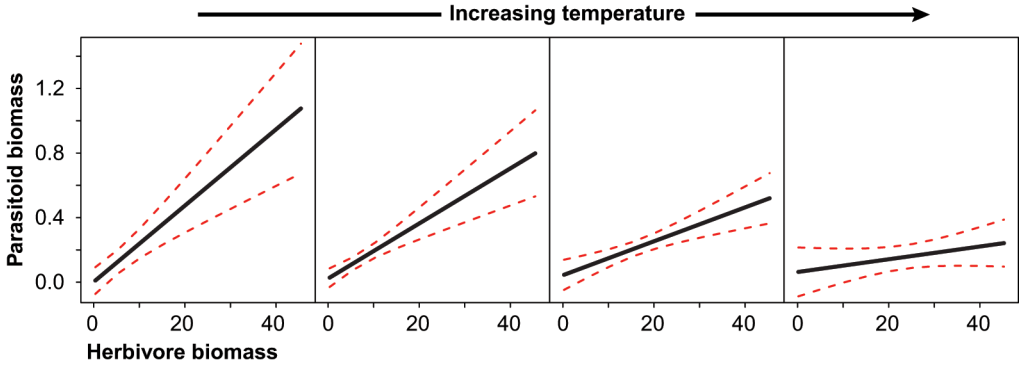

Figure 3.1: The correlation between herbivore biomass and parasitoid biomass along the temperature gradient. Panels are ordered from left to right. Thus, the left plot represents the herbivore-parasitoid biomass relationship at the lowest temperature and the right plot is for the highest temperature. Black lines represent fitted values from the mixed effects model, dashed lines show the upper and lower range of the standard error. The biomass relationship curve flattens as temperature increases, indicating an increasingly weaker increase in parasitoid biomass per unit of herbivore biomass increase.

3.4.2 Warming experiment

In the warming experiment, relative biomass responses to each driver differed across the different trophic levels (Table 3.2, Figure 3.2). There was no significant relative change in plant biomass at high temperature, but there was a significant increase in the nitrogen treatment (both in the absolute biomass, Table 3.2A, and in the mean (± SE) percent change relative to control = +63.8% ± 24.9, P = 0.016), which remained when temperature and nitrogen were combined (+ 59.9% ± SE 15.2, non-significant warming x nitrogen interaction: Table 3.2 A). In contrast, herbivore biomass on average doubled in response to temperature (relative change of + 102% ± 18.6, P = 0.006, for absolute change in total biomass see Table 3.2 B) and was marginally higher in the nitrogen treatment (+ 64.7 ± SE 32.9, P = 0.062), with combined treatments showing a weakly sub-additive effect (+ 88.1% ± SE 33.1, P = 0.095).

Herbivore total biomass was positively correlated with plant biomass but, nevertheless, retained a positive effect of temperature (Table 3.2 B), consistent with the altitudinal gradient experiment. In contrast, the marginally-significant (P = 0.062)

main effect of nitrogen on herbivores disappeared after plants were included in the model, providing some evidence that nitrogen effects on herbivores were indeed bottom-up.

Finally, parasitoid relative biomass did not differ from control under any treatment combination ($P > 0.1$ in all cases). After including herbivore biomass alongside the treatments predicting total parasitoid biomass, we found a positive correlation between resource and consumer biomass and only a trend ($P < 0.1$) towards a negative effect of temperature on parasitoid biomass after controlling for the effect of herbivore biomass (Table 3.2 C).

Overall, the observed changes in biomass at higher temperatures led to an increase in the biomass ratio between herbivores and plants ($t = 2.50$, $P = 0.023$) and a tendency towards a decrease in the ratio of parasitoid to herbivore biomass ($t = -1.78$, $P = 0.093$). In contrast, we found no effect of the drivers on parasitism rates in the warming experiment.

Table 3.2: Artificial warming experiment: coefficient table for the combined effect of the drivers on A) plant biomass, and the effect of the drivers and resource (plant or herbivore host respectively) biomass on total biomass of B) herbivores and C) parasitoids. Asterisks indicate level of significance (. ≤ 0.1, * ≤ 0.05, ** ≤ 0.01, *** ≤ 0.001).

	Value	Std.Error	df	t-value	P-value	
A) Plants						
(Intercept)	2036.54	377.49	12	5.39	<0.001	**
Warming	751.10	480.56	12	1.56	0.144	
Nitrogen	1299.46	480.56	12	2.70	0.019	*
Warming : nitrogenn	-828.69	679.62	12	-1.22	0.246	
B) Herbivores						
(Intercept)	-0.18	1.37	15	0.13	0.898	
Plant biomass	0.002	0.0005	15	3.68	0.002	**
Warming	2.84	1.22	15	2.33	0.034	*
Nitrogen	0.15	1.34	15	0.16	0.910	
Warming : nitrogen	-1.70	1.69	15	-1.01	0.330	
C) Parasitoids						
(Intercept)	0.03	0.04	15	0.62	0.548	
Herbivore biomass	0.02	0.007	15	2.84	0.012	*
Warming	-0.08	0.05	15	-1.81	0.098	.
Nitrogen	-0.03	0.04	15	-0.60	0.556	
Warming : nitrogen	0.06	0.06	15	0.95	0.358	

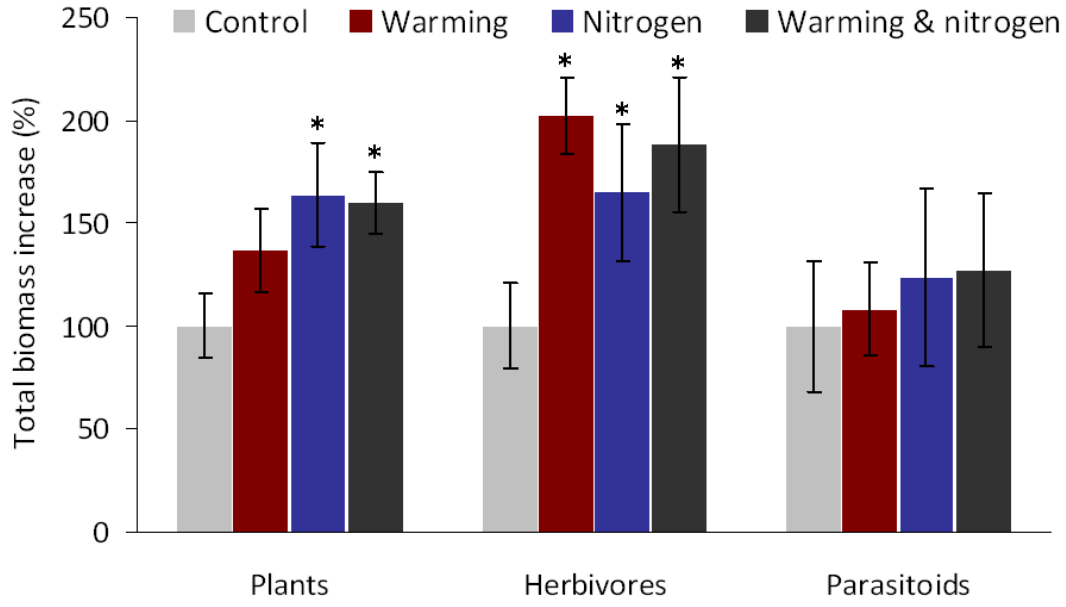

Figure 3.2: The effect of the global change drivers (warming, nitrogen and their combination) on the percentage increase in biomass relative to the control treatment for plants, herbivores and parasitoids. As the percentage increase is scaled within trophic levels, this graph allows a direct comparison of the effect of the drivers within and across trophic levels (plant, herbivores and parasitoids). Asterisks depict significant differences within groups.

3.5 Discussion

We found distinct responses of biomass at different trophic levels under elevated temperature and nitrogen and, overall, these results were consistent between two experiments that strongly differed in spatial scale and design. In particular, herbivore biomass increased significantly more than plant or parasitoid biomass at higher temperature, and this generated an increased ratio of herbivore to plant biomass with warming. Our findings of greatly increased herbivore biomass at higher temperature support hypotheses of increased herbivory based on data from agricultural systems (reviewed by Rustad et al. 2001, Bale et al. 2002 and Throop and Lerdau 2004) and paleological records (Wilf and Labandeira 1999, Currano et al. 2008).

In contrast to warming, the strength of the nitrogen direct effect on relative biomass change decreased from plants (strong positive response) to herbivores (marginally-significant positive response) to parasitoids (no response), suggesting

that bottom-up effects, or increases in resource availability, had decreasing strength or efficiency moving up the food chain. The importance of bottom-up effects was emphasized by the significant effect of plant on herbivore, and herbivore on parasitoid biomass in both experiments, and after controlling for these effects, nitrogen had no significant effect on herbivore or parasitoid biomass. In light of these findings, the role of natural enemies in controlling herbivore populations is likely to be strongly impaired by both drivers, an hypothesis supported by the significant reduction in parasitism rates under elevated temperature or nitrogen. These results are consistent with previous findings of larger herbivore population and no response by parasitoids (Haddad 2000). Leaf chewers such as caterpillars can often compensate for reduced food quality by increasing their consumption, which may reduce performance by leading to greater intake of secondary allelochemicals (Slansky and Wheeler 1992). However, this mechanism is likely to play a more important role in plants that, unlike grasses, are usually chemically defended.

In addition to biomass, rates of herbivory are also predicted to increase at higher levels of nitrogen availability, which could in turn support larger herbivore populations (Throop and Lerdau 2004). Although there may have been a top-down reduction in plant biomass due to elevated herbivory, this was not sufficient to outweigh the effect of nitrogen on plant growth, a finding congruent with that of Throop (2005), who showed that positive impacts of N on shoot biomass were not significantly suppressed by herbivory. In our experiments, we found that both plants and herbivores substantially gained biomass under elevated nitrogen, indicating a generally more productive system at the plant and herbivore level, but not at the parasitoid level.

Interestingly, under elevated levels of both temperature and nitrogen, we observed a higher increase in biomass of herbivores than parasitoids, whilst the increase of herbivores and plants was qualitatively similar. In other words, the presence of nitrogen as a second driver mitigated the strong difference in response between plants and herbivores at higher temperatures. This result highlights the importance of considering the co-occurrence of global change drivers; under a scenario of global warming with no increase in nitrogen deposition, herbivores showed a clearly stronger response than did plants and parasitoids. However, under a

realistic scenario of co-occurring drivers (Didham et al. 2007, Tylianakis et al. 2008, Gilman et al. 2010), the difference in response, particularly between plants and herbivores, may be less than expected when considering each driver in isolation.

However, we found strong evidence in both field experiments that natural enemies were not able to respond as positively to increased herbivore (host) resource availability under a changing environment. Parasitoid biomass did not significantly increase under any treatment and, importantly, showed a significantly lower response than herbivores at higher temperature. Moreover, both experiments qualitatively showed a net negative effect of temperature on parasitoids. Once we had accounted for the predictable correlation between herbivore biomass and parasitoid biomass, we found a trend for a negative effect of temperature on parasitoid biomass in the artificial warming experiment. Similarly, in the elevation gradient experiment, we found that the biomass correlation between parasitoids and herbivores was weaker at higher temperatures, and led to a decreasing parasitoid-herbivore biomass ratio. A conceivable interpretation of these results is that parasitoids were not able to counteract the strong response of herbivores (perhaps because parasitoid population responses were too slow), and this effectively generated a situation of predator-release under elevated temperature. This view is also supported by the significantly lower rates of parasitism found in the gradient experiment. Even though parasitoids attacked significantly more hosts under elevated temperature (results not shown), this increase was not proportionate to the increase in host abundance, which generated a lower proportion of hosts parasitised. It must also be noted that parasitism rates did not differ significantly across treatments in the artificial warming experiment. However, due to the small distance between plots, it is plausible that parasitoids could display behavioral choices to attack hosts across different treatments depending on their availability at a given time. Therefore, the parasitism results in the artificial warming experiment should be taken cautiously and should not undermine the validity of the results we obtained under natural field conditions in the elevational gradient study.

Our results contrast with those of Andrew and Hughes (2005), who found no evidence for increased ratios of herbivores to parasitoids and other natural enemies along a latitudinal gradient. However, the scope and methodology of their study

shows substantial differences from ours. Andrew and Hughes (2005) sampled all arthropods by knocking them down from the host plant using a pyrethrum/water solution. They thus obtained data on abundance and biomass of the major insect taxa sorted into feeding groups, but would have also included 'tourist' species, which may not have been feeding on the plants or herbivores. In contrast, we reared parasitoids from living hosts, which incorporates host-selection effects on parasitoids, and provides a measure of biomass that directly relates to the ecosystem function of parasitism. Thus, our estimate of biomass is obtained from parasitoids that are not merely present, but also able to interact successfully with their host. Nevertheless, the results of Andrew and Hughes imply that sampling of free-living adult parasitoids could lead to different results.

Strengthened top-down control by generalist predators observed under warming in terrestrial systems (Barton and Schmitz 2009) suggests that more specialized natural enemies such as parasitoids may be less responsive than generalists. Temperature is known to increase metabolic rates of mobile predators such as spiders (Rall et al 2011) whilst, in contrast, parasitoid development is dependent on their host, which may constrain (e.g., through changes in host phenology or quality) their ability to adapt to change. Previous studies on tri-trophic food chains concluded that parasitoids are unlikely to effectively counteract the response of herbivores to climate change (Hoover and Newman 2004), and specifically suggested that bottom-up forces may be more important than top-down control by the parasitoids (Tuda et al. 2006). Our findings are congruent with this suggestion, and show a severe limitation in the ability of parasitoids to effectively control herbivore populations. Our findings have concerning implications for biological control of herbivore pests, and suggest that herbivores will be the most likely to benefit and thrive in a changing environment.

CHAPTER IV

Bottom-up effects mediate response of food-web structure and resilience to climate change and nitrogen deposition

4.1 Abstract

Environmental changes, such as climate, affect species abundances, distribution and diversity, thereby affecting communities and ecosystems. Furthermore, the effects of global change drivers such as climate may depend on other drivers acting simultaneously. However, the role of species interactions in responding to and mediating such changes is largely unknown. Networks of feeding interactions (food webs) describe the underlying structure of ecological communities and are considered to be important factors determining ecosystem function and stability. In this study, we analyze the response of 50 quantitative herbivore-parasitoid food webs in a grassland system to the separate and combined effects of temperature and simulated nitrogen deposition. We experimentally applied nitrogen in both an altitudinal gradient and an artificial warming experiment, then used path analysis to disentangle the direct vs. bottom-up effects of temperature and nitrogen on food webs.

We found that food-web structure was altered by profound changes in the abundance and diversity of interacting species, which responded to an increase in basal resources. In contrast, the direct effects of the drivers on food-web structure (e.g., through phenological mismatch) were negligible, and the overall effects of temperature and nitrogen were buffered by the opposing direction of different effect pathways. However, we found that temperature and nitrogen sub-additively caused a decrease in mean food chain length, driven by a disproportionate increase in the abundance of herbivores compared with natural enemies. This loss of energy transfer

to higher trophic levels supports general concerns about increases in herbivory under climate change. We then used a model to determine the effect of food-web structure on its equilibrium stability, and found that stability remained similar under both driver effects.

Our results emphasize that bottom-up pathways play an important role in mediating the response of food webs to climate change, and that the effects of climate can interact with those of other drivers. We found no evidence of changes to the stability of food webs; nevertheless, bottom-up resource-driven changes in interaction distribution and strength are likely to bear consequences on future biodiversity.

4.2 Introduction

Human impacts on Earth's ecosystems are widely recognized, and there is little doubt that multiple global environmental changes (GEC), triggered by the dominance of humans on earth (Vitousek et al. 1997b), are driving widespread biodiversity loss (Chapin et al. 2000, Pimm and Raven 2000). Climate change and anthropogenic nitrogen deposition have been identified as major drivers of biodiversity loss (Sala et al. 2000, Walther et al. 2005), and both drive changes in plant primary production (Reich et al. 2006a), which could indirectly affect entire ecosystems. Moreover, rising temperatures are known to alter the abundance and distribution of species (Walther et al. 2002, Parmesan and Yohe 2003), as well as the timing of crucial life-history events (Menzel and Fabian 1999, Menendez et al. 2007, van Asch and Visser 2007).

A large body of research has demonstrated effects of climate on population abundances, organismal physiology, species richness and composition (Sala et al. 2000). However, it is less clear how responses of individual species to climate change will percolate through ecological communities via their interactions with other species. Recent studies highlight that biotic interactions are likely to be impacted by climate, and that many important effects of climate change will not result from the direct effects of rising temperature on species, but rather from the impact of temperature on species interactions (Tylianakis et al. 2008, Berg et al.

2010). Furthermore, biotic interactions, such as predation, play an important role in the maintenance of biodiversity, mediation of ecosystem responses to GEC (Suttle et al., 2007, Brooker, 2006), and stability of ecosystem services (Dobson et al. 2006). In particular, networks of interactions between species at different trophic levels have been shown to have emergent properties (such as resistance to perturbation), that result from their overall architecture, and can only be identified by analyzing the structure of the community as a whole (Dunne et al. 2002b, Bascompte et al. 2006, Stouffer and Bascompte 2010, 2011). The critical importance of these interaction networks (McCann 2000, Montoya et al. 2006, Rooney et al. 2006) has prompted numerous authors to call for the conservation of interactions in addition to biodiversity (McCann 2007, Tylianakis et al. 2010).

Studies that have investigated the response of food webs to anthropogenic disturbance have found complex responses that go beyond the presence or absence of species. Some have shown that species interact differently in response to disturbance such as land-use change and species invasions (Tylianakis et al. 2007, Aizen et al. 2008), whilst others have found food-web structure to be resilient to changes in species composition following habitat fragmentation (Kaartinen and Roslin 2011).

Despite the obvious importance of temperature as a change driver, climate-induced effects on food-web structure are largely unknown. In a pioneering study, Petchey et al. (1999) showed severe effects of temperature on a microcosm community, which occurred through a disproportionate loss of top predators. More recently, Petchey et al. (2010) added temperature dependence of foraging traits to an existing model of food-web structure, and predicted potentially large negative effects of temperature on complexity, highlighting the need for empirical studies to test how temperature affects community structure and dynamics.

Further to changes to metabolism, growth and performance, temperature can also indirectly affect species and their interactions through bottom-up changes in the abundance and quality of basal resources (Bale et al. 2002, Shaw et al. 2002, Zavaleta et al. 2003, Stiling and Moon, 2005, Van Nouhuys and Laine, 2008 Bukovinszky et al. 2008, Tylianakis et al., 2008). Moreover, it has been shown that trophic levels can respond differently to climate (Voigt et al. 2003), and different phenological responses of species at different trophic levels can disrupt the

synchrony of predators and prey (Visser and Holleman 2001, van Asch and Visser 2007, Klapwijk et al. 2010, Miller-Rushing et al. 2010), with unclear consequences at the network level.

In addition, other co-occurring global change drivers may synergistically interact with climate (Didham et al. 2007, Tylianakis et al. 2008). For instance, biologically reactive nitrogen in the bioshpere has doubled since the introduction of synthetic fertilizers and fossil fuels (Vitousek et al. 1997; M.E.A 2005). Nitrogen deposition typically alters plant net primary production, but it is also known to cause shifts in competitive advantages among plant (Reich et al. 2006b) and animal species (Tylianakis et al. 2008), and to drive loss of biodiversity (Stevens et al. 2004, Clark and Tilman 2008), and these effects may be temperature dependent (Reich et al. 2006a). Changes in basal plant resources usually benefit herbivores, and some evidence suggests that natural enemies can also benefit from increased nutrients (Moon and Stiling 2000, Bukovinszky 2008). However, the relative interplay of bottom-up vs. direct effects of temperature and nitrogen on the structuring of communities and their interactions remain virtually unknown (but see Richardson et al. 2002).

Here, we combine tri-trophic-interaction data, from two field experiments in a grassland system of plants, caterpillar herbivores and their natural enemies (parasitoids), to investigate for the first time the effects of global warming and nitrogen deposition on quantitative food webs. We utilize structural equation modeling to partition the bottom-up and direct effects of the drivers at each trophic level, and to ultimately determine their impact on network structure and resilience.

4.3 Material and Methods

We used data from both an altitudinal gradient and an artificial warming experiment. The experimental design for these experiments is described in detail in Chapters 2 and 3 and in Chapter 3, respectively, but summarized again here.

4.3.1 Study sites and sampling: altitudinal gradient experiment

We established five vertical transects of three plots, each at 150 m intervals of elevation, such that there was a total of 300 m difference in altitude between the lowest and the highest plot in each transect. This provided a total temperature gradient of 2.83 °C across all plots. We further subdivided each plot into two 12 x 12 m subplots, and randomly assigned one of these to a nitrogen addition treatment (addition or control with no added N). We used nitrogen fertilizer in the form of Calcium Ammonium Nitrate granules (Ravensdown LTD, New Zealand). This form of fertilizer combines fast and slower release of biologically available nitrogen, and has been used previously to simulate atmospheric deposition (Clark and Tilman 2008). We added a total of 50 Kg ha^{-1} yr^{-1} with evenly-distributed applications during the rest of the year; this rate of deposition falls within the current range of globally-observed rates of atmospheric deposition (M.E.A 2005).

We started sampling in October 2008, and continued at monthly intervals, whenever possible, until December 2009, completing a total of 11 sampling rounds successfully. To minimize disturbance and depletion of caterpillars in the experimental area, we subdivided each 12 x 12 m subplot into 4 strips of 3 x 12 m each, and sequentially sampled one strip only during each sampling round. We first sampled two randomly-positioned 1 m^2 quadrats from each subplot, where we searched all above-ground vegetation for Lepidoptera larvae and recorded the host plant for each larva. This search also provided a standardized measure of herbivore density per unit area that was used for the abundance analyses. To yield higher numbers of larvae, we then searched all the tussocks within the 3 x 12 m strip. Plant searches involved thorough teasing apart of denser vegetation to locate any hidden larvae.

Using natural-gradient studies has limitations in the ability to explain the response of communities to temperature changes, as populations may already have adapted to the different conditions (Hodkinson 2005); similarly, rapid evolutionary adaptation may occur, and such response could lead to conservative results. Additionally, changes to mean temperatures following global warming may be strongly influenced by changes to frequency and magnitude of extreme temperature events (IPCC 2007), which remain unaccounted for in our study.

4.3.2 Study sites and sampling: artificial warming experiment

We set up an artificial warming experiment adjacent to the University of Canterbury field station at Cass in the Waimakariri River catchment, South Island of New Zealand. The experiment comprised a 2 x 2 factorial design, with warming and nitrogen as treatments with two levels each (control and elevated) and five true replicates per treatment combination, totaling 20 plots of 3.5m length and width (12.25 m^2).

We generated the warming treatment by installing underground heating cables, pairing each warming plot with a control plot to keep the warmed treatments at 3°C above ambient; the warming treatment was first activated in April 2009. Heating power totaled 940 Watts per plot or a power density of 76W/ m^2. Similar power output has been recommended (Peterjohn et al. 1993) and successfully used in previous underground heating experiments (Melillo et al. 2002).

We planted each plot with four species of tussock grasses, which were common to the area and were also found in the altitudinal gradient experiment (*50x Poa cita*, 50x *Festuca novae-zelandiae*, 12x *Chionochloa rigida* and 12x *Chionochloa flavecens* per plot), in a consistent plant composition and layout; as a result, each plot was planted with 144 individual plants, amounting to 2880 tussocks in total. We started the nitrogen treatment application shortly after planting (Jan 2009), using the same type of fertilizer (CAN) at the same simulated deposition rate (50 Kg ha^{-1} yr^{-1}) as in the altitudinal gradient experiment.

We began sampling insects in January 2010, that is, a full year after plot establishment and planting, and continued at monthly intervals until June 2010 (i.e. mid winter, when snow cover made sampling impractical), and resumed at four

weeks intervals from September to December 2010, totaling 11 sampling rounds. To minimize disturbance and depletion of caterpillars in the experimental area, we sampled half of each plot during each round, alternating between the two halves.

Artificial warming experiments such as the one presented in this study can be criticized for the necessarily small scale, and limitations of any heating method used in simulating global change (Kimball 2008, Peterjohn et al. 1993). However, most experiments to date have used either experiments or natural gradients. In this study, we used both a large-scale field experiment combined with a manipulative controlled field experiment, and find largely consistent results between the two.

4.3.3 Insect identification and quantification of feeding links

We excluded all caterpillars that died during rearing. In the gradient experiment, rearing allowed the collection of 4225 data points (adult Lepidoptera or adult parasitoid). In the artificial warming experiment, rearing allowed the identification of 983 specimens. A full species list is provided in Appendix 3.1.

To establish plant-herbivore-parasitoid feeding links, we recorded the host plant and parasitoid emergence for each sampled herbivore larva. We defined a host plant as the plant from which we collected the caterpillar. When a caterpillar was found on the ground (soil), we assigned a plant in the immediate vicinity as a host if we could observe feeding signs. For individuals where there was no host plant association evident, we assigned a "ground" category, and discarded them from the plant-herbivore networks (though they were included in the herbivore-parasitoid networks). Host-parasitoid association links were assigned by individually rearing caterpillars, and recording the identity of each parasitoid emerging.

We sought the expertise of two taxonomists to help with the identification: John S. Dugdale (Landcare Research, Nelson) confirmed the lepidopteran ID, helped with developing a larval key and identified all the tachinid flies. Jo Berry (MAF Biosecurity, Wellington) validated hymenopteran morphospecies and formally identified all known species.

4.4.4 Data analysis

Food-web analyses

We conducted all analyses in R 2.12.0 (R Development Core Team; 2010) and calculated food-web metrics using the bipartite package (Dormann et al. 2008). For both experiments, we constructed sample- (plot or subplot) level quantitative host-parasitoid matrices (20 for the artificial warming experiment, 30 for the altitudinal gradient experiment), pooling the samples through time for each plot. To describe the parasitoid-host food webs, we calculated weighted, quantitative versions of Connectance, Vulnerability and Generality, based on information theory (Bersier et al. 2002, Banasek-Richter et al. 2004) and following Tylianakis et al. (2007). Connectance is a widely-used measure of complexity (Dunne et al. 2002a), which can be correlated with network stability or robustness (McCann 2000, Dunne et al. 2002b), although its role in the stability of trophic (predator-prey) networks is debated (Thebault and Fontaine 2010). Vulnerability (the weighted average number of parasitoid species attacking each host species) can be important for prey suppression, because high parasitoid diversity can promote high rates of parasitism (Tylianakis et al. 2006). Generality, measured as the weighted average number of host species used by each parasitoid species, describes whether the food web is dominated by generalists or specialists. Changes in nutrient availability and temperature can alter quality and growth rates of herbivores (Bale et al. 2002, Throop and Lerdau 2004), both of which could affect parasitoid choice and behavior (Muller and Schmidhempel 1993, Hance et al. 2007), which could alter the above metrics. Furthermore, altered phenology of hosts and parasitoids could uncouple specialist interactions, and favor generalists within the web. In addition to the above metrics, which measure the extent to which species at each trophic level are connected, we also calculated quantitative mean food chain length (Bersier et al. 2002) for the tri-trophic food web involving plants, herbivores and parasitoids. Mean chain length provides a measure of efficiency in energy transfer to higher trophic levels.

These quantitative metrics are weighted to incorporate the total inflow and outflow of biomass (which, in the case of parasitoid-host networks is the number of individuals) per species (Bersier et al. 2002). Quantitative metrics are more robust to sampling differences than their qualitative counterparts, and their lower sensitivity

makes them more conservative when comparing webs across treatments (Banasek-Richter et al. 2004).

Ultimately, we measured network resilience following De Ruiter et al.(1995) and Neutel et al. (2007). With this method (based on measuring interaction strengths and the community matrix according to May's definition (May 1973)), we make the assumption of observing the networks and abundances at equilibrium, and estimate the probability that these are stable.

Parasitism rates of pairwise interactions

We determined whether the strength (i.e. interaction-specific parasitism rate) of any given interaction was affected by the drivers and, if so, whether this effect was direct or bottom-up. This measure of host-parasitoid interaction strength, helped to clarify the mechanisms behind any changes in quantitative connectance. We tested the interaction strength for each pairwise interaction (i.e. each unique host-parasitoid combination) as a response variable in a generalized linear mixed model with a binomial error structure in the lme4 package (Bates and Maechler 2010). We also included the identity of each pairwise interaction as a random effect. In the altitudinal gradient experiment, this was crossed with transect and plot as nested random factors to accommodate the split-plot design. We initially included temperature, nitrogen and their interaction as predictors to obtain the overall effect of the drivers. Subsequently, we added individual interactions' asynchrony, plot-level herbivore and parasitoid richness, and herbivore and parasitoid composition (PCA scores, see below), to highlight whether any significant effect of the drivers was mediated by bottom-up changes to the herbivore or parasitoid community structure. In the maximal model, we also included interactions between the drivers and all other variables. We used maximum likelihood (ML) to fit the model (Bolker et al. 2009), which we simplified by removing non-significant terms and assessing changes in model fit using AIC scores, until we obtained the minimal adequate model. The final model was fitted using restricted maximum likelihood (Bolker et al 2009).

Path analysis

To disentangle the direct and indirect channels through which warming and nitrogen deposition affect food-web structure and resilience, and to test the relative influence of plant resource availability on measures of abundance and richness at higher trophic levels, we used generalized multilevel path models (Shipley 2009). The rationale for the choice of variables and pathways included in the initial model is detailed in Figure 4.1. For each experiment, we also constructed a path model to test the effects of resource availability and diversity and abundance of consumers on quantitative mean food chain length.

Testing the validity of a generalized multilevel path model consists of (Shipley 2009): (1) finding the 'basis set' of independence claims implied by a directed acyclic graph (i.e. a box-and-arrow causal diagram that involves no feedback loops) that, together, expresses the full set of dependence and independence claims implied by the graph, (2) obtaining the probability p_i associated with each of the k independence claims in the basis set, using appropriate statistical tests (note that we used three different approaches: general linear models for the warming experiment and linear mixed models for the elevation-gradient data (because of the split-plot design), and multivariate permutational anova for multivariate data in both datasets, as described below), (3) combining the pi using $C = -2\sum k\ i=1\ \ln(p_i)$, and (4) comparing the C-statistic to a chi-square (χ^2) distribution with 2k degrees of freedom to assess the overall fit of the model as a whole (Shipley 2009). This latter test gives the probability (P value) for a null hypothesis that the data do not depart significantly from what would be expected under such a causal model (Shipley 2009). A model can be rejected if the P-value derived from the C statistic is less than the specified α-level (in our case $\alpha = 0.05$; as an example, see Appendix 3.2 for the full basis set of independence claims of the final model for the altitudinal gradient experiment). If the model is not rejected, the dependence claims are then tested separately to confirm the significance of each path.

Model variables

We constructed a maximal path model, including all potential logical pathways among the following variables (described in detail below) at different trophic levels:

at the plant level, we measured plant resource availability and quality; at the herbivore and parasitoid levels, we included measures of species richness, abundance, and composition. Further, we included interaction asynchrony, quantitative food-web metrics (Connectance, Vulnerability and Generality) and network resilience as final downstream variables (Figure 4.1). The measure of asynchrony (see below for details) did not fit the data (i.e. did not predict any downstream variables) and was therefore removed during model simplification. All the remaining variables listed above were retained in the final model (see Appendix 3.3 Figure S9 for the final path model of both experiments).

In the path models for mean food chain length, we included resource availability and quality at the plant level, and abundance at both herbivore and parasitoid levels, with quantitative mean chain length as the final downstream variable. Here, we initially included resilience as the final model variable, but then removed it as we found no significant correlation with chain length.

We calculated resource availability differently for the two experiments, reflecting the different sampling regimes. In the altitudinal gradient experiment, we searched all vegetation in a standardized area, and therefore calculated resource availability as the proportion of known food-plant cover over the total vegetation cover (a food plant was defined as any plant species that we observed caterpillars feeding on, and was confirmed by feeding trials). In the artificial warming experiment, we sampled herbivores from a standardized tussock composition. Therefore, to reflect effects of the drivers on plants as food resources, we estimated changes in tussock biomass as a measure of resource availability. Furthermore, we measured the percentage nitrogen in leaf tissue as an indicator of plant quality in both experiments.

For both herbivores and parasitoids, abundance and richness were calculated as total counts of individuals and species, pooled over all sampling rounds for a given plot or subplot. To account for potential changes in the identity of species, even if diversity did not change significantly, we included herbivore and parasitoid community composition in the path models for both experiments, and tested these paths using multivariate permutational Anova in Primer V6 (version 6.1.11) and the Permanova+ package (version 1.0.1, Anderson et al. 2008). The split-plot design

used in the altitudinal gradient experiment required us to include transect and plot as random factors, whilst the artificial warming experiment did not require random factors. To test the response of herbivore and parasitoid community composition in both experiments, we obtained a community dissimilarity matrix based on the Modified-Gower distance with base 10 (Anderson et al. 2006). This distance measure considers an order-of-magnitude change in abundance (e.g., from 0.01 to 0.1) equal to a change in composition (i.e. from 0 to 1 species), and therefore accounts for the changes in relative abundance of species in addition to changes in the community composition *per se.*

To include community composition in the upstream independence and dependence claims, i.e when using community composition as predictor, we used a principal components analysis (PCA) to reduce the variability in herbivore and parasitoid community composition to a set of orthogonal axes (Hirst and Jackson 2007). We included in the analyses all principal component axes that explained more than 5% of the variation in herbivore or parasitoid community composition (Altitudinal gradient: 3 axes explaining herbivore composition, cumulative variation = 97.8%; 3 axes explaining parasitoid composition, cumulative variation = 89.7%; Artificial warming experiment: 4 axes explaining herbivore composition, cumulative variation = 91.6%; 3 axes explaining parasitoid composition, cumulative variation = 93.8%.

For each pathway leading from community composition in our path models, we tested a full model including all of these PCA axes that explained more than 5% of the variation alongside other upstream variables, and then simplified it to the minimal number of axes showing a significant effect. In other words, a significant path (dependence claim) from community composition represented the combined effect of all significant PCA axes explaining composition. When including PCA axis scores as conditioning variables in independence claims, we initially included all axes in a maximal model, then removed all non-significant axis scores (except for one) if all were non-significant. This procedure gave the most statistical power to the variable being tested in the independence claim, and was thereby as conservative as possible in assigning independence to unconnected variables in the path model.

Phenological synchrony of species at different trophic levels is known to be an important factor shaping population dynamics, particularly under climate change (Sparks and Yates 1997, Virtanen and Neuvonen 1999, Visser and Holleman 2001, Stenseth and Mysterud 2002, van Asch and Visser 2007). To account for potential mismatches between hosts and parasitoids, we defined asynchrony as the difference in timing of peak abundance between each parasitoid and its host. We then calculated, for each plot, the mean interaction asynchrony as:

$$A_k = \frac{\sum_{1}^{N} \left| \left(H_{ik} - P_{jk} \right) \right|}{N}$$

where A_k is the asynchrony in plot k, H_{ik} is the time (month) of peak abundance of host i in plot k, P_{jk} is the time of peak abundance of parasitoid j in plot k, and N is the total number of pairwise interactions (i.e. food web links) in plot k. In other words, mean asynchrony would increase if hosts were on average peaking earlier or later than their parasitoids. To test the validity of this measure, we used a linear mixed model (with binomial errors and the identity of the interaction as a random effect) to test whether the asynchrony of each interaction $|(H_{ik} - P_{jk})|$ predicted the proportion of individuals of that host that were parasitized (i.e. interaction strength, see above). We found a negative effect of asynchrony on interaction strength ($Z = -5.22$, $P < 0.0001$), indicating that it was a valid measure of the temporal co-occurrence of parasitoids and hosts, and of their ability to interact.

For the altitudinal gradient experiment, we tested all normally-distributed response (i.e. endogenous) variables in the path model using linear mixed effects models within the nlme package (Pinheiro et al. 2011) for R. For count data (species richness and abundance), we used generalized linear mixed effects models in the lme4 package (Bates and Maechler 2010), with a Poisson error distribution. All models included plots nested in transect as random factors. To fit the individual path coefficients leading to endogenous variables (measured variables within the model that have arrows leading to them), we used restricted maximum likelihood (REML) estimation. All models were tested for overdispersion and, wherever necessary, overdispersion was taken into account using a Poisson log-normal distribution, whereby an observation-level vector is included as a random effect (Bolker et al.

2009). For the artificial warming experiment, which had a fully-factorial design, we used linear models for normal data and generalized linear models with a Poisson error structure for count data (i.e. richness and abundance).

Standardized path coefficients, visually represented by the arrow width in the model, allow assessment of the relative effect strength of each predictor on endogenous variables, as the units of change are expressed in units of standard deviation and can be directly compared across pathways (irrespective of the units of the variables themselves). We calculated standardized coefficients as described by (Aiken and West 1991), i.e taking the mean-centered ($x - \bar{x}$) values of each predictor and dividing these by the standard deviation. However, this method of standardization is not suitable for models using a Poisson distribution, because mean centering of variables inevitably produces negative or non-integer values that are not in keeping with a Poisson distribution. Therefore, for the calculation of standardized path coefficients, we modeled count data as normally distributed (Gaussian error), and used a square root transformation prior to mean centering and division by the standard deviation. A procedure to calculate standardized coefficients for multivariate data is not available; therefore, we estimated standardized coefficients for community composition (as both a predictor and response variable) by using the coefficients from the PCA scores in linear, general linear and mixed effect models.

Given these approximations, the standardized path coefficients should be interpreted with caution, although we believe that they still provide a useful comparison of the relative strength of effects between different variables. Note, however, that statistical significance and unstandardised path coefficients were calculated using the appropriate distribution or multivariate analysis on untransformed data, so the above caveats do not apply. Similarly, R-square values were obtained directly from the linear and general linear models. For mixed models, we used an R^2 statistic developed specifically for linear mixed models (Kramer 2005). While acknowledging that the calculation and interpretation of R^2 statistics for mixed models are still under debate, we still believe that they provide a reasonable indication of how the model for each path fits the data.

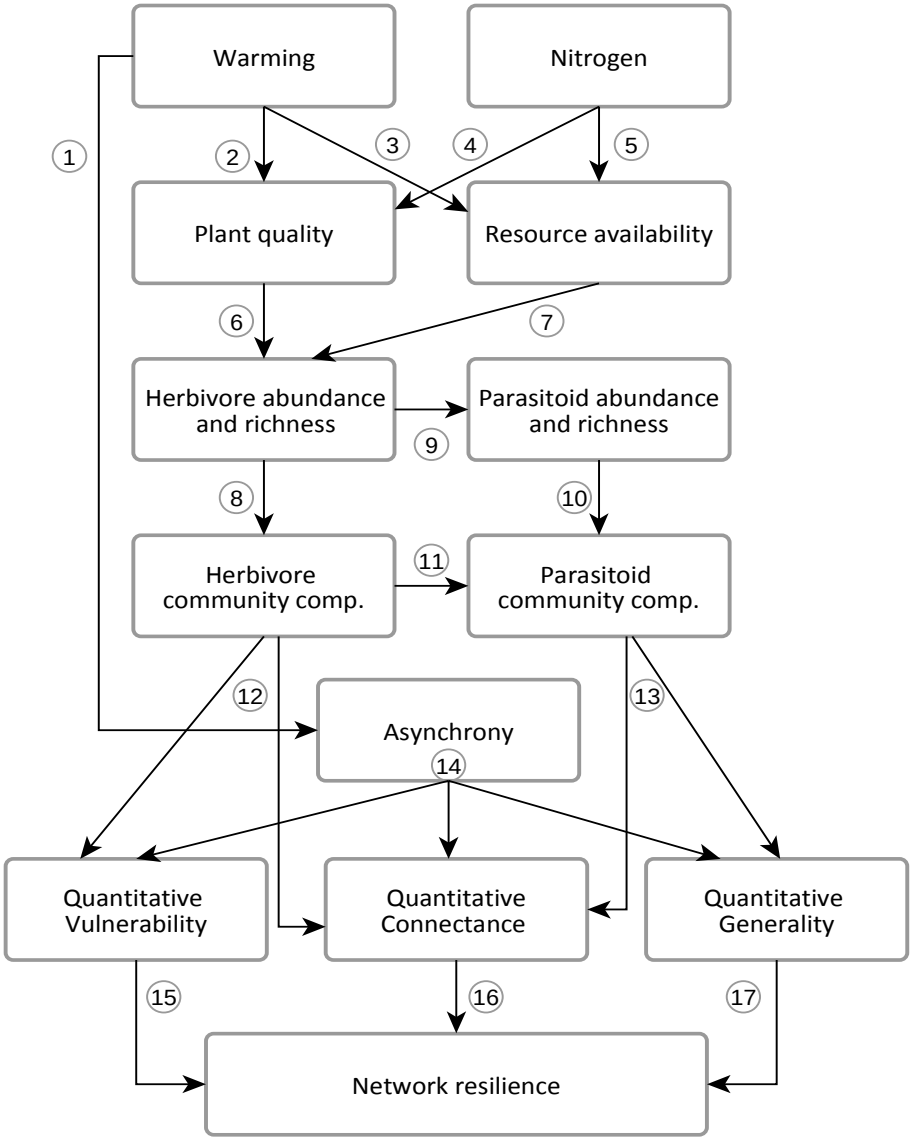

Figure 4.1: Path model rationale and construction. Global environmental changes can affect species interactions directly (e.g., by altering encounter probabilities or frequencies) or indirectly through changes in the composition of communities at different trophic levels (Tylianakis et al., 2007, Albrecht et al., 2007, Bukovinszky, 2008, Petchey et al., 1999, Tylianakis et al., 2008). Therefore, we used generalized multilevel path models (Shipley 2009) to test the relative influence of plant resource availability alongside measures of abundance and richness at each trophic level, as indirect pathways against which to compare the direct effects of the drivers on food-web structure. The hypothesis tested for each numbered pathway or set of paths is as follows:

1) It has been suggested that climate-induced changes in phenology of different species can lead to a situation of trophic mismatch where prey and predators are phenologically decoupled (Gutierrez et al. 2008, Both et al. 2009, Klapwijk et al. 2010, Miller-Rushing et al. 2010, Yang and Rudolf 2010).

2) Warmer temperatures have been shown to alter plant nutrients (leaf nitrogen) positively (Bezemer and Jones 1998) or negatively (Reich et al. 2001, Flynn 2006).

3) If not too extreme, warming is often also associated with higher net primary production (i.e. higher basal resource availability) (Rustad et al. 2001).

4) Effects of nitrogen fertilization on plants are well known. However, the effects of low-levels (compared to agricultural fertilization) of nitrogen deposition resulting from human activities are less well resolved. Studies on experimental grass communities have nonetheless shown that nitrogen deposition promotes higher plant quality (lower C/N ratio) (Reich et al. 2006a, Peters 2007), in addition to

5) promoting increases in biomass and changes to composition and diversity (Reich et al. 2001, Richardson et al. 2002, Clark and Tilman 2008).

6) Plant quality is likely to affect herbivores, which can benefit from higher nutrient content and exhibit higher population densities (Sudderth et al. 2005, Krauss et al. 2007), altered fecundity (Awmack and Leather 2002), or increased consumption and development rates (Hattenschwiler and Schafellner, 1999, Kerslake et al., 1998, Prudic et al., 2005). However, increasing plant quality has also been shown to negatively affect herbivore performance by increasing defensive compounds (Erelli et al. 1998).

7) Herbivore abundance is likely to scale with resource availability, measured as the quantity of plant material available to the herbivores. Increased resource availability could sustain larger herbivore populations, and alter herbivore diversity, e.g., by shifting competitive balance (Richardson et al. 2002, Tylianakis et al. 2008).

8) Herbivore species composition (presence or absence of different species and their relative abundances) strongly depends on overall species abundance and/or richness (Hartley et al. 2003, Thompson et al. 2008); however, composition can also be directly affected by warming (Zhang et al., 2005, Yang et al., 2011, Chapther 2).

9, 10 and 11) Shifts in herbivore diversity, abundance, and community composition can affect the abundance and species richness of their natural enemies (Muller et al. 1999, Tylianakis et al. 2006). If parasitoid species are highly host-specific, parasitoid abundance and species richness could be directly affected by changes in the abundance and richness of herbivores. Alternatively, parasitoids could change their behaviour and preference under different environmental conditions (Awmack et al. 2004, Griswold and Lounibos 2005, Tylianakis et al. 2007), which could in turn affect parasitoid richness, abundance and composition independently from the response of herbivores.

12 and 13) Overall changes in community composition can lead to changes in network structure, as different species may attract different interactions, or parasitoids may react to changes in host density (Chapter 5). Bottom-up changes in resources can percolate up to food-web structure (Bukovinszky 2008). Generality and vulnerability, measured quantitatively as the weighted mean number of hosts per parasitoid and the mean number or parasitoids per host,

respectively, could respond to changing host availability through density-dependent attack rates (a parasitoid chooses a given prey species only if it is readily available, but does not specifically search for it). Generality would increase if parasitoids attack more prey species as a consequence of increased thermal budget (search behavior) or host palatability (e.g., increased host body size). Alternatively, changes in generality and vulnerability could indicate shifts in the parasitoid community (e.g., loss of specialist parasitoids, or increase in generalist species). Connectance can be altered directly by temperature (Petchey et al., 1999, Petchey et al., 2010) and changes in community composition (Aizen et al. 2008), although it has been shown to remain stable in the face of changes in the identity of species in the community (Kaartinen and Roslin 2011). The effect of temperature on the connectance of a host-parasitoid system is unknown.

14) Asynchrony can affect food-web structure if herbivores and parasitoids differ in their phenological response to climate (Van Nouhuys and Lei 2004) leading to loss of interactions, which would affect overall connectance, and decrease vulnerability. The effect of asynchrony on generality would depend on which particular parasitoid species are affected. If specialists are more strongly dependent on synchrony with their prey, we could expect that they would be the first to suffer any temporal mismatch, which could cause them to be lost from the network, thus leading to an overall increase in network-wide generality. Moreover, a recent study suggests that phenology is a major determinant of food-web structure, and contributes to both topology and stability (Encinas-Viso et al. 2012).

15, 16 and 17) We hypothesize that changes to food-web structure will result in changes to the stability (resilience) of the network. A relationship between complexity and stability has been suggested by decades of theoretical work (Yodzis, 1981, May, 1973, MacArthur, 1955, Pimm and Lawton, 1978). Whilst in randomly-assembled food webs complexity is usually linked to instability (May 1973), it is clear that food-web structure is non random (Allesina and Pascual 2008), and its topology can strongly increase stability (McCann 2000, Montoya et al. 2006, Neutel et al. 2007, Otto et al. 2007), as can changes in the relative strength of interactions (McCann et al. 1998). Although higher diversity and connectance can promote the persistence and resilience of mutualistic networks, these attributes have been suggested to destabilize trophic networks (Thebault and Fontaine 2010).

4.4 Results:

In the altitudinal gradient experiment, we analyzed 30 quantitative host-parasitoid food webs, comprising 4225 caterpillars (39 species of Lepidoptera) and 980 parasitism events with 41 species of parasitoids (27 Hymenoptera species and 14

Diptera species) comprising 106 links (unique host-parasitoid interactions). In the artificial warming experiment, we described 20 food webs, utilizing 983 herbivores (from 27 Lepidoptera species) and 333 feeding interactions with 21 parasitoid species (10 Hymenoptera and 11 Diptera), comprising 41 links.

In the altitudinal gradient experiment (AGE), temperature ($Z = -3.21$, $P = 0.001$) and nitrogen ($Z = -1.99$, $P = 0.046$) had a negative, sub-additive (interaction: $Z = 1.87$, $P = 0.061$) effect on the strength (host-specific parasitism rate) of each interaction, leading to a net overall negative effect on quantitative connectance. However, the effect of the drivers on interaction frequency was mediated by the community composition of herbivores ($|Z| > 3.41$. $P < 0.001$ for the first two PCA axes), parasitoid composition ($Z = 5.59$, $P < 0.0001$ for the first PCA axis), host abundance ($Z = -6.22$, $P < 0.0001$) and asynchrony ($Z = -4.36$, $P < 0.0001$). As expected, species asynchrony, measured as the difference between the times of peak abundance of a parasitoid species and its herbivore host, had a strong negative effect on interaction strength ($Z = -5.22$, $P < 0.0001$). Interestingly, a sub-additive interaction with temperature ($Z = 4.10$, $P < 0.0001$) showed that differences in the timing of peak host and parasitoid abundance inhibited interactions most strongly at low temperature. However, asynchrony was not directly affected by temperature or nitrogen ($t < 1$, $P > 0.1$ in both cases). These results were supported by the artificial warming experiment (WE), where we found that interaction strength was negatively affected by host herbivore abundance ($Z = -3.18$, $P = 0.002$) and, congruently, it responded to herbivore ($Z = 2.80$, $P = 0.005$ for the first PCA axis) and parasitoid composition ($Z = 4.98$, $P < 0.0001$ for the first PCA axis). Asynchrony only had a marginally non-significant negative effect ($Z = -1.84$, $P = 0.067$) on interaction strength in the WE, and there was no effect of the drivers after controlling for composition and asynchrony. Similarly, asynchrony was not affected by warming or nitrogen, and for this reason it was removed from the path model.

These results indicate that the lower average strength of each interaction at higher temperatures was mediated by changes to community composition at both the herbivore and parasitoid levels. Moreover, we found no effect of asynchrony and/or the drivers on the proportion of interactions that were present/absent (i.e. binary connectance, all $P > 0.1$).

Using a path analysis (see Figure 4.2 for a result summary, Figure S12 in Appendix 3.3 for the full best-fitting path models), we found that the drivers promoted changes in the organization of the multitrophic assemblage and their interaction structure via bottom-up pathways (Figure 4.2; all tests for the AGE are reported in Table 4.1 A), tests for the WE are reported in Table 4.1 B)). Temperature and nitrogen promoted higher resource availability, and nitrogen addition increased plant quality (Figure 4.2, Step 1). These increases in resource availability led to strong increases in herbivore abundance, whilst higher leaf nitrogen content had a negative effect on herbivore counts (Figure 4.2, Step 2). In AGE, we also found a direct effect of temperature and nitrogen on herbivore abundance, beyond the measured plant-mediated effects. Herbivore richness was not affected directly by the drivers, but generally increased as a result of increasing herbivore abundance. Note that, in the WE, herbivore richness was not correlated with herbivore abundance and did not respond to the drivers. Together, changes in abundance and richness predicted a significant change in the composition of the herbivore assemblage. In both experiments, increased herbivore abundance was associated with an increase in parasitoid abundance and, directly as an abundance effect or through herbivore species richness, also promoted increasing parasitoid richness (Figure 4.2, Step 3). Whilst temperature had a slight positive direct effect on parasitoid abundance in the AGE, we found a strong negative effect of warming on parasitoid richness in the WE. However, as a consequence of the strong effect of herbivores on parasitoid abundance and richness, changes in parasitoid composition were driven directly by herbivore abundance and richness in the WE. Conversely, in the AGE, shifts in parasitoid composition were explained by changes in abundance of both herbivores and parasitoids, and were also correlated with herbivore composition, rather than richness *per se*.

We found little or no direct effect of the drivers on network metrics, though quantitative food-web structure responded indirectly to changes in species abundance and richness at both herbivore and parasitoid levels. In the AGE, we found a weakly significant, positive direct effect of temperature on food-web generality that was not mediated by changes in the parasitoid community (e.g., a selective shift toward more generalist species); this result was not consistent with the WE, and was the only

direct effect of temperature. In contrast, we found a suite of bottom-up effects on web metrics (Figure 4.2, Step 4). Vulnerability was affected by parasitoid composition in both experiments. Moreover, connectance responded to increasing richness in the food webs. In the AGE, parasitoid richness had a strong negative effect on connectance alongside an effect of herbivore composition that strongly depended on the abundance of the dominant herbivore species (the first PCA axis had a significant effect, and the species that made the greatest contribution to this axis were the three most abundant species in the system). In the WE, connectance responded negatively to both herbivore and parasitoid richness. Interestingly, there was a positive effect of herbivore abundance after accounting for the effect of richness, likely indicating positive density-dependent parasitism.

In the AGE, the resilience of the network responded negatively to nitrogen addition ($t = -2.33$, $P = 0.036$), and this effect was weaker at higher temperatures (temperature x nitrogen interaction: $t = 2.21$, $P = 0.045$). However, we found no such effect in the WE, where overall stability did not respond directly to warming or nitrogen (all $P > 0.1$). In both experiments, the resilience of the network was unaffected by changes in species richness, abundance or composition (Figure 4.2, step 5). Rather, we found that network resilience was strongly correlated with the web metrics, in particular showing a strong negative correlation with connectance. In contrast, both vulnerability and generality had a positive effect on network resilience.

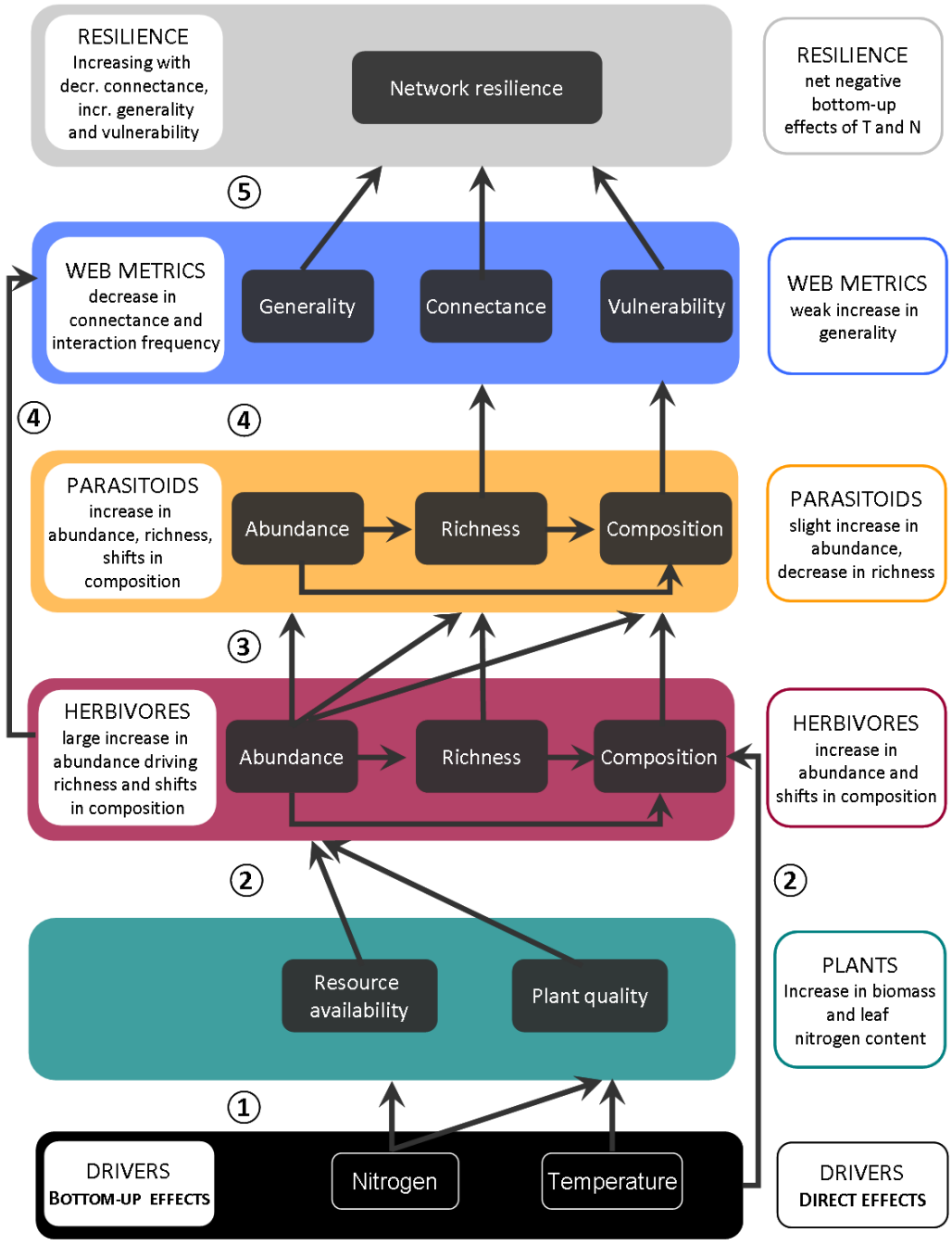

Figure 4.2: Flow diagram synthesizing the results of the path analyses for both the altitudinal gradients and artificial warming experiments. Arrows represent significant effects between variables (dark boxes within the larger, colored boxes represent the hierarchical structure of the model). Text boxes within the colored boxes (left margin) summarize the overall bottom-up effects. Standalone text boxes (right margin) summarize the main direct effect of the drivers. We found that both drivers altered plant availability and/or quality (Step 1). Both drivers also increased plant availability and strongly affected herbivore abundance and composition (Step 2). In turn, changes to herbivores cascaded to increase parasitoid abundance and alter their community composition (Step 3). The web metrics responded to changes in the abundance, richness and composition of species at both herbivore and parasitoid levels (Step 4). Finally, network resilience was sensitive to changes in web structure.

In addition to 'horizontal' measures of food-web complexity, we measured quantitative mean food chain length (Bersier et al. 2002) as a measure of vertical complexity. In the WE, the drivers showed a weakly sub-additive (interaction: t = 1.88, P = 0.092) negative effect of temperature (t = -2.38, P = 0.041) and nitrogen (t = -2.13, P = 0.062) on mean chain length, indicating an overall decrease in energy transfer to higher trophic levels. However, the direct effects of the drivers were overridden by the indirect pathways, specifically a strong positive effect of parasitoid abundance (t = 11.47, P < 0.0001) and a negative effect of herbivore abundance (t = -9.84, P < 0.0001). In the WE, we found a similar effect of consumer abundance (parasitoid: positive effect, t = 12.88, P < 0.0001; herbivores: negative effect, t = -7.27, P < 0.0001), and no direct effect of the drivers (See Figure 4.3 for path models involving food chain length).

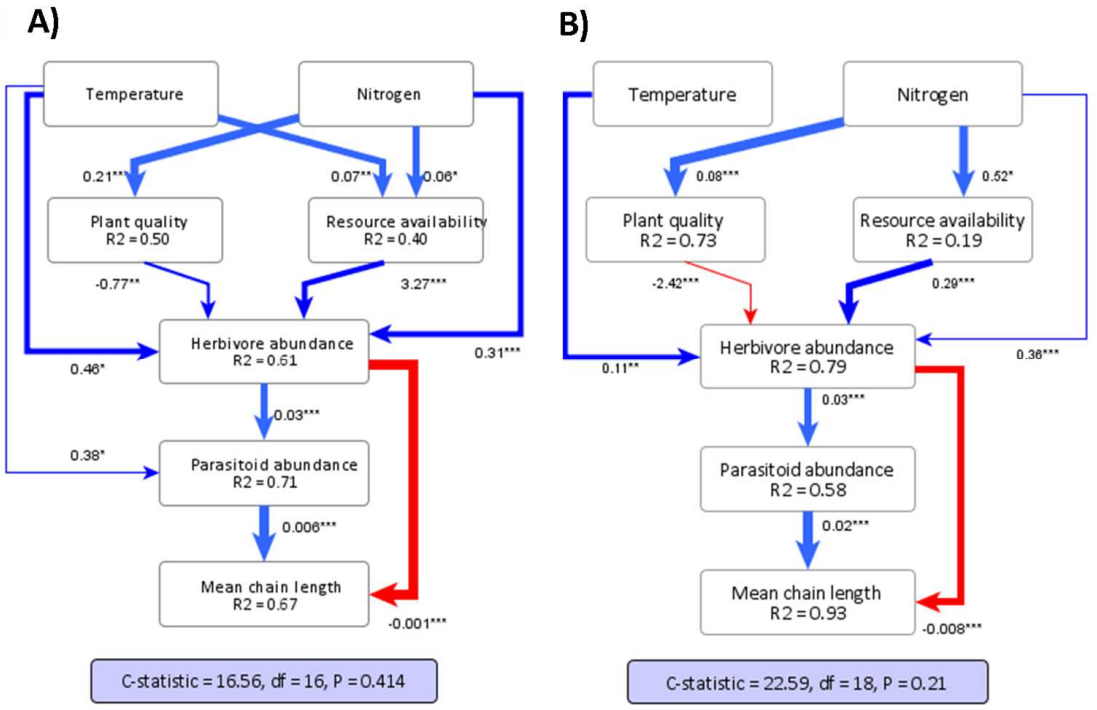

Figure 4.3: Confirmatory path analysis (Shipley et al. 2009) showing the cascading effects of temperature and nitrogen on mean food chain length for A) Altitudinal gradient experiment and B) Artificial warming experiment. Arrows represent flow of causality. Blue arrows depict a positive effect, red arrows a negative effect. Arrow width is scaled to the standardized path coefficients. Unstandardised path coefficients are given with each path. Asterisks indicate level of significance *P < 0.05, **P < 0.01, ***P < 0.001.

Table 4.1: Coefficient tables for the causal pathways in A) Altitudinal gradient experiment and B) Artificial warming experiment. Depending on the error structure, t-values are given for Gaussian data, Z-values for Poisson (count) data, and Pseudo-F tests based on permutational Anova (Anderson et al. 2008) are given for community composition. R-square values give an indication of fit of each pathway. Asterisks indicate level of significance (. ≤ 0.1, * ≤ 0.05, ** ≤ 0.01, *** ≤ 0.001

A)

Response variable	Predictors	Estimate	Std. Error	Test statistic	Test value	P-value		R-square
Resource availability	(Intercept)	0.070	0.127	T	0.55	0.588		
	Temperature	0.077	0.023	T	3.26	0.001	**	
	Nitrogen	0.062	0.023	T	2.72	0.017	*	0.40
Plant quality	(Intercept)	1.103	0.049	T	22.11	<0.0001	***	
	Nitrogen	0.210	0.039	T	5.34	<0.0001	***	0.50
Herbivore abundance	(Intercept)	1.253	1.232	Z	1.02	0.309		
	Temperature	0.461	0.225	Z	2.06	0.039	*	
	Nitrogen	0.318	0.061	Z	5.15	<0.0001	***	
	plant quality	-0.773	0.279	Z	-2.77	0.006	**	
	Resource availability	3.275	0.396	Z	8.28	<0.0001	***	0.61
Herbivore richness	(Intercept)	2.21	0.093	T	23.49	<0.0001	***	
	herbivore abundance	0.001	0.0004	T	3.61	0.0003	***	0.47
Herbivore composition	(transect)	0.042		Pseudo-F	1.65	0.001	**	
	Temperature	0.048		Pseudo-F	3.78	0.0001	***	
	herbivore richness	0.077		Pseudo-F	5.87	0.0001	***	0.40
Parasitoid abundance	(Intercept)	-0.521	1.449	Z	-0.46	0.649		
	Temperature	0.397	0.206	Z	1.93	0.054	.	
	herbivore richness	0.087	0.032	Z	2.66	0.008	**	
	herbivore abundance	0.002	0.0009	Z	2.38	0.017	*	0.77

Parasitoid richness	(Intercept)	0.12	0.39	Z	0.31	0.755		
	parasitoid abundance	0.02	0.006	Z	3.89	0.0001	***	
	herbivore richness	0.11	0.03	Z	3.74	0.0002	***	
	herbivore composition (1st PCA axis)	0.01	0.002	Z	2.72	0.007	**	
	herbivore composition (2nd PCA axis)	-0.005	0.001	Z	-2.71	0.007	**	0.83
Parasitoid composition	(transect)			Pseudo-F	1.58	0.001	**	
	herbivore abundance	1.08		Pseudo-F	1.72	0.027	*	
	parasitoid abundance	0.19		Pseudo-F	2.67	0.0001	***	
	herbivore composition (1st PCA axis)	0.95		Pseudo-F	1.66	0.037	*	
	herbivore composition (2nd PCA axis)	0.02		Pseudo-F	1.72	0.032	*	
	herbivore composition (3rd PCA axis)	0.04		Pseudo-F	1.78	0.018	*	0.96
Generality	(Intercept)	0.77	0.28	t	2.72	0.019	*	
	Temperature	0.10	0.05	t	1.88	0.093	.	0.13
Vulnerability	(Intercept)	1.41	0.12	t	11.89	<0.0001	***	
	parasitoid composition (1st PCA axis)	0.03	0.009	t	2.99	0.017	*	
	parasitoid composition (2tndPCA axis)	0.05	0.02	t	2.74	0.025	*	0.40
Connectance	(Intercept)	0.09	0.006	t	13.49	<0.0001	***	
	herbivore composition (2nd PCA axis)	0.0002	0.001	t	2.83	0.022	*	
	herbivore composition (3rd PCA axis)	0.0003	0.001	t	3.24	0.017	*	0.77
	parasitoid richness	-0.002	0.001	t	-5.42	0.0006	***	
Resilience	(Intercept)	0.98	0.07	t	-14.78	<0.0001	***	
	Connectance	-3.96	0.61	t	-6.49	0.0002	***	
	Generality	0.05	0.03	t	1.93	0.081	.	
	Vulnerability	0.09	0.03	t	3.24	0.012	*	0.66

Table 4.1 A) continued

Table 4.1 B)

Response variable	Predictors	Estimate	Std. Error	Test	Test value	P-value		R-square
Resource availability	(Intercept)	-39.56	23.86	t	-1.66	0.115		
	Nitrogen	79.11	33.74	t	2.35	0.031	*	0.19
Plant quality	(Intercept)	-0.04	0.01	t	-4.60	0.0002	***	
	Nitrogen	0.08	0.01	t	6.51	<0.0001	***	0.69
Herbivore abundance	(Intercept)	3.67	0.05	Z	72.12	<0.0001	***	
	Warming	0.19	0.07	Z	2.90	0.004	**	
	plant quality	-1.53	0.74	Z	-2.08	0.037	*	
	resource availability	0.003	0.0001	Z	6.66	<0.0001	***	0.79
Herbivore composition	herbivore richness	0.002		Pseudo-F	2.31	0.006	**	
	herbivore abundance	0.01		Pseudo-F	2.12	0.016	*	
Parasitoid abundance	(Intercept)	1.58	0.22	Z	7.29	<0.0001	***	
	herbivore abundance	0.03	0.004	Z	6.12	<0.0001	***	0.58
Parasitoid richness	(Intercept)	0.73	0.38	Z	1.91	0.056	.	
	herbivore abundance	0.02	0.01	Z	2.68	0.007	**	
	Warming	-0.42	0.22	Z	-1.90	0.058	.	0.42
Parasitoid composition	herbivore abundance	0.04		Pseudo-F	1.94	0.033	*	
	parasitoid richness	0.02		Pseudo-F	1.72	0.063	.	
	herbivore richness	0.03		Pseudo-F	2.36	0.011	*	
	Warming	0.06		Pseudo-F	2.02	0.034	*	
Generality	(Intercept)	0.0001	0.12	t	0.001	1		
	parasitoid composition (1st PCA axis)	0.06	0.02	t	2.56	0.02	*	0.23
Vulnerability	(Intercept)	0..001	0.06	t	0.001	1		
	herbivore composition (1st PCA axis)	-0.05	0.01	t	-5.08	<0.0001	***	
	parasitoid composition (1st PCA axis)	-0.04	0.01	t	-3.26	0.005	**	0.58
Connectance	(Intercept)	0.09	0.03		2.88	0.011	*	
	herbivore richness	-0.01	0.004	t	-2.83	0.012	*	
	herbivore abundance	0.001	0.001	t	2.56	0.021	*	
	parasitoid richness	-0.01	0.003	t	-3.97	0.001	**	0.48
Resilience	(Intercept)	-1.41	0.19	t	-7.63	<0.0001	***	
	Connectance	-8.51	1.47	t	-5.80	<0.0001	***	
	Generality	0.41	0.08	t	4.83	0.0002	***	
	Vulnerability	0.49	0.11	t	4.51	0.0004	***	
	parasitoid composition (1^{st} PCA axis)	-0.04	0.007	t	-5.10	0.0001	***	0.72

4.5 Discussion

We investigated the direct and indirect effects of temperature and nitrogen deposition on the structure and resilience of a grassland host-parasitoid food web, and found that changes in food-web structure were largely mediated by indirect (bottom-up) effects on communties at each trophic level. In particular, both drivers increased plant resource availability and quality, which drove increased herbivore and parasitoid abundances. Changes in the abundance of both herbivores and natural enemies were also associated with higher species richness under the treatments, resulting in substantial shifts in herbivore and parasitoid composition. The changes at the herbivore level had important bottom-up effects on the natural enemies. In particular, parasitoid abundance and richness strongly depended on the abundance and richness of herbivores, but did not show any significant direct response to the drivers. Ultimately, changes in composition at both herbivore and parasitoid trophic levels altered the structure of the food web.

Changes in richness promoted a strong decrease in web complexity (connectance), whilst food-web vulnerability responded to changes in composition (i.e. in the relative abundance of species). Importantly, the structure of the food web responded to bottom-up effects, but the only direct effect of the drivers was a slight increase in generality at higher temperatures. This more generalist feeding by parasitoids suggests that parasitoids were able to find more suitable hosts from their potential range, possibly due to reduced thermal constraints on foraging ability (Sutterlin and vanLenteren 1997). An alternative interpretation could be that the parasitoid community was shifting to include more generalist species, but the independence of generality from richness and species composition suggests that this interpretation is unlikely.

Stability responded strongly to differences in web complexity and generality, and in accordance with classic theory (May 1973), network stability was negatively correlated with connectance. Higher connectance promotes the persistence and resilience of mutualistic networks, but destabilizes trophic networks (Thebault and Fontaine 2010). However, connectance is known to decrease rapidly with richness in trophic webs (Thebault and Fontaine 2010). With our data, we found strong evidence

for a negative effect of connectance on resilience, but we did not find support for a negative relationship between web size and connectance. Species-rich webs showed similar connectance to smaller webs, which implies a shift from fewer, stronger interactions to more, weaker links (resulting in an increase in generality). Thus, changes to the distribution and strength of interactions, suggested as a stabilizing force in food-web dynamics (McCann et al. 1998), counterbalanced the effect of increasing richness on connectance, and finally resulted in similar stability of the food web between the different treatments.

Whilst metrics of complexity such as connectance have been widely used to infer stability of static (equilibrium) webs, evidence from dynamic simulations suggests that other metrics such as compartmentalization may be more important in predicting the persistence of a network in the long term (Thebault and Fontaine 2010, Stouffer and Bascompte 2011). Therefore, applying dynamic models to our empirical data could increase our understanding of whether food-web attributes other than measures of complexity play an important role in the persistence of interactions and the species involved.

Parasitoids did not appear to directly benefit from either of the treatments, and this stark contrast to the herbivore's response likely played a major role in the changes to the trophic balance (Chapter 3). We found that parasitism rates of individual interactions (interaction strength) decreased under both temperature and nitrogen treatments. As we hypothesized, temporal asynchrony between the herbivore and parasitoid involved in any particular interaction had a negative effect on its strength. However, our measure of asynchrony (both the plot-averaged measure and the measure of asynchrony of individual interactions) did not respond to the treatments, which was surprising for temperature in particular. Although herbivore phenology was altered by temperature and nitrogen, this was not sufficient to decouple their temporal synchrony with their parasitoids (Klapwijk et al. 2010), as has been predicted for pollination networks (Memmott et al. 2007). Therefore, a direct effect of temperature on species phenology was not a likely mechanism for the reduction in interaction strength under all treatment combinations. However, we cannot exclude that phenological asynchrony had some influence in our study, as the temporal scale at which we measured peak abundances (every month) may be

insufficient to capture asynchrony at smaller timeframes that could nevertheless play an important role. Furthermore, the pooled food-web data over time may conceal phenological effects that could be most relevant at the extremes of the growing season (Menzel and Fabian 1999, Wallisdevries and Van Swaay 2006). Interestingly, the effect of asynchrony on interaction strength was greatest in the cooler plots. This was likely because peaks of abundance of hosts are much narrower at colder temperatures (Chapter 2, Fig. 2.2), which increases the necessity of parasitoids to match their peak abundance with the short window of host availability. Conversely, in warmer sites, host abundance tends to remain high for several months, such that differences in the absolute peak of abundance between parasitoids and hosts may not be so critical

The decrease in interaction strength under elevated temperature and/or nitrogen led to shorter average food chains. Pimm (2002) (building on the seminal work of Lindeman (1942), proposed that food chains are limited by the amount of energy transferred from one trophic level to the next, such that chain length is limited by primary productivity and/or energy transfer efficiency. Congruent with this hypothesis, we found a positive bottom-up chain of effect; higher resource availability drove higher herbivore abundance, which promoted higher parasitoid abundance, and parasitoid abundance had a positive effect on mean chain length. However, a negative effect of herbivore abundance on mean chain length likely signified a limitation in energy transfer efficiency between herbivores and parasitoids, perhaps because parasitoid populations could not keep up with the higher population growth of their hosts under the simulated global changes. It has also been proposed that food webs with longer food-chains might be more susceptible to shortening by disturbance (Jenkins et al. 1992) and might re-assemble more slowly after disturbance than would food webs with shorter food chains (Pimm and Kitching 1987). However, this dynamical constraint hypothesis was based on spatially- and temporally- localized effects, and it has been argued that there is no strong theoretical or empirical evidence to directly support this idea (Post 2002). In our system, chain length was negatively (albeit sub-additively) affected by the drivers in the gradient experiment. This overall effect was explained by a stronger increase in herbivore abundance relative to parasitoid abundance, an effect that we found in both

experiments. However, this reduction in food chain length did not affect food-web stability.

Overall, our results were consistent between two experiments that strongly differed in design and spatial scale. Both experiments showed strong bottom-up effects of plants on herbivores; in the gradient experiment, nitrogen had a direct effect on herbivore abundance after controlling for plant measures, whilst plant measures absorbed the effect of nitrogen in the warming experiment. This discrepancy likely reflects that, in the gradient experiment, a more variable and complex plant community may have had effects on herbivores beyond resource availability. Such effects could include increased palatability of multiple food sources (Siemann 1998), or asymmetric effects of nitrogen on phenology of plant species (Wallisdevries and Van Swaay 2006). Bottom-up effects of herbivores strongly determined the response of parasitoids in both experiments. It must be noted, however, that in the artificial warming experiment we found a strong negative effect of temperature on parasitoid richness. Given the small spatial scale of the experiment, we cautiously infer that this difference in species richness was likely due to parasitoid behavior and choice rather than physiological effects of temperature.

Our results highlight the importance of species interactions in mediating effects of global environmental changes. We showed that the response of herbivores to temperature and nitrogen was a combination of plant-mediated and direct effects whilst, in contrast, parasitoids were largely affected by bottom-up changes in resources but did not respond to the drivers directly. Importantly, we also show that food-web structure changed in response to the altered trophic communities, shifting from fewer, strong interactions to more, weaker links, although its complexity and stability remained relatively unchanged. In conclusion, our approach allowed us to disentangle the influence of different pathways with contrasting effects, which would not have been detected by simply looking at overall network responses, but are likely to have implications on the long-term persistence of both the interactions and the species involved.

CHAPTER V

Warming and nitrogen affect size-structuring of a host-parasitoid food web

5.1 Abstract

Body size is a major factor constraining the trophic structure and functioning of ecological communities. Food webs are known to respond to changes in basal resource levels, and climate change can initiate compounding bottom-up effects on food-web structure through altered resource availability and quality. However, the effects of climate and co-occurring global changes, such as nitrogen deposition, on the density and size relationships between resources and consumers are unknown, particularly in host-parasitoid food webs, where size structuring is less apparent than other terrestrial and aquatic systems. We use a Bayesian modelling approach to explore the role of consumer and resource density and body size on host-parasitoid food webs assembled from a field experiment with factorial warming and nitrogen treatments. We show that these drivers increase resource (host) availability and quality (size), leading to measureable changes in parasitoid feeding behavior. Temperature and nitrogen had a negative effect on interaction evenness: parasitoids interacted less evenly within their host range and increasingly focused on abundant and high-quality (i.e., larger) hosts. Our results also suggest a less pronounced direct response of parasitoids to higher temperatures through increased thermal budgets. In summary, we present evidence that climate-mediated bottom-up effects can significantly alter food-web structure through both density- and trait- (e.g., body-size) mediated effects.

5.2 Introduction

Body size is a fundamental trait that characterizes species and individuals. Many other characteristics of species, such as growth rates, bioenergetic needs, dispersal, longevity, and population densities, are strongly related to body size (Weitz and Levin 2006). There is growing recognition that body size can constrain who will interact with (i.e., eat) whom and, consequently, that allometric relationships play an important role in population dynamics and in determining food-web structure (Yodzis and Innes 1992, Cohen et al. 2003, Emmerson and Raffaelli 2004, Loeuille and Loreau 2005, Woodward et al. 2005, Weitz and Levin 2006). There is increasing awareness that trophic interactions occur between individual organisms of each species, rather than between species *per se* (Woodward and Warren 2007, Stouffer 2010). As a consequence, the conventional approach to documenting food webs, which has traditionally focused on taxonomic entities (species, etc.), may conceal much of this information on size structure at the individual level (Woodward et al. 2010).

Recent studies have suggested that general rules related to morphological, metabolic, or foraging constraints, many of which are closely correlated with body size, can capture the complexity of feeding interaction networks (Williams and Martinez 2000, Petchey et al. 2008). In particular, studies on stream macroinvertebrate food webs showed that community niche space may be collapsed into a single axis given by body size, in which case, characterizing the size distribution within a food web would capture much of the biologically-meaningful variation between species (Woodward et al. 2005).

Size structuring is usually strongest where organisms are gape-limited (Woodward and Warren 2007), whereas body size may not be such a critical determinant in systems where gape is not a factor, such as those in mutualistic plant-pollinator networks (Ings et al. 2009). Considering these two examples as the polar ends of size structuring of communities, terrestrial predator-prey interactions seem to cover a broad spectrum of size-structuring strength and magnitude. Animal consumers are often considerably larger than their prey (Cohen et al. 1993), whereas parasites and pathogens are usually smaller than their resources (Memmott et al. 2000). However, rules relating to size structuring may be broadly applicable to

predator–prey interactions in general (Brose et al. 2006), particularly if metabolic constraints rather than gape limitation drive these patterns.

Insect parasitoids complete their larval development feeding on one single host, and therefore represent a distinct feeding class. They are often similar in size to their insect hosts (Cohen et al. 2005), and thus lie between the extremes described above. Therefore, some general rules that apply to predator-prey dynamics (both aquatic and terrestrial) may not be best suited to describe body size relationships of host-parasitoid systems or to inform on the role of size-structure in their food webs. Understanding the factors governing host-parasitoid interactions is important both because of the ubiquity of these interactions in nature, and because of the widespread use of parasitoids in biological pest control (Godfray 1994).

Previous research has shown some size structuring of host-parasitoid interactions, with parasitoid individuals consistently scaling to the body lengths of their individual aphid hosts (Cohen et al. 2005). However, it has also been shown that bottom-up forces may play a role in mediating interactions involving plants, herbivores and their parasitoids (Harvey et al. 2003), wherein larger hosts can be preferred because they provide a better quality resource (Mackauer et al. 1996). Furthermore, recent studies showed that bottom-up changes to host and parasitoid body size contributed, alongside density-mediated effects, to overall changes in food-web structure (Bukovinszky et al. 2008, Laliberte and Tylianakis 2010). In addition to size-related host preferences, parasitoid body size may affect dispersal and search ability, whereas host body size can be inversely correlated with abundance (Woodward et al. 2005), and these two factors may affect encounter rates and food-web structure (Laliberte and Tylianakis 2010).

Climate warming is known to alter herbivore population growth (Bale et al. 2002) in addition to affecting individual body size (Awmack et al. 2004). Despite the important implications of these changes for interaction dynamics, empirical evidence on the effects of raising temperatures on host-parasitoid systems, and their size structuring, is currently lacking. Moreover, it is important to understand how co-occuring global change drivers (Didham et al. 2007, Tylianakis et al. 2008), such a nitrogen deposition (Vitousek et al. 1997a, Sala et al. 2000), may compound the effects of temperature to promote changes to basal resources that can further exacerbate or mitigate any effect on food-web structure.

In this study, we use an artificial field warming experiment with factorial temperature and nitrogen treatments to test effects on prey and consumer density and body size relationships using a grassland caterpillar-parasitoid system. We hypothesize that changes in density and body size of some hosts will augment community-wide differences in host quality, and induce parasitoid interactions to shift towards more profitable hosts (i.e., those that became more abundant and/or larger), thereby altering the structure and complexity of the food web.

5.3 Material and Methods

For this Chapter, I used data from the artificial warming experiment only. This is because the controlled settings and the scale of the experiment were best suited to detect behavioral responses of parasitoids (i.e selection of host based on traits such as body size) leading to changes in food-web structure, unrestricted by other ecological constraints that may be at play on a large-scale experiment in a semi- natural landscape.

5.3.1 Study site and experimental set up

Full details on the experimental set up are provided in Chapter 3, but summarized here. We set up an artificial warming experiment adjacent to the University of Canterbury field station at Cass in the Waimakariri River catchment, South Island of New Zealand. The experiment comprised a 2 x 2 factorial design, with warming and nitrogen as treatments with two levels each (control and elevated) and five true replicates per treatment combination, totaling 20 plots of 3.5 m length and width (12.25 m^2).

We generated the warming treatment by installing underground heating cables. We dug a 24 m by 19 m experimental area in October 2008, to a depth of 20 cm to establish the 20 plots, each separated by a 1m corridor. We then leveled the ground and installed custom-made electric heating cables (Argus Heating Ltd, Christchurch, New Zealand: coiled copper wire on fiberglass core and silicon coating) in half of the plots, and dummy cables in the remaining (unheated) plots. Heating power totaled 940 Watts per plot or a power density of 76W/m^2 (see Appendix 2.1 for details).

Similar power output has been recommended (Peterjohn et al. 1993) and successfully used in previous underground heating experiments (Melillo et al. 2002).

We paired each warming plot with a control plot to keep the warmed treatments at 3°C above ambient, logging the temperature of all thermocouples every minute using two Campbell CR1000 (Campbell Scientific, USA) data loggers. The average temperature of the thermocouples in the warming plots is used against the control plot to switch the power on and off as required. The warming treatment was first activated in April 2009.

We planted well-established individuals of four species of tussock grasses in a consistent composition and layout for each plot. This resulted in each plot being planted with 144 individual plants, amounting to 2880 tussocks in total.

We started the nitrogen treatment application shortly after planting (Jan 2009). We used nitrogen fertilizer in the form of Calcium Ammonium Nitrate granules (Ravensdown LTD, New Zealand). This form of fertilizer combines fast and slower release of biologically available nitrogen, and has been used previously to simulate atmospheric deposition (Clark and Tilman 2008). We added a total of 50 Kg ha^{-1} yr^{-1} using evenly-distributed applications during the rest of the year, with the exception of three winter months, for both 2009 and 2010.

We began sampling insects in January 2010, that is, a full year after plot establishment and planting. Sampling continued at monthly intervals until June 2010 (i.e. mid winter, when snow cover made sampling impractical), and resumed at monthly intervals from September to December 2010, totaling 11 sampling rounds. To minimize disturbance and depletion of caterpillars in the experimental area, we sampled half of each plot during each round, alternating between the two halves. This ensured a time window of at least 8 weeks before re-sampling of the same section. Sampling entailed visually searching for caterpillars on tussock plants, teasing apart the dense vegetation to find any hidden larvae. The standardized plant composition in each plot provided a standardized measure of insect abundance per unit area, unconfounded by differences in host plant availability. Although the scale of the experiment suggests caution in the interpretation of community-wide effects, we believe that it provides an ideal system to study the behavioral response of insects to changing environmental conditions.

5.3.2 Insect identification and body size measures

To allow collection of parasitoids, we individually reared all larvae to maturity (emergence of the adult moth or parasitoid) in a climate-controlled room, with a constant temperature of 16 degrees, relative humidity of 60% and a light cycle of 16L:8D. All parasitoids were identified to species level where possible, and to morphospecies for organisms lacking a recognized classification. We sought the expertise of two taxonomists to help with the identification: John S. Dugdale confirmed the lepidopteran ID, helped with developing a larval key and identified all the tachinid flies. Jo Berry validated hymenopteran morphospecies and formally identified all known species.

We excluded from analyses all caterpillars that died during rearing. Successful rearing allowed the identification of 983 herbivores (27 Lepidoptera species) and 333 interactions with 21 parasitoid species (10 Hymenoptera and 11 Diptera). We weighed the caterpillars (Mettler Toledo analytical balance accurate to 0.0001g) directly after collection for all samples. Unlike herbivore mass, parasitoid body mass can only be measured at emergence, and could therefore be strongly determined by the age at which the host larva was brought into the laboratory for rearing, and the laboratory food provided to the growing larva. Additionally, the host larval mass represents the mass of the individual engaging in the interaction. In contrast, the parasitoid mass represents the offspring of the individual engaging in the interaction, and although parasitoid offspring quality will in part reflect maternal quality, offspring mass could also be influenced by host mass (Cohen et al. 2005). Therefore, to avoid the possibility that these effects could generate spurious differences in parasitoid size across treatments, we calculated parasitoid body size as the average weight of that species. We obtained each species average by weighing 20 adult individuals of each species across all treatments, or all individuals for the rarer species (less than 20 individuals).

5.3.3 Data analysis

Community composition

As a first step to identify changes to community structure, we tested the effect of the drivers on herbivore and parasitoid community composition. A substantial shift in herbivore composition would influence the ability of parasitoids to interact with particular hosts, and could therefore affect the role of body size (e.g., if the herbivore community shows a remarkably different size distribution under different treatments) and host abundance. Conversely, shifts in parasitoid composition (e.g., if a subset of parasitoid species became dominant under the elevated treatments) could also generate changes in the architecture of interactions, and potentially override changes in host selection and size structuring within species.

We tested herbivore and parasitoid community composition using permutational distance multivariate ANOVA carried out with the PRIMER V6 software and the PERMANOVA package (Clarke and Gorley 2006, Anderson et al. 2008). We used two different dissimilarity measures, one accounting for species composition and abundance (Modified Gower base 10) and one focusing on species presence/absence (Jaccard dissimilarity). The Modified Gower distance measure considers an order-of-magnitude change in abundance (e.g., from 1 to 10) equal to a change in composition (i.e. from 0 to 1 species), and therefore accounts for the changes in the relative abundance of species in addition to changes in the community composition alone. This approach of using two dissimilarity measures allowed us to specify explicitly the relative importance given to changes in species relative abundance vs. changes in composition in the analysis (Anderson et al. 2006). In these analyses, we used herbivore or parasitoid composition as response variables, predicted by warming, nitrogen addition, and their interaction as fixed factors.

General linear models and generalized linear mixed models

We carried out univariate analyses using R version 2.12.0 (R Development Core Team; 2010). In addition to the composition tests, we also tested how the total and relative abundance of species changed under the drivers. We tested total abundance as total insect counts per plot, and this was predicted by warming and nitrogen in a generalized linear model (using the glm function in the base package in

R), with a Poisson error structure and log link function. To test changes in abundance within species, we used generalized linear mixed effects models (Bolker et al. 2009) in the lme4 package (Bates and Maechler 2010) in R. These models were the same as that for abundance above, but included species identity as a random effect to test for changes within each species. Together, these two tests allowed us to discern whether there was an overall difference in herbivore abundance and, if so, whether these differences were caused by a similar response by any given species, or if the total abundance was driven by a subset of species showing a particular strong increase.

To verify the overall size structuring in our system, we tested how parasitoid size responded to herbivore size in a linear mixed model, which included interaction, herbivore, and parasitoid identities as crossed random effects.

Body size and parasitism change metric

To test whether increases in body size of host larvae under the treatments led to higher attack rates (i.e. preferential choice by parasitoids), we examined the relationship between body size and any change in parasitism as follows: i) for each species, we calculated an average body size in the control (C) and under each treatment combination (T), and calculated a size change metric S=(T-C)/C; ii) for each species, we then obtained total parasitism rates for control plots (P) and each treatment (Q); iii) we used the same change metric in (i) to calculate a comparable change in parasitism rate for each host, R=(Q-P)/P; and iv) used a linear regression of R vs. S. If R increases with S, it would suggest that those hosts experiencing the largest increases in body size also attracted the greatest increase in numbers of parasitoids.

Model construction

Our Bayesian approach to modeling interaction counts uses models common to regression analysis, and for simplicity we assume that interaction counts are Poisson-distributed. As with most large food webs, the data display overdispersion with large numbers of zero values (missing interactions), and so a Zero-Inflated Poisson (ZIP) model is appropriate (Martin et al. 2005). In a ZIP model, two generalised linear models are used to explain the data: a logit part for the binary presence-absence of an interaction, and a Poisson part for its magnitude (i.e., frequency). For our study

system, the logit part can be understood as generating structural zeros: mainly, whether there is sufficient density of species for a given host-parasitoid encounter to take place. The Poisson part then gives an indication of the preference (or strength) for an interaction once the encounter has taken place. More formally, the number of interactions between host *i* and parasitoid *j* is given by a set of explanatory variables x*ij*:

$$Y_{ij}|\underline{x}_{ij} \sim \begin{cases} 0, & with\ \ probability\ \ 1-p_{ij} \\ Poisson(\lambda_{ij}), & with\ \ probability\ \ p_{ij} \end{cases}; \quad (1)$$

where λ_{ij} is the expected value of a Poisson distribution. The probability of an interaction being present is modeled using a logistic regression:

$$\text{logit}(p_{ij}) = \ln\left(\frac{p_{ij}}{1-p_{ij}}\right) = \alpha_0 + \alpha_1 x_{ij1} + ... + \alpha_k x_{ijk},, \quad (2)$$

with regression parameters α_k. The expected value for the Poisson distribution is given by

$$\ln(\lambda_{ij}) = \beta_0 + \beta_1 x_{ij1} + ... + \beta_k x_{ijk}, \quad (3)$$

with regression parameters β_k. Any combination of explanatory variables (ecological covariates) can be specified independently for the logit and Poisson parts. In this study, the logistic part always contained an intercept and parameter associated with host density; the Poisson part contained an intercept and one of eight combinations of parameters associated with six ecological covariates: host density (HD), parasitoid density (PD), host body size (HBS), parasitoid body size (PBS), nitrogen treatment (N), and site temperature (T). The eight combinations used in the Poisson part are listed below:

- HD PD
- HD PD N
- HD PD T
- HD PD N T
- HD PD HBS PBS
- HD PD HBS PBS N
- HD PD HBS PBS T
- HD PD HBS PBS N T

Three components are required to conduct a Bayesian analysis: i) data, ii) a model, and iii) prior distributions for parameters. In order to remain conservative in our analysis, we set uninformative priors for each of the regression parameters (α_k and β_k): specifically, normal distributions with extremely large variance (Hilborn and

Mangel 1997, Clark 2007). As is commonly done, Markov Chain Monte Carlo (MCMC) runs were used to sample from the full posterior distributions for all parameters (Robert and Casella 2004, Clark 2007). All simulations were run in R using the R2jags package (Su and Yajima 2012) that interfaces with JAGS 2.2.0 with the following settings: 50,000 iterations after a burn-in of 50,000 iterations, three chains, thinning = 20. Convergence was assessed using the Gelman-Rubin diagnostic function (Gelman and Rubin 1992) provided in JAGS 2.2.0.

We used the deviance information criterion (DIC) as measure of model fit, because it is easily calculated from the samples generated by an MCMC simulation (Spiegelhalter et al. 2002). DIC is a generalization of the Akaike Information Criterion (AIC) and the Bayesian Information Criterion (BIC) and, as with AIC and BIC, it penalises more complex models (typically those with large numbers of parameters) (Ellison 2004). Thus, models with lower DIC are preferred to models with larger DIC.

Assessed within a given model, each posterior distribution indicates the extent to which its associated covariate explains the presence of an interaction (logit part) or contributes to the magnitude of the interaction (Poisson part). If the credible interval of a posterior distribution contains zero, then its associated covariate explains little of the empirical data. The width of the distribution denotes the confidence one can have in a parameter estimate (mean, median or mode) given the empirical data: a wide distribution indicates greater uncertainty in its value. Posterior distributions are obtained automatically as part of the Bayesian analysis, which contrasts with frequentist methods that usually only provide point estimates for model parameters. Very often, if the same analysis is run using a Frequentist approach and a Bayesian approach, the mean (or median, or mode depending on the prior used) of the resulting posterior distribution is very close to the single frequentist estimator (Hilborn and Mangel 1997, Clark 2007).

ZIP model posterior distributions were used to measure the influence of the various ecological covariates on pairwise interaction count under different experimental treatments involving temperature and nitrogen content. The twenty food-web replicates were evenly divided into four treatment classifications: control nitrogen and control temperature; control nitrogen and elevated temperature; elevated nitrogen and control temperature; and elevated nitrogen and temperature. The food-

web data were studied at three resolutions: coarse, medium and fine. At the coarse resolution, all replicate webs were grouped together; at the medium resolution, replicate webs were grouped by nitrogen treatment and grouped by temperature treatment; at the fine resolution, replicate webs were grouped by nitrogen and temperature distinguishing the four treatment combinations. No single resolution is inherently better or more informative than another: the resolutions account for the trade off between more data but less environmental-discriminatory power. At the coarse resolution, we obtained well-resolved behaviour for each ecological covariate over all the data. Yet as a consequence, we cannot comment on how the influence of host or parasitoid density might change under the treatments. To do so, one must partition the data, as we have done for the fine resolution, but this comes at the expense of incorporating fewer data into the analytical models. The basic effect of an ecological covariate on interaction count can be determined from the sign and magnitude of its associated parameter estimate (obtained from the posterior distribution) within a grouping. The effect of a treatment on interaction count can be determined by comparing parameter estimates between groupings at the same resolution. We incorporated host body size (HBS) into the model in two ways: i) for each species, the average HBS across all individuals in all treatments was used; and ii) for each species, the average HBS across all individuals within each treatment combination (Control, N, warming, N + warming) was used. The use of these two approaches allowed us to compare the effect of driver-induced changes in host body size brought about by the altered relative abundance of smaller and larger species (i) with the effect of changes in body size of individuals within species (ii).

5.4 Results

Herbivore and parasitoid species composition did not differ significantly under the global change treatments. Specifically, we found no treatment effect on composition, either including the relative abundance of species ($F_{1,19} < 1.60$, $P > 0.1$ for both herbivores and parasitoids) or simply using the presence/absence (composition) of species ($F_{1,19} < 1.24$, $P > 0.1$ in all cases). In contrast, we found that total herbivore abundance increased strongly under warming ($Z = 4.56$, $P < 0.0001$) and nitrogen treatments ($Z = 3.28$, $P = 0.001$), with a sub-additive effect of the drivers (warming x nitrogen interaction: $Z = -2.86$, $P = 0.004$). We found support for

this result also when using abundance data per species, rather than total abundance; temperature and nitrogen had a strong positive effect ($Z = 4.33$, $P < 0.0001$ and $Z = 2.74$, $P = 0.006$, respectively), and their combination was sub-additive (interaction: $Z = -2.78$, $P = 0.005$). Overall, we found that host body mass and parasitoid mass were strongly correlated, ($t = 2.82$, $P = 0.005$), which suggests that some general size structuring is present in the relationship between host and parasitoid species within the community. We explored this further using the Bayesian analysis.

In our Bayesian model, at the coarse resolution with all data grouped together, the data were best explained by the model that included all covariates (Table 5.1). Pairwise interaction count depended on both host and parasitoid density and body size. In particular, we found that elevated temperature or nitrogen had a negative effect on host-parasitoid interaction counts—this is signified by the negative sign of values in the posterior distributions of the parameters associated with temperature and nitrogen (Figure 5.1).

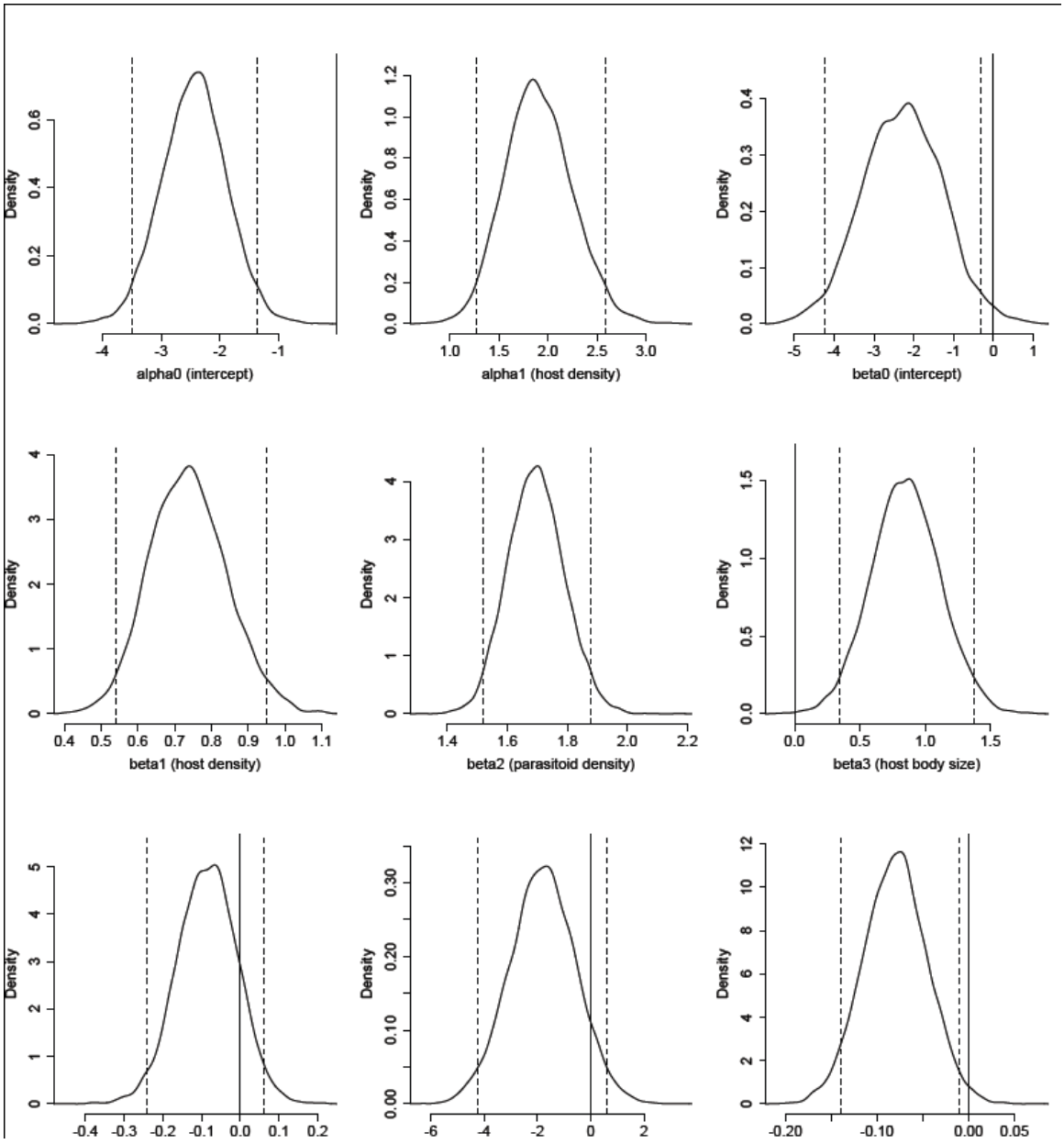

Figure 5.1: Posterior distributions for the most complex ZIP model at the coarse resolution. alpha0 and beta0 are intercepts in the logistic and Poisson parts, respectively. The other parameters are associated with ecological covariates and follow the nomenclature given in Equations 1 through 3, main text. Positive parameter values indicate a positive contribution to interaction count. Vertical dashed lines indicate Bayesian credible intervals (95%-level), and distributions including zero (vertical solid line) are considered less significant.

At the medium resolution, with no added nitrogen, elevated temperature had a negative effect on pairwise interaction count; however, at higher nitrogen, elevated temperature did not have any further effect. Similarly, the nitrogen treatment had a negative effect on interaction counts at control temperature, but not at elevated

temperature (Figure 5.2). Additionally, we found that the influence of both herbivore and parasitoid density changed under the treatments. An increase in temperature or nitrogen caused the influence of both host density and parasitoid density to become more positive. This suggests that interactions by parasitoid species were becoming less even, and they were typically with more abundant host species (Figure 5.2).

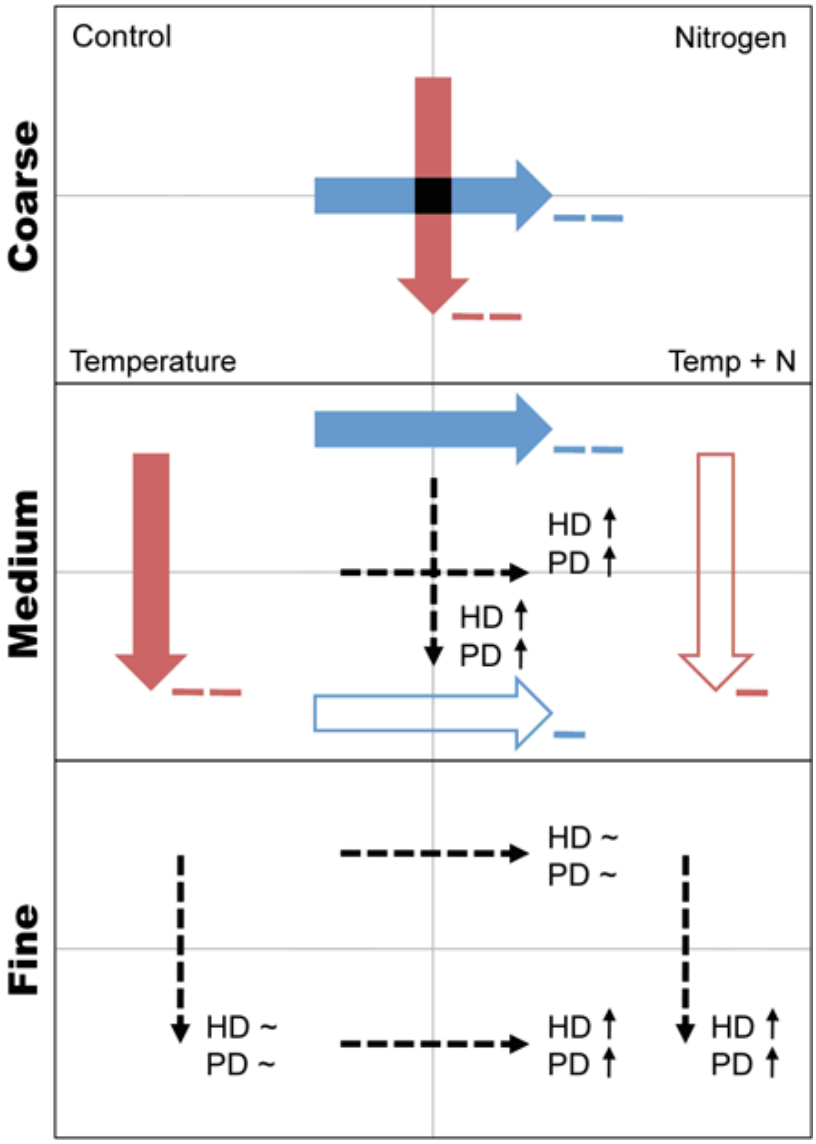

Figure 2: Effect of temperature (red arrows) and nitrogen (blue arrows) treatments on interaction count at coarse, medium and fine resolutions. Sub panels represent treatment combinations (control, top left; nitrogen, top right; temperature, bottom left; and temperature and nitrogen, bottom right). Thick arrows reference *within*-grouping behavior of posterior distribution sign in response to nitrogen and temperature; for example, at medium resolution the effect of the nitrogen treatment is more strongly negative (--) on interaction count when temperatures are low compared to when temperatures are high (-). Dashed arrows reference *between*-grouping changes in host density (HD) and parasitoid density (PD) posterior distribution magnitude (up-arrows indicate increasing influence, tilde indicate non-significant influence); for example, at fine resolution when temperatures are low, the influence on interaction count attributable to host and parasitoid density does not change as nitrogen levels are increased, however, when temperatures are higher, the influence of host and parasitoid density are are higher, the influence of host and parasitoid density are increasingly positive as nitrogen levels rise.

We also found that increasing temperature or nitrogen led to larger host species being favored, i.e., being attacked more often. Additionally, we found a positive relationship ($t = 2.20$, $P = 0.047$) between the increase in body size and the increase in parasitism rates for each species between control and treatments, supporting the results from the Bayesian analysis.

At the fine resolution, within treatment combinations, differences in nitrogen or temperature had no detectable effect on interaction count. We also found that, under either elevated temperature or elevated nitrogen, there was no significant change in the effect of host or parasitoid density on interaction count. However, at simultaneously elevated levels of both treatments, the influence of both host density and parasitoid density became more positive. The latter result suggests that, under the combined effect of temperature and nitrogen, interactions by parasitoid species were becoming less even, and they were typically with more abundant host species (Table 5.2, Figure 5.2). We found that larger host species were preferred by parasitoids when each treatment was applied individually, although the results were not statistically significant. However, we found a significant preference for larger body size when both treatments were applied together (Table 5.2, Figure 5.3). Results were qualitatively similar whether we used host body size averages calculated across all treatments (the entire data set), or averaged separately within each treatment combination (Fig. 5.3 A and B).

To further investigate the role of host body size, we looked at patterns at the species level. In the control treatment, we found that the two most abundant host species, *Persectania aversa* and *Tmetolophota unica*, attracted the most parasitism events (they comprised 76% of the available host abundance and together yielded 86% of parasitoid interactions)—a pattern that primarily reflects density effects. Under the elevated global change treatments, these two species increased in abundance, although to a comparatively lesser extent than other host species. However, their share of parasitism became disproportionately high: under simultaneously elevated temperature and nitrogen, these two species combined attracted almost the same fraction of interactions despite making up 13 percentage points less of the available resources (63% of the available host abundance and together garnering 84% of parasitoid interactions). Interestingly, this increase in proportional parasitism was accompanied by a 52% (*P. aversa*) and 41% (*T. unica*) increase in average size.

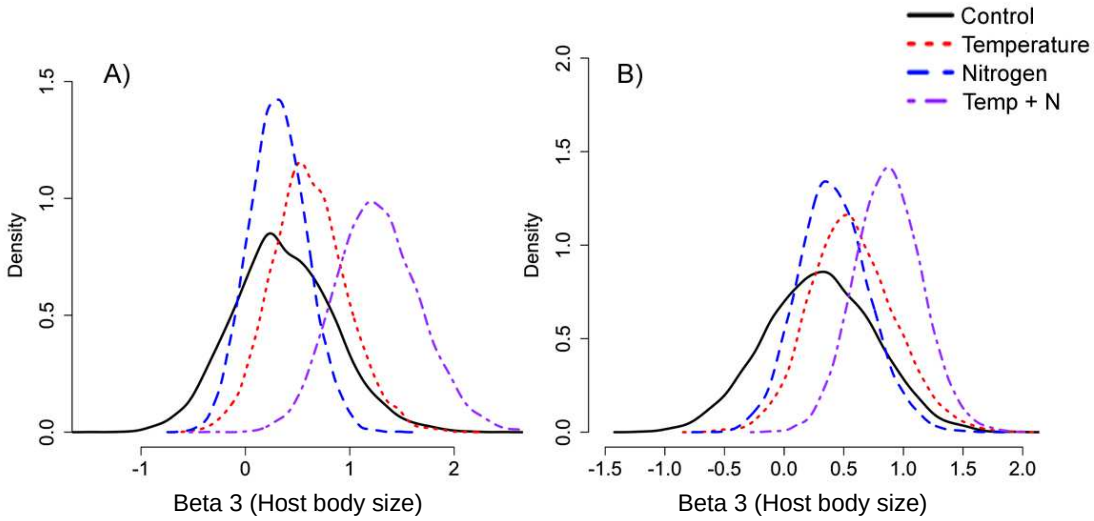

Figure 5.3: Influence of host body size on interaction count at fine resolution. A: host body size uses average values across all treatments, and B: host body size uses four treatment-specific sets of point estimates. Both figures show posterior distributions of the parameter associated with host body size under four treatments: control (solid line, black); control nitrogen and elevated temperature (dotted line, red); elevated nitrogen and control temperature (dashed line, blue); and elevated nitrogen and elevated temperature (dot-dashed line, purple). Host body size positively influences interaction count most strongly when nitrogen and temperature treatments are applied together. Note that the x-axis scale is parameter value, and does not represent a biological measurement.

Table 5.1: DIC values for eight ZIP models across three data-grouping resolutions. Models with lower DIC are preferred to models with higher DIC, and numerical comparisons should only be made within columns (best-fit model in bold). Twenty food-web replicates were evenly divided into four treatment classifications: control nitrogen and control temperature; control nitrogen and elevated temperature; elevated nitrogen and control temperature; and elevated nitrogen and elevated temperature. The food-web data were studied at three resolutions—coarse, medium and fine—according to how the food-web replicates were grouped (see main text). Some models were not applicable to certain groupings (denoted by X). Simulations were run in R using the R2jags package that interfaces with JAGS 2.2.0 with 50,000 iterations after a burn-in of 50,000 iterations, three chains, thinning = 20. Convergence was assessed using the Gelman-Rubin diagnostic function (40) provided in JAGS 2.2.0.

Model	Coarse	Medium				Fine			
	All	Control N	N	Control T	T	Control	Temperature	Nitrogen	Temp-N
HD PD	944.8	434.1	526.1	498.9	466.5	**201.7**	244.6	**298.1**	231.5
HD PD N	943.3	X	X	498.9	469.1	203.6	246.0	299.5	233.0
HD PD T	940.8	**428.1**	526.5	X	X	205.0	246.1	299.6	228.0
HD PD N T	938.8	X	X	X	X	205.2	247.3	299.2	230.7
HD PD HBS PBS	935.7	432.0	**519.6**	498.5	**464.7**	204.5	**243.8**	300.8	223.6
HD PD HBS PBS N	935.1	X	X	**496.5**	472.6	207.1	244.7	303.0	224.5
HD PD HBS PBS T	932.2	443.6	521.5	X	X	206.9	246.3	302.2	**218.5**
HD PD HBS PBS N T	**932.0**	X	X	X	X	207.8	248.0	303.7	219.6

Table 5.2: Parameter estimates (mean value of posterior distributions) for Zero-Inflated Poisson model explaining interaction counts at fine resolution. Parameter estimates in bold are statistically significant. Values in parentheses are standard deviations of the parameter estimate; values in brackets are 95% credible intervals. Bayesian posteriors were calculated in R using the R2jags package that interfaces with JAGS 2.2.0 with 50,000 iterations after a burn-in of 50,000 iterations, three chains, thinning = 20. The model includes seven parameters (alpha0 and alpha1 are omitted for clarity) and is run separately for each combination of treatments. Convergence was assessed using the Gelman-Rubin diagnostic function (Gelman and Rubin 1992) provided in JAGS 2.2.0.

Parameter	Control	Temp	Nitrogen	Temp-N
Intercept: β_0	**-6.81 (1.51) [-9.99, -4.01]**	**-6.74 (1.40) [-9.56, -4.05]**	**-6.11 (1.09) [-8.31, -4.07]**	**-4.77 (1.32) [-7.48, -2.28]**
Host density: β_1	**1.21 (0.15) [0.91, 1.53]**	**1.17 (0.14) [0.91, 1.46]**	**1.26 (0.17) [0.92, 1.61]**	**1.43 (0.14) [1.15, 1.72]**
Parasitoid density: β_2	**1.64 (0.22) [1.24, 2.09]**	**1.57 (0.17) [1.25, 1.94]**	**1.54 (0.16) [1.23, 1.88]**	**1.79 (0.18) [1.45, 2.17]**
Host body size: β_3	0.36 (0.48) [-0.53, 1.36]	0.61 (0.36) [-0.07, 1.36]	0.31 (0.27) [-0.21, 0.87]	**1.27 (0.40) [0.52, 2.11]**
Parasitoid body size: β_4	-0.21 (0.18) [-0.58, 0.14]	-0.21 (0.17) [-0.55, 0.10]	-0.06 (0.12) [-0.32, 0.18]	0.02 (0.16) [-0.30, 0.33]

5.5 Discussion

We examined the effect of climate and nitrogen deposition on a size structured host-parasitoid food web. The community composition of both herbivores and natural enemies (parasitoids) was not significantly affected by higher temperatures or nitrogen deposition. However, host total abundance was higher under all treatments relative to the control, and this effect was consistent across species. Thus, this system proved well suited for disentangling the effects of host body size and density on community structure, without the confounding issue of communities differing significantly in their species composition (and therefore pairwise interactions) or strongly shifting toward a subset of heavier (or lighter) species, which would render the role of body size more difficult to interpret.

The negative sign of the posteriors associated with the global change treatments at the coarse scale suggests that warmer temperatures and more nitrogen led parasitoids to feed more generally, i.e to attack more different host species. We found congruent results at the medium resolution; increasing temperature or nitrogen led to more general feeding by parasitoids, but increasing both drivers simultaneously did not yield any further increase in host-use generality beyond that observed for each driver in isolation. It is therefore likely that both nitrogen and temperature altered the availability or palatability of different hosts, making a wider range of host resources available to parasitoids. Thus, both treatments stimulated more generalist feeding by parasitoids; however, parasitoids could not keep up with the increasing host abundance under elevated N and temperature, so they became saturated and per capita attack rates on each host declined.

At medium resolution (grouping by nitrogen or temperature treatments), each driver moderated the influence of host and parasitoid density on interaction counts. In particular, an increase in temperature or nitrogen was associated with an increasing influence of both host and parasitoid density. These effects can be interpreted as interactions becoming less even, and shifting towards more abundant host species. Nitrogen and temperature are known to generally favor herbivore populations, as well as individual, growth (Bale et al. 2002, Throop and Lerdau 2004), and an increasing influence of host density supports the hypothesis that resource abundance has a strong effect on interaction frequencies. These results are

consistent with the increased unevenness of host-parasitoid interactions observed when certain hosts become more abundant (and disproportionately attacked) following land-use intensification (Tylianakis et al. 2007).

Parasitoids favored larger hosts under high temperature or nitrogen treatments. It is conceivable that parasitoids, given the increase in resource availability and quality, had the opportunity to be more specific (through improved searching efficiency) in their host selection, favoring hosts that grew larger and were more abundant (Stiling 1987, Walde and Murdoch 1988). In addition to the parameter estimate for the host-body-size effect becoming larger under the effect of the drivers, the posterior distributions also narrowed relative to the control (Fig. 3), indicating that parasitoids were less variable in their use of larger hosts. At higher temperature, this behavior could also be explained by an increased thermal budget for searching suitable hosts (Sutterlin and vanLenteren 1997, Zilahi-Balogh et al. 2009), a view that is supported by the higher mean of the posterior distribution for the temperature treatment relative to control and nitrogen. The low average annual temperatures that characterize our system also make it possible that parasitoids could respond positively to an increased thermal budget. This host-selective behavior could bear important implications for the future of host-parasitoid interactions: if parasitoids become consistently able to choose “ideal” hosts in a warmer world, it is likely that this would lead to increased parasitoid fitness in the generations to come, which could counteract the negative effect of the drivers on parasitism rates, and potentially generate strong selection pressures against preferred host species.

We found that herbivore abundance reacted strongly to the treatments, and previous work in the same system has shown that changes to plants can play a primary role in mediating herbivore responses to global environmental changes (Chapter 2). In fact, grasslands are known to respond rapidly and strongly to changes in abiotic conditions (Bloor et al. 2010), and therefore provide a strong change in basal resources than can be utilized by herbivores. In contrast, we found that, overall, parasitoids did not respond as strongly as herbivores to any of the treatments.

Host body size had a significant influence on interaction counts only under elevated temperature and nitrogen. A deeper examination of parasitism of the two most common species revealed that, despite the increase in average body size being similar under all treatments, they were attacked disproportionately more under both

treatments in combination. Therefore, results at the finer resolution also supported the view that temperature and nitrogen increased the importance of host body size by increasing overall availability (choice pool) and individual body size. Most importantly, higher temperatures allowed parasitoids to exploit more efficiently these changes in the host resource.

Consistent with our hypothesis, the drivers impacted host-parasitoid food-web structure by altering the response of parasitoid species to host density and size structuring. In particular, we observed a shift of interactions toward abundant and heavier host species. Host body size was coupled with changes in species abundance under all treatments but, interestingly, its effect on interaction structure emerged clearly only under the combined effect of temperature and nitrogen. These results carry important implications for the evolution of host-parasitoid communities under climate change. Optimal foraging theory suggests that food-web interactions depend on the body sizes of predators and prey (Beckerman et al. 2006), and we found that this size-dependence (and the likely foraging benefit of specializing on certain hosts) is altered by global environmental changes. Thus, our observations suggest that food webs will increasingly become characterized by fewer, stronger links between relatively abundant species, likely resulting in a decrease in web complexity, with unclear consequences for stability (Thebault and Fontaine 2010). In the face of global change, bottom-up effects on resource quality and body-size preferences are likely to have strong impacts on ectotherm community structure and the arrangement of interactions within food webs.

CHAPTER VI

Discussion

Human activities since industrialization have deeply transformed ecosystems more rapidly and extensively than during any comparable period in human history (Tilman et al. 1994, Pimm et al. 1995, Vitousek et al. 1997b, Chapin et al. 2000, Pimm and Raven 2000, Walther et al. 2002). These changes to ecosystems have contributed to substantial net gains in short-term human well-being and economic development (Raudsepp-Hearne et al. 2010). However, such gains have been achieved at growing costs in the form of degradation of many ecosystems, reduction in ecosystem services (Foley et al. 2005, M.E.A 2005) and diversity loss (Pimm and Raven 2000, Sala et al. 2000). The degradation of ecosystems is likely to worsen significantly during the first half of this century (Thuiller et al. 2004, IPCC 2007). Thus, ecologists are presented with the urgent and complex challenge of understanding the mechanisms through which global environmental change (GEC) influences species, communities, ecosystem functioning, and consequently, the delivery of services on which we all depend (Chapin et al. 2000).

The decline in biodiversity over the recent decades has motivated researchers to investigate the relationship between species richness (biodiversity) and ecosystem function. This resulted in one of the largest and most heated debates in ecological research, commonly referred as the "Biodiversity and Ecosystem Function Debate" (Schulze and Mooney 1993, Naeem et al. 1994, Naeem 2000, Wardle et al. 2000, Loreau et al. 2001, Hooper et al. 2005). Although the debate continues (Thompson and Starzomski 2007), there is a general consensus that ecosystem properties depend greatly on biodiversity and the functional characteristics of organisms present in the ecosystem (Hooper et al. 2005). Species loss does affect the functioning of a wide variety of organisms and ecosystems, but the magnitude of these effects is partly determined by the identity of species that are going extinct (Cardinale et al. 2006),

and the environmental context under which species partition their resources (Tylianakis 2008b, Griffin et al. 2009, Hiddink et al. 2009).

However, one of the most relevant and acknowledged limitations in this wide and productive field of research is that multiple trophic levels have been understudied in biodiversity/ecosystem functioning research (Duffy 2002, Ives et al. 2005, Cardinale et al. 2006). All organisms are embedded in a web of interactions with other organisms, and these mutualistic, competitive, predation, or parasitic relationships between individuals of species and species at different trophic levels are not only crucial components of ecosystems, but they comprise many of the ecosystem functions/services (e.g., pollination, biological pest control) on which humans depend. The response of ecosystem properties to varying composition and diversity of consumers is much more complex than responses seen in experiments that vary only the diversity of one trophic level (usually primary producers).

The importance of ecological interactions as a key component of ecosystems has been suggested for decades (Janzen 1970, Janzen et al. 1976, Harrington et al. 1999), but their role has received disproportionately little attention, and much of it only in recent years (McCann 2007). However, new understanding points at interactions as a driving force of biodiversity (Bascompte and Jordano 2007, Encinas-Viso et al. 2012), ecosystem responses to GEC (van der Putten et al. 2004, Suttle et al. 2007, Tylianakis et al. 2008) and stability of ecosystem services (Dobson et al. 2006).

Thus, there is a need in ecological research to improve our understanding of the response of biotic interactions to global environmental changes, and their role in mediating the ecosystem response to change drivers. This was the underlying focus of my thesis. In particular, I studied the effects of global warming and nitrogen deposition on a sub-alpine grassland system comprising plants, herbivores and their natural enemies. I aimed to integrate increasing biotic complexity throughout the different chapters, in a bid to highlight the importance of studying communities rather than species, multiple trophic levels rather than resources or consumers, and finally to specifically consider the complex networks of interactions between organisms at the different levels, and how changes at each level may impact the network as a whole. This thesis was divided into four main chapters. In this

concluding chapter, I summarize the main findings for each of them, and highlight how they improve our current understanding. I then highlight possible avenues for future research, and finally present a general conclusion from the thesis.

6.1 Summary

6.1.1 Plant-mediated and non-additive effects of two global change drivers on an herbivore community

In Chapter 2, I explored the effects of temperature and nitrogen on the composition and phenology of a herbivore community. The key findings of this chapter were:

- phenology: herbivore abundance and development through time were strongly influenced by the drivers, which promoted higher abundance and earlier seasonality at the community level. Contrasting responses of species likely contributed to:
- changes in composition: in response to the drivers, the herbivore community changed drastically in its overall composition and its turnover through time.
- Interactions between global change drivers: the drivers showed frequent non-additive effects on phenology, total and relative abundance. However, the effect of the drivers on herbivores was largely explained by:
- plant-mediated effects: Changes in herbivore composition and abundance were mediated by changes in the plant community, with increased non-native grass cover under high treatment levels being the strongest determinant of herbivore abundance. Nevertheless, temperature was directly associated with
- biotic homogenization: herbivore communities in warmer conditions were, on average, more similar to each other than at colder temperatures.

It is generally recognized that herbivore populations can increase at higher temperatures and nitrogen availability (Bale et al. 2002, Throop and Lerdau 2004).

However, I am not aware of any studies that previously showed the response of entire herbivore assemblages to these drivers, including important phenological effects (van Asch and Visser 2007). This study also highlights the importance of plant-mediated effects on herbivores; had the plant community responded differently (e.g., if no exotic grasses were present in the study area), the results would have potentially been very different. In the general context of my thesis, this result provides the first contribution to highlight the importance of trophic (plant-herbivore) interactions in mediating system-wide response to GEC. Finally, I am not aware of any previous studies reporting temperature-driven biotic homogenization at the consumer level. This result therefore adds a potentially important mechanism to the known effects of climate change on ecosystems.

6.1.2 Climate change disproportionately increases herbivore over plant or parasitoid biomass

In Chapter 3, I examined how biomass allocation at three trophic levels responded to the effects of temperature and nitrogen, thereby expanding on the previous chapter by including natural enemies in addition to plants and herbivores. With this study, I found that:

- at high temperature, herbivore biomass increased dramatically more than plant or parasitoid biomass. However:
- the positive response of plants to nitrogen implied that, under co-occurring drivers, the response of plants and herbivores did not differ significantly but, in contrast:
- natural enemies (parasitoids) did not show any significant response to the treatments, and therefore showed a weaker response than herbivores under all treatment combinations.

It has previously been suggested that different trophic levels may be differentially sensitive to climate (Voigt et al. 2003); however, very few studies have specifically tested this hypothesis and, to my knowledge, none have empirically tested the response of a plant-host-parasitoid system in this sense. I showed that higher temperatures and elevated nitrogen generated a multitrophic community that

was increasingly dominated by herbivores. These results contribute to the concern that parasitoids are likely to be inefficient in exerting top-down control of herbivores under a scenario of climate change. This concern was first raised by Hoover and Newman (2004) by means of a mechanistic mathematical model, but my study provides much-needed empirical support.

6.1.3 Bottom-up effects mediate the response of food-web structure and resilience to climate change and nitrogen deposition

In Chapter 4, I examined in more detail the general findings of the previous chapter. In particular, I scale up the complexity of the study system by testing the effects of the GEC drivers on the structure of host-parasitoid food webs and, using path analyses, identified the main effect pathways. The key findings of this chapter were:

- Food-web structure responded to profound changes in resource availability, herbivore and parasitoid composition, whilst the direct effects of the drivers were negligible.
- The drivers did not alter food-web stability, as the opposing direction of different effect pathways buffered against large changes in food-web structure and resilience.
- Temperature and nitrogen sub-additively caused a decrease in mean food chain length, indicating a proportionate reduction energy transfer to higher trophic levels.
- Interaction strength increased with temporal synchrony of the host and parasitoid, though synchrony was not significantly affected by the drivers.

The response of food webs to global warming has been as argued to be an urgently needed area of research (Petchey et al. 2010, Woodward et al. 2010). Petchey et al. (2010) predicted potentially large effects of temperature on connectance (often used as a measure of complexity); however, empirical evidence was lacking. I showed that bottom-up resource-driven changes played a primary role in mediating the response of food webs to temperature and nitrogen. Thus, this chapter further builds on the findings of the previous chapters, specifically

highlighting that interactions between species play a crucial role in the ecosystem response to global environmental changes, and the pathways through which this occurs. Furthermore, the loss of energy transfer to higher trophic levels supports general concerns about increases in herbivory under climate change.

6.1.4 Climate and nitrogen affect size-structuring of a host-parasitoid food web

In Chapter 5, I used body size as a trait that plays an important role in structuring pairwise interactions, and investigated the influence of temperature and nitrogen on the host-parasitoid density and size relationships. Through this study, I showed that:

- increased resource (host) availability and quality (size) led to measureable changes in parasitoid feeding behavior; in particular,
- the drivers altered the relationship between host and parasitoid density and
- Parasitoids increasingly focused on abundant and larger hosts.

Body size is correlated with a suite of species traits that can affect the structure and dynamics of food webs and other ecological networks, across multiple scales of organization (Woodward et al. 2005, Brose et al. 2006, Brose 2010). This chapter showed that parasitoids interacted less evenly within their host range and increasingly focused on abundant and high-quality (i.e., larger) hosts under the influence of the global change drivers. These results imply that global change-mediated bottom-up effects can significantly alter food-web structure through both density- and trait- (e.g., body size) mediated effects. With this study, I showed that traits of species, rather than species *per se*, can provide a useful tool to examine, and eventually predict, how ecological interactions are affected by GEC, and how they mediate effects of GEC on ecosystems.

6.2 Outlook

Global environmental changes present an enormous and time-pressured task to ecologists. The main difficulty in tackling this challenge stems from an historical weakness affecting ecological research, which is its limited track record as a predictive science. There are multiple reasons that contribute to this fact but, arguably, the primary reasons that impede ecology in the context of global environmental changes are, at a coarse resolution, quite simple. Conducting experiments at a global scale to predict nature's response is simply not feasible. Research at the broadest scale is, typically, descriptive of what *happened*, e.g., describing climate-driven range shifts, as did some of the seminal studies of global warming effects on biota (Parmesan 1996, Parmesan et al. 1999). On the other hand, experiments at the small scale that are suited to discovering underlying mechanism and responses to change usually have little power to generalize to different taxa, regions or ecosystems. Developing ways to integrate local with global scale patterns and mechanisms is therefore a necessary yet complex step to accomplish. As a step in that direction, solid advances have been made in predicting and modeling shifts in range and distribution of species (Guisan and Zimmermann 2000, Guisan and Thuiller 2005, Elith et al. 2006, Phillips et al. 2006).

Climate envelope models, combined with empirical evidence, make a compelling case for shifting species ranges, or the inability to do so, as crucial mechanisms through which climate change induces biodiversity loss (Walther et al. 2002, Wilson et al. 2005, Parmesan 2006). However, an obvious limitation of climate envelope models is that distributions of species also reflect the influence of interactions with other species, so predictions based on climate envelopes may be very misleading if the interactions between species are altered by climate change (Davis et al. 1998). Thus, to successfully understand and predict future species ranges, and the response of whole communities, biotic interactions need to be incorporated into future predictions. Network theory provides a conceptual framework to assess the consequences of perturbations at the community level (Bascompte 2009). However, the response of different network attributes to biotic and abiotic changes must be understood and generalized, before they can be successfully integrated into broad-scale projections through space and time (such as

climate envelope models). Steps in this direction are being taken, and there is a growing body of literature rapidly identifying fundamental building blocks of ecological networks that are essential to stability and biodiversity maintenance (Bascompte and Jordano 2007, Stouffer and Bascompte 2010, 2011). Recent theoretical studies suggest significant dynamic reasons underpinning empirically-observed food-web structure (Stouffer and Bascompte 2010, Thebault and Fontaine 2010), and these provide a framework and hypotheses that need to be tested on real ecosystems. Theoretical and empirical approaches therefore need to be co-evolving, and wherever possible integrated in a more fluid dialogue (e.g., using large empirical data sets for dynamic modeling). Furthermore, examining the mechanisms (e.g., body-size changes, altered community composition) through which global change affects interaction networks will provide greater ability to generalize than the simple description of observed network changes.

Expanding our understanding of interaction structure beyond individual species identities could present ecologists with a rewarding avenue towards generalization of key patterns in biotic interactions. Networks of interactions are composed of nodes and links between nodes. Much of the food-web research to date has considered species as unit of study, and therefore as the nodes comprising food webs. Some advances are already being made by using key traits of species that are thought to be important for the dynamics and persistence of food webs. Conceptually, this can be expanded to consider community-wide mean-trait values as nodes, allowing the horizontal generalization of food web analyses across widely different taxonomic groups and ecological systems. Developments in this direction are technically envisageable, and could help to transition our understanding of local mechanisms to global solutions.

6.3 Final conclusions

A very large body of literature addressing the effects of global environmental changes, warming in particular, has focused on individual species. The number of studies addressing the response of multiple species declines proportionally (or perhaps exponentially) with the complexity of the system studied. The aim of this thesis was to study the effects of co-occurring warming and nitrogen deposition on complex multitrophic communities. I used a grassland system comprising plant, caterpillar herbivores and parasitoids as natural enemies. I based my thesis on the combination of data from an altitudinal gradient experiment and an artificial warming experiment.

I found that, overall, results were very consistent between these two experiments, which differed considerably in their set up and spatial scale, and across the different response measures used. This consistency gives me a good degree of confidence that the caveats and limitations associated with both experiments (or for that matter, any ecological experiment) did not distort the biological relevance of the findings and the overall validity of my results.

Both global change drivers studied were responsible for strong bottom-up effects that percolated asymmetrically through the community. More specifically, the herbivore assemblage showed a strong phenological response to the drivers. However, the predominant increase in abundance and biomass was attributed to plant-mediated effects and, to a lesser extent, a direct effect of the drivers, temperature in particular. These findings were consistent when considering biomass as a response variable. Although plants showed some positive response to the drivers, herbivores showed a stronger response that, importantly, was not matched by parasitoids. These important differences in response to the drivers at the different trophic levels were ultimately the primary cause for shifts in the food-web structure. In fact, bottom-up effects altered the trophic balance and shifted interaction preference and choice by parasitoids. Changes in interaction strength and evenness may have consequences on the long-term persistence of the food web (Thebault and Fontaine 2010), although stability, as measured by our equilibrium model, was not affected.

The common themes underlying my data suggested that i) trophic interactions largely mediated the effect of nitrogen and, less intuitively, also the effects of temperature. ii) Herbivores responded most strongly to the drivers, by virtue of benefiting from plant-mediated effects as well as some direct effects. The parasitoid response, in contrast, was almost exclusively dependent on herbivores, though generally weaker. Therefore, in the tri-trophic system studied in this thesis, herbivores seemed most capable of thriving under the simulated global change scenario of higher temperatures and nitrogen availability. iii) In this system, temperature and nitrogen behaved predominantly sub-additively. The effect of nitrogen and temperature in isolation were often very similar, and their combination was less than the sum of their isolated effects.

By examining the effects of multiple drivers on communities at different trophic levels, the work presented in this thesis highlights the potential of species interactions, food webs in particular, for understanding and perhaps one day forecasting the likely impact of global environmental changes on complex communities and ecosystems.

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Appendix 1

Appendix 1.1: Supplementary field methods

Study site

The climate of the study region is cool and humid, with a mean annual rainfall of 1560 mm and a mean annual temperature of 9.1°C (Williams & Courtney 1995). With the exception of high alpine areas (elevation > 1,300 m, ≈ 15% of the landscape), the region comprised continuous southern beech (*Nothofagus* spp.) forest prior to human settlement (Ewers et al. 2007). Following land clearing by fire in the mid 1800s, it is now occupied by semi-natural tussock grassland, dominated by the tussock species *Poa cita*, *Festuca novae-zelandiae*, and *Rytidosperma setifolium*, which are typical of semi-arid to humid, montane and subalpine zones in New Zealand (Rose et al. 2004). The inter-tussock ground is generally dominated by stock-palatable Eurasian species (particularly *Agrostis capillaris, Anthoxanthum odoratum, Trifolium* spp.), which were over-sown after forest clearing. The mouse-ear hawkweed *Hieracium pilosella* was accidentally over-sown in contaminated seed and now has a variable, patchy distribution across New Zealand, including the study area. Native herb species such as *Leucopogon* spp., *Celmisia* spp., and *Acaena* spp. show a patchier distribution, and are generally less abundant in the study site. At present, the area is farmed at very low intensity, with a stock density of less than 1 sheep per hectare, and no nitrogen fertilizer is applied outside of our experimental plots.

Site locations and details

Transects were at least 600 m apart (twice the vertical length of each individual transect). All plots had a similar incline and vegetation type, and faced north or north-west. To maintain this similarity of characteristics, transects were not all positioned at exactly the same elevation, so plots ranged from 650 m at the lowest point to 1073 m a.s.l at the highest (423 m of total elevation span). Temperature was recorded in each plot from February to December 2009 using Hobo series ProV2 dataloggers, logging temperature at 1h intervals protected by a sun shield and placed

at ca. 80cm above ground to capture the near-ground air temperature that is likely to most affect both caterpillar and adult Lepidoptera.

Table S1: Sampling location details. Sampling was conducted along 5 transects, each with 3 different elevations (plots) and a nitrogen subplot at each elevation point. Subplots are not separated here, as values apply to the entire plot. Coordinates are based on a GPS New Zealand map with EGS84 reference grid.

Transect	Elevation	Altitude (m.a.s.l)	Coordinates		Mean
			North (N)	East (E)	Temperature (°C)
DE	Bottom	650	N 584 3746	E 246 5971	6.72
	Mid	790	N 584 3468	E 246 5740	5.61
	Top	940	N 584 3367	E 246 5739	5.00
KE	Bottom	732	N 583 9796	E 245 8308	5.31
	Mid	891	N 583 9287	E 245 8289	5.79
	Top	1031	N 583 9021	E 245 8168	5.03
DW	Bottom	724	N 584 3754	E 246 4325	6.55
	Mid	880	N 584 3364	E 246 4341	6.00
	Top	1009	N 584 3109	E 246 4357	4.49
NS	Bottom	743	N 584 0489	E 245 8540	5.64
	Mid	883	N 584 0796	E 245 8657	5.08
	Top	1050	N 584 1071	E 245 8817	4.03
LZ	Bottom	792	N 5840276	E2456733	5.29
	Mid	937	N 5840655	E 2456562	4.79
	Top	1073	N 5840791	E 2456751	3.89

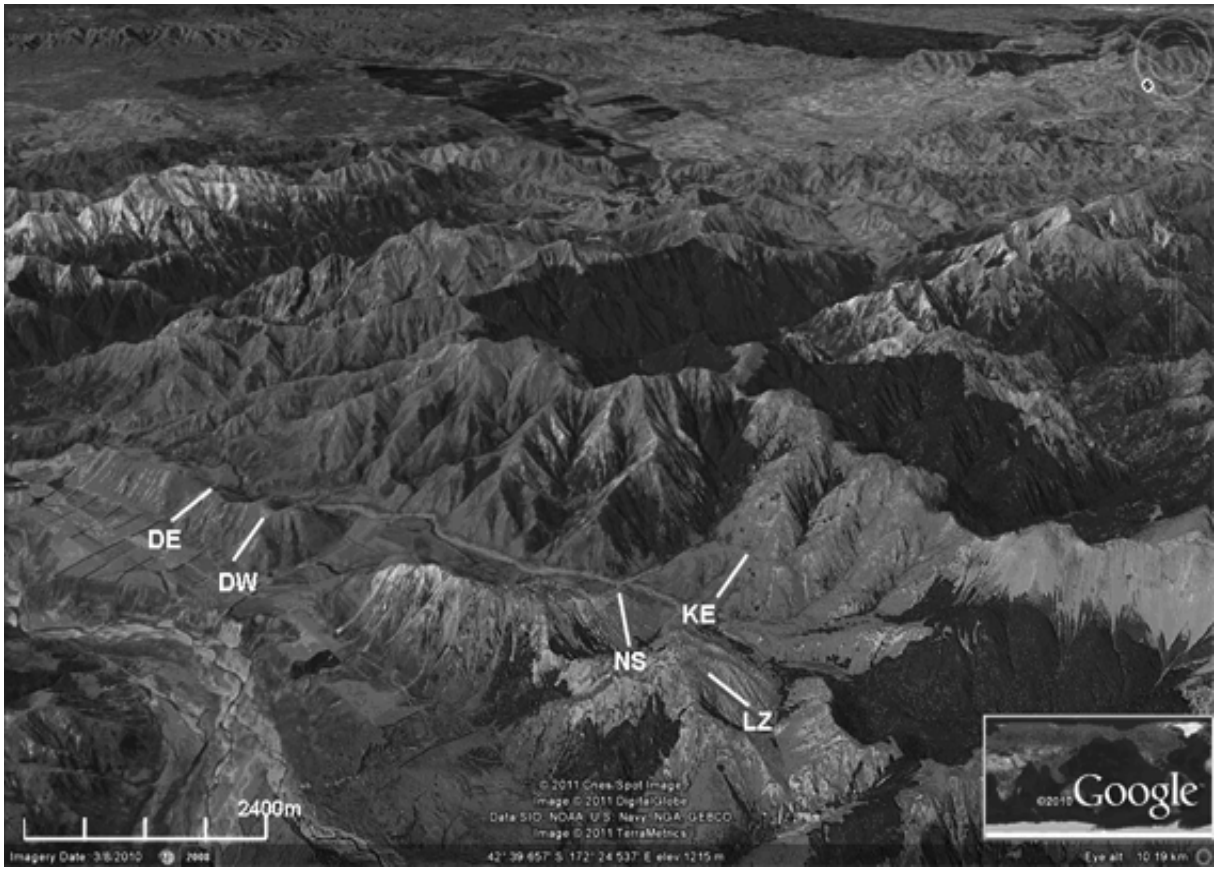


Figure S1: Satellite imagery of the experiment area of the Hope River, South Island, New Zealand (Source: Google Earth), showing locations of the transects (white lines) and their code (See Table S1 for topographic details and coordinates).

Experimental sampling and rearing

Sampling began with the visual search of two randomly-positioned 1 m^2 quadrats from each subplot, where we searched all above-ground vegetation for Lepidoptera larvae. This provided a standardized measure of herbivore density per unit area that was used for the abundance analyses. However, larval densities were generally higher on tussock plants than in the inter-tussock areas. Thus, to yield higher numbers of larvae for the community composition analysis, we searched all the tussocks within the 3 x 12 m strip.

We collected and identified each individual larva to morphospecies level, then to confirm identification we reared specimens to maturity in a climate-controlled room, with a constant temperature of 16 degrees, relative humidity of 60% and a light cycle

of 16L:8D. The exact rearing protocols varied depending on the species requirements. However, we kept most species in 1oz (30ml) plastic cups with a ca. 0.5 cm layer of vermiculite at the bottom, filled with clipped grass (*Poa cita*) from our greenhouse seed-grown plant cultures as food. The diet of the more voracious species was supplemented with small cubes of artificial beet-based diet designed for Lepidoptera (Bio Serv, US). We reared specialist forb feeders in the same way, with artificial diet to supplement their specific host plant. Once per week we supplied all caterpillars with clean cups and fresh food, and checked their development. We maintained individuals of rare species, and species that showed poor performance with the above method, on living plants potted in PVC cylinders, covered with a clear plastic cylinder with holes and fine mesh for ventilation.

Individuals that died during rearing and could not be identified (n = 46) were kept in the dataset for abundance analysis, but discarded from community composition analysis. One morphospecies comprised two very cryptic species (*Tmetolophota propria* and *Tmetolophota atristriga*), which we were unable to separate as larvae. These species are thought to be a very recent radiation, and have virtually identical ecology (J.S. Dugdale, personal communication); therefore, we treated them as a single species complex in the community analyses. To estimate effects of temperature and N on larval biomass, we weighed the caterpillars directly after collection for all samples from September 2009 to December 2009 (i.e. after one year of N fertilization).

Nitrogen treatment application

Precise N deposition rates for the study region are not known, but expansion of dairy farming across New Zealand is driving rapid increases in N fertilizer application (Austin et al. 2007), which will likely impact adjacent semi-natural grasslands. Nitrogen fertilizer was applied in the form of Calcium Ammonium Nitrate (CAN) granules (Ravensdown LTD, New Zealand). This form of fertilizer combines fast and slower release of biologically available nitrogen, and has been used previously to simulate atmospheric deposition (Clark & Tilman 2008).

We began N addition in September 2008, by adding 40% of the total year budget (20 Kg ha^{-1} yr^{-1}, 1066 g CAN per subplot), and applied the remaining 60% in 4 pulses, evenly distributed over the next 12 months, sprinkling the dry granules throughout

the treated subplot. Fertiliser addition continued at a rate of 50 Kg $ha^{-1}yr^{-1}$ until sampling was completed in December 2009. To verify that the nitrogen treatment was taken up and caused significant changes to plant nitrogen levels - the most likely pathway of effect on the insect herbivore community (Tylianakis et al. 2008) - we analyzed the total N content of clippings of *Festuca novae-zelandiae* harvested from the plots in December 2009. Analyses were carried out by the University of Waikato Stable Isotope Unit, Hamilton, New Zealand, using a Dumas elemental analyser interfaced with an isotope mass spectrometer (Europa Scientific 20-20 Stable Isotope Analyser, Europa Scientific Ltd, Crewe, U.K). Additionally, we visually separated all dead leaf material from green leaf material, and used the relative proportion of each to estimate the proportion of tussock biomass that was actually available as a food source for caterpillars. This measure was intended to remove a possible bias that could arise from a simple measure of total biomass, if slower decay and decomposition of old leaf material in colder conditions led to tussock retaining more dead standing vegetation. Including elevation and/or temperature or their interaction with the nitrogen treatment significantly reduced the fit of a model testing the effect of nitrogen treatment on leaf nitrogen, indicating that N treatments were unlikely to have been confounded by underlying nutrient gradients in the landscape that were correlated with the elevation gradient. This also excludes the possibility of any differential uptake of nitrogen by the plants at different elevations.

Vegetation survey

We measured plant species composition by visually estimating the percentage cover of all plant species within five 1 m^2 quadrats, and classed their abundance according to a seven point semi-quantitative scale: 1, ≤0.1%; 2, 0.1-0.9%; 3, 1-5%; 4, 5-25%; 5, 26-50%; 6, 51-75%; 7, 76-100% (Mueller-Dombois & Ellenberg 2003). We included both rooted and overhanging species, thus total percentage cover could be more than 100%. Plant species present within the experimental area but not within the search quadrats were arbitrarily assigned to the lowest cover class. Mean percent cover per species per subplot was calculated by taking the median of the cover class for each species in all five quadrats, then averaging across these quadrats.

To estimate tussock biomass, we counted all tussocks in the subplot, distinguishing between the two species present, *Poa cita* and *Festuca novae-zelandiae.* We then randomly selected a number of tussocks to assess their size. This was achieved by running a line along the diagonal of the subplot and measuring the first 15 tussocks either side of the subplot's centre point, totaling a maximum of 30 tussocks per subplot. We obtained the size estimate (volume) by multiplying the basal circumference by the height from ground level to the tip of the highest leaf of the tussock. Finally, we multiplied the average tussock biomass estimate from these 30 plants by the total count of tussocks to estimate the total tussock biomass per subplot.

Appendix 1.2: Data analysis

Dissimilarity measures

We conducted two sets of analyses, each based on a different dissimilarity measure. The first used only species presence-absence data, with the Jaccard dissimilarity, to focus strictly on changes in community composition. The second test used the Modified-Gower distance with base 10 (Anderson *et al*. 2006). This distance measure considers an order-of-magnitude change in abundance (e.g., from 0.01 to 0.1) equal to a change in composition (i.e. from 0 to 1 species), and therefore accounts for the changes in relative abundance of species in addition to changes in the community composition *per se*. This approach allowed us to specify explicitly the relative importance given to changes in species relative abundance vs. changes in composition in the analysis (Anderson et al. 2006).

Plant composition in the herbivore community composition analyses

To include the effect of plant composition relative to the experimental drivers, we performed a Principal Component Analysis (PCA) and extracted the scores of the four PCA axes that each explained more than 5% of the variation in plant composition (see Table S2). Together, these 4 axes explained 75.9% of the total variation in plant community composition and were included in the model as fixed effects alongside temperature and nitrogen. We tested the maximal model first, and then removed all non-significant terms until the best-fitting model was obtained.

In both plant and herbivore analyses, the effect of temperature was significant even when it entered the model after elevation, indicating that temperature had an effect on plant community structure even after controlling for other effects correlated with elevation (e.g., radiation, partial gases concentration). In contrast, elevation was not significant, even when it entered the model before temperature ($P > 0.05$).

Table S2: Factor loadings of the Principal Component Analysis on plant composition, showing the relative (% Variation) and cumulative (Cum. % Variation) contribution of the individual axes (PC) to the explained variation.

PC	Eigenvalues	%Variation	Cum.%Variation
1	21500	35.5	35.5
2	12200	20.1	55.6
3	7820	12.9	68.5
4	4490	7.4	75.9

Test for biotic homogenization

This test computes a distance matrix between the species composition of groups (in our case, between the coldest, mid, and warmest plot in each transect), and the individual distances from each site to its group centroid are then used in a one-way permutational ANOVA to test for differences in multivariate dispersion between groups (Anderson et al. 2006). In other words, it tests whether, for example, the warmest sites in each transect were on average more similar to each other in their composition than were the coldest sites, or vice versa. Note that this required us to treat temperature as a categorical factor for this analysis (to have groups within which to assess similarity), rather than a variate as in all our other analyses.

Mixed effects models

To test the effect of vegetation composition on total herbivore abundance and biomass (using a Poisson error), we included the first four axes of the plant composition PCA in the initial model, and subsequently removed all non significant scores.When testing species richness, we included the total sample size as an additional covariate, to determine whether changes in richness were simply driven by changes in sample size.

All initial models were fitted using maximum likelihood estimation, then simplified by removing non-significant interaction terms and then main effects (at alpha = 0.05) until no further reduction in residual deviance (measured using the Akaike Information Criterion, AIC) could be obtained. We removed non-significant terms sequentially, re-testing the effect of removal on other non-significant terms before

any further simplification. Final simplified models were then fitted using restricted maximum likelihood (REML), as recommended by Bolker et al. (2009), and tested for overdispersion.

For models using a Poisson error (abundance data), we directly tested the coefficients of our fixed effects (as recommended by Bolker et al. 2009). Due to issues associated with calculating P values from mixed effects models with a Gaussian error structure (Bolker et al. 2009), we used Markov Chain Monte Carlo (MCMC) resampling to estimate P values from Gaussian models. The MCMC procedure was carried out using the pvals.fnc function in the languageR package (Baayen 2010) for R.
We used the same approach for all analyses, however, in the case of the models that included time, the large number of coefficients being tested (11 levels of the time factor, plus interactions) makes interpretation difficult. Therefore, for clarity, we also tested fixed effects in these models by removing the factor then comparing the two models using a likelihood ratio test (Crawley 2007). This provides a single, more-easily interpretable Chi square statistic and probability (P) value for the overall effect of time, though we present the full coefficients table of each model (including a test for the coefficient of each factor level) in Appendix 5 and 8.

Table S3: Comparison of the maximal models in our phenology analyses, using either temperature or elevation as predictor. Asterisks (*) between two or more predictors signify that the model included main effects of each predictor and all possible interactions between them. A colon (:) between two predictors indicates an interaction effect between them. In all cases, temperature provided a better fit than elevation. The maximal is simplified to a reduced best model if necessary. We provide a P value from a Likelihood Ratio test between the elevation and temperature model, and between the initial and the best model (where applicable). Lower values of the Akaike Information Criterion (AIC), indicate better fit.

Response variable	**Model**	**Predictors**	**AIC**	**L.R Test**
Herbivore abundance through time	Elevation	elevation*nitrogen*time	873.48	
	Temperature	temperature*nitrogen*time	818.21	P = 0.001
	Best final model	temperature*nitrogen*time	818.21	
Herbivore bodyweight through time	Elevation	elevation*nitrogen*time	35673	
	Temperature	temperature*nitrogen*time	35666	P = 0.004
	Best final model	Temperature*nitrogen*time	35666	
Herbivore biomass through time	Elevation	elevation*nitrogen*time	2271.5	
	Temperature	temperature*nitrogen*time	2242.5	P = 0.001
	Best final model	temperature+nitrogen+time+temp:time	2233.4	P = 0.030

Appendix 1.3: Supplementary Results

Collinearity between the effects of global change drivers and plant composition on the herbivore assemblage

Both temperature and nitrogen had significant effects when they entered their respective model first ($F_{1,13}$ = 9.53, P = 0.0001 and $F_{1,28}$= 4.05, P= 0.0004, respectively), whereas plant composition had no significant effect when it entered the model after the drivers (P > 0.05 in all cases). This picture reversed when plant composition entered first, causing it to assume the shared variance, and the effect of both drivers to become non-significant (temperature model: $F_{1,13}$ > 6.22, P < 0.005 for the first two axes, $F_{1,13}$ =1.28, P = 0.259 for temperature; nitrogen model: $F_{1,28}$> 2.94, P < 0.004 for the first two axes, $F_{1,28}$= 0.70, P = 0.715 for nitrogen. Note that only the first two plant composition PCA axes were significant and retained in the models). Although this strongly suggests that the effects of temperature and nitrogen on the herbivore community may have been mediated via plant community shifts, we cannot objectively attribute this shared variance to either of the collinear predictor variables with certainty.

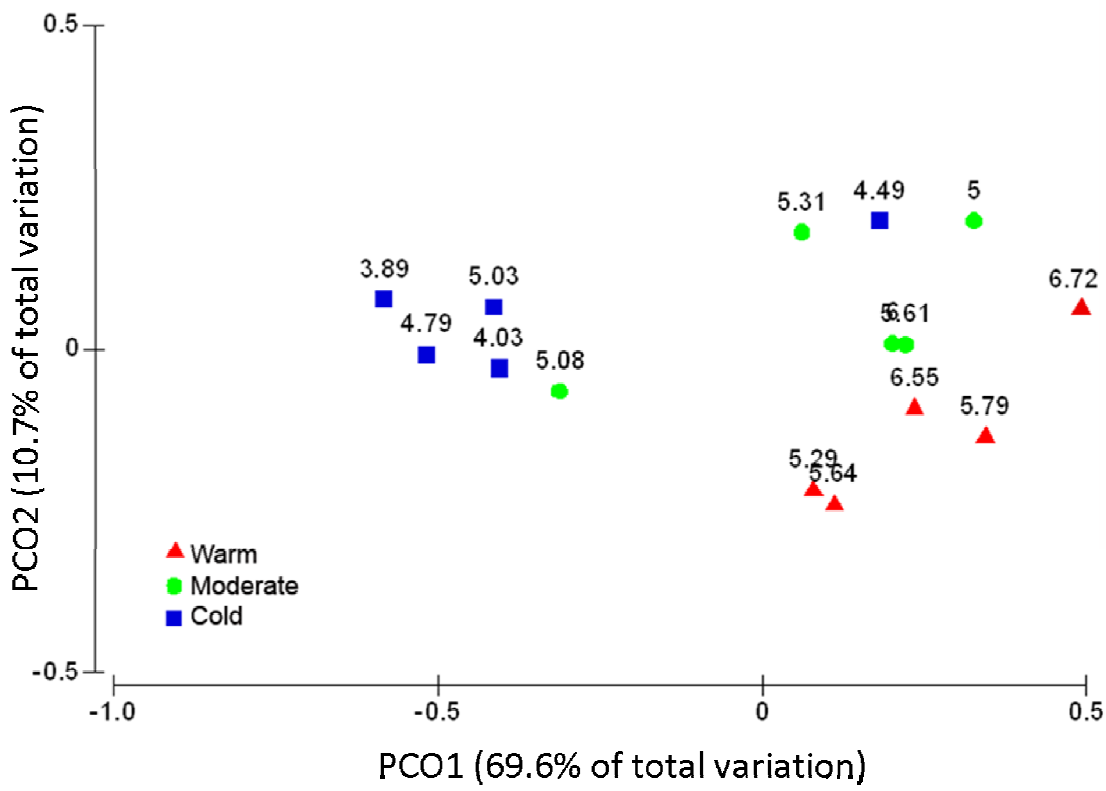


Figure S2: Effects of the global change drivers (temperature and nitrogen) on the herbivore assemblage and multivariate dispersion of herbivore community across sites: Principal Coordinates ordination showing differences in assemblage composition, based on the modified Gower base 10 dissimilarity (Anderson et al. 2006), across plots. For visual clarity and the homogeneity of dispersion analyses, plots are split into three temperature categories (red triangles = warm, green circles = moderate and blue squares = cold plots from each transect), but analyses of driver effects on composition treated temperature as a variate (mean temperature values are given above each point), blocked by transect.

Table S4: Results of permutational distance-based multivariate ANOVA (Anderson et al. 2006), using the Modified Gower distance base 10 dissimilarity to compare changes in the relative abundance of species. Error structure followed a split-plot design. Asterisks indicate level of significance (. ≤ 0.1, * ≤ 0.05, ** ≤ 0.01, *** ≤ 0.001).

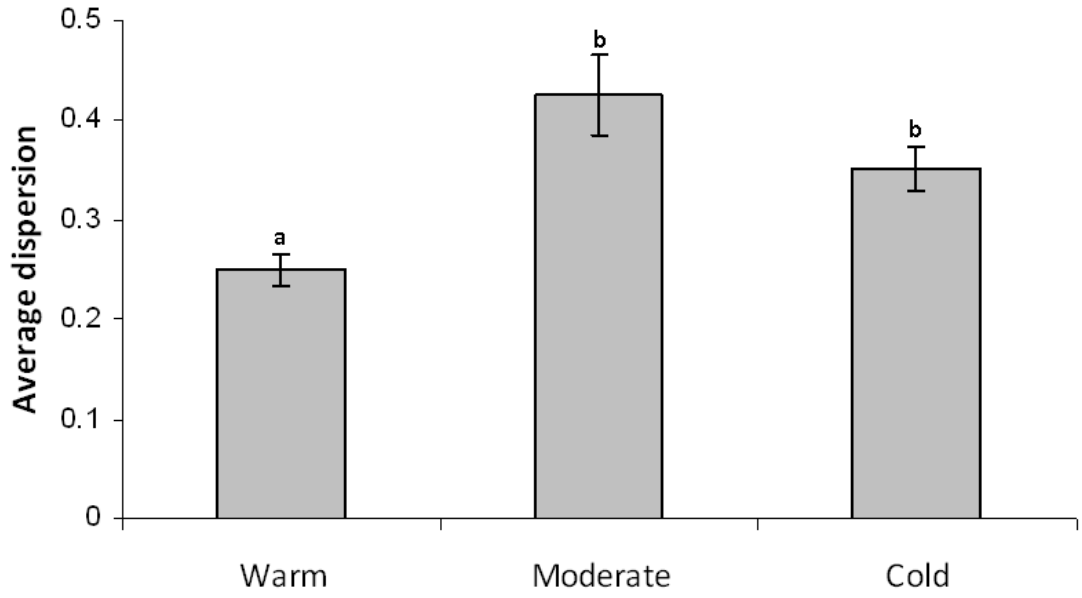
Error: Transect							
Source	Df	SS	MS				
Residuals	4	0.58	0.14				
Error: Plot in Transect							
Source	Df	SS	MS	Pseudo-F	P(perm)	perms	
Temperature	1	0.97	0.97	8.00	<0.001	9946	***
Elevation	2	0.17	0.08	0.80	0.575	9935	
Residuals	7	0.74	0.11				
Error: Subplot in Plot in Transect							
Nitrogen	1	0.16	0.16	3.69	0.001	9924	**
Temperature x nitrogen	1	0.06	0.06	1.50	0.169	9941	
Residuals	13	0.56	0.04				
Error: Sampling dates in Subplot in Plot in Transect							
Time	10	49.07	4.91	11.99	0.0001	9730	***
Temperature x time	10	13.40	1.34	3.27	0.0001	9774	***
Nitrogen x time	10	4.11	0.41	1.01	0.470	9769	
Temperature x nitrogen x time	10	3.68	0.37	0.90	0.810	9746	
Residuals	260	106.43	0.41				
Total	329	239.24					

Table S5: Results of permutational distance-based multivariate ANOVA (Anderson et al. 2006), using the Jaccard dissimilarity to compare changes in the presence/absence of species, ignoring changes in relative abundances. Error structure followed a split-plot design. Asterisks indicate level of significance (. ≤ 0.1, * ≤ 0.05, ** ≤ 0.01, *** ≤ 0.001).

Error: Transect							
Source	Df	SS	MS				
Residuals	4	2283.50	570.88				
Error: Plot in Transect							
Source	Df	SS	MS	Pseudo-F	P(perm)	perms	
Temp	1	2860.00	2860.00	5.75	< 0.001	9935	***
Elevation	2	710.61	355.00	0.96	0.470	9925	
Residuals	7	2585.40	369.35				
Error: Subplot in Plot in Transect							
Nitrogen	1	564.01	564.01	2.12	0.059	9942	.
Temp x Nitrogen	1	459.66	459.66	1.73	0.117	9949	
Residuals	13	3454.50	265.73				
Error: Sampling dates in Subplot in Plot in Transect							
Time	10	2219.30	221.93	9.96	0.0001	9786	***
Temp x Time	10	468.13	4681.30	2.10	0.0001	9778	***
Nitrogen x time	10	224.29	2242.90	1.01	0.461	9752	

Appendix 1.4: Homogenisation of the herbivore community under warmer temperatures

Figure S3: Bar plot summarizing the multivariate dispersion (dissimilarity in community composition across plots, mean +/- SE) for three temperature categories. Letters show significant differences across groups following a permutation-based test for homogeneity of multivariate dispersions (Anderson et al. 2006). Warmest sites show lowest dispersion, indicating that warm sites were on average more similar to each other than moderate or cold sites.



Appendix 1.5: Changes in herbivore abundance and biomass through time in response to temperature and nitrogen addition

Table S6: Results table from a generalized linear mixed effects model (Poisson errors) with the total abundance of larvae as the response. This minimum adequate model was generated by removing non-significant parameters from the maximal model (see main text) to provide the best fit (lowest Akaike Information Criterion, AIC score). Each fixed effect was tested here by removing it from the model and testing for a change in unexplained deviance using a likelihood ratio test. Individual coefficients and tests of their significance are shown below. Asterisks indicate level of significance (.≤ 0.1, * ≤ 0.05, ** ≤ 0.01, *** ≤ 0.001).

	Df	AIC	ΔAIC	Chisq Chi	P	
Full model	10	818.21				
3 way interaction	10	858.90	40.69	60.68	<0.0001	***
Temperature x nitrogen	1	859.10	0.20	2.21	0.118	
Nitrogen x time	10	896.96	38.06	58.06	<0.0001	***
Temperature x time	10	903.48	44.58	64.58	<0.0001	***
Nitrogen	1	914.76	11.28	21.18	0.001	***
Temperature	1	907.01	10.05	7.57	0.022	*
Time	10	1306.34	411.24	386.15	<0.0001	***

Table S7: Full coefficient table for model of changes in larval abundance through time with Poisson error distribution. A colon between two variables indicayes an interaction effect. Asterisks indicate level of significance (. ≤ 0.1, * ≤ 0.05, ** ≤ 0.01, *** ≤ 0.001).

	Estimate	Std.Error	Z value	P (>|z|)	
(Intercept)	-0.59	1.54	-0.38	0.703	
Temperature	0.37	0.28	1.29	0.199	
Nitrogen	-0.75	1.36	-0.57	0.578	
time2	-2.38	1.30	-1.83	0.068	.
time3	-1.15	1.45	-0.79	0.428	
time4	-2.72	1.65	-1.65	0.100	
time5	2.46	2.77	0.89	0.375	
time6	1.25	3.48	0.36	0.720	
time7	-3.03	1.89	-1.60	0.109	
time8	-5.74	1.59	-3.60	0.0003	***
time9	-1.50	1.40	-1.08	0.282	
time10	-3.50	1.44	-2.43	0.015	*
time11	-2.83	2.77	-1.02	0.306	
temp:nitrogen	0.14	0.25	0.56	0.575	
temp:time2	0.47	0.23	2.01	0.045	*
temp:time3	0.16	0.26	0.63	0.530	
temp:time4	0.38	0.30	1.27	0.206	
temp:time5	-0.85	0.54	-1.58	0.116	
temp:time6	-0.71	0.67	-1.06	0.290	
temp:time7	0.36	0.34	1.07	0.284	
temp:time8	0.95	0.28	3.40	0.0006	***
temp:time9	0.26	0.25	1.01	0.312	
temp:time10	0.60	0.26	2.34	0.020	*
temp:time11	0.15	0.50	0.31	0.758	
Nitrogen:time2	5.23	1.82	2.87	0.004	**
Nitrogen:time3	3.89	1.93	2.01	0.044	*
Nitrogen:time4	5.94	2.45	2.42	0.015	*
Nitrogen:time5	-0.13	3.48	-0.04	0.970	
Nitrogen:time6	-1.19	4.21	-0.28	0.777	
Nitrogen:time7	1.77	2.45	0.72	0.471	
Nitrogen:time8	0.95	2.14	0.45	0.656	
Nitrogen:time9	4.67	1.90	2.45	0.014	*
Nitrogen:time10	3.78	1.90	1.99	0.046	*
Nitrogen:time11	-0.69	3.15	-0.22	0.827	
temp:nitrogen:time2	-0.95	0.33	-2.86	0.004	**
temp:nitrogen:time3	-0.63	0.35	-1.77	0.076	.
temp:nitrogen:time4	-1.16	0.45	-2.55	0.011	*
temp:nitrogen:time5	0.16	0.67	0.24	0.812	
temp:nitrogen:time6	0.40	0.80	0.49	0.621	
temp:nitrogen:time7	-0.22	0.44	-0.50	0.614	
temp:nitrogen:time8	-0.11	0.37	-0.31	0.760	
temp:nitrogen:time9	-0.81	0.35	-2.32	0.020	*
temp:nitrogen:time10	-0.57	0.34	-1.68	0.093	.
temp:nitrogen:time11	0.43	0.57	0.75	0.458	

Table S8: Results of a linear mixed effects model with Gaussian errors and total herbivore biomass through time as the response. This minimum adequate model was generated by removing non-significant parameters from the maximal model (see main text) to provide the best fit (lowest Akaike Information Criterion, AIC score). Each fixed effect was tested by removing it from the model and testing for a change in unexplained deviance using a likelihood ratio test. Individual coefficients and tests of their significance are shown in below. Asterisks indicate level of significance (.≤ 0.1, * ≤ 0.05, ** ≤ 0.01, *** ≤ 0.001).

	Df	AIC	ΔAIC	Chisq Chi	P-value	
Best model	3	2233.4				
Temperature	1	2266.3	32.9	21.49	<0.0001	***
Nitrogen	1	2250.6	17.2	5.81	0.055	.
Time	3	2243.7	10.3	2.84	0.584	
Temperature x time	3	2248.8	15.4	21.42	<0.0001	***

Table S9: Full coefficients table of model for changes in herbivore biomass through time with Gaussian error distribution. A colon between two variables indicayes an interaction effect. Asterisks indicate level of significance (. ≤ 0.1, * ≤ 0.05, ** ≤ 0.01, *** ≤ 0.001).

	Estimate	Std.Error	t value	pMCMC	
(Intercept)	-16068.08	4300.71	-3.74	0.001	**
Temperature	3594.87	802.60	4.48	0.002	**
Nitrogen	7794.26	5431.11	1.46	0.188	
time9	6049.21	5300.90	1.14	0.302	
time10	494.99	5300.90	0.09	0.935	
time11	12380.58	5300.90	2.37	0.035	*
temp:nitrogen	1742.91	1017.14	1.71	0.118	
temp:time9	-1168.03	992.75	-1.18	0.284	
temp:time10	79.12	992.75	0.08	0.933	
temp:time11	-2508.84	992.75	-2.53	0.022	*
Nitrogen:time9	7936.48	7496.61	1.06	0.347	
Nitrogen:time10	8633.13	7496.61	1.15	0.300	
Nitrogen:time11	9042.91	7496.61	1.21	0.278	
temp:nitrogen:time9	-1568.76	1403.96	-1.12	0.314	
temp:nitrogen:time10	-1856.53	1403.96	-1.32	0.229	
temp:nitrogen:time11	-1650.01	1403.96	-1.18	0.288	

Appendix 1.6: Effect of temperature and nitrogen on herbivore phenology (i.e. three-way interaction between temperature, nitrogen and time)

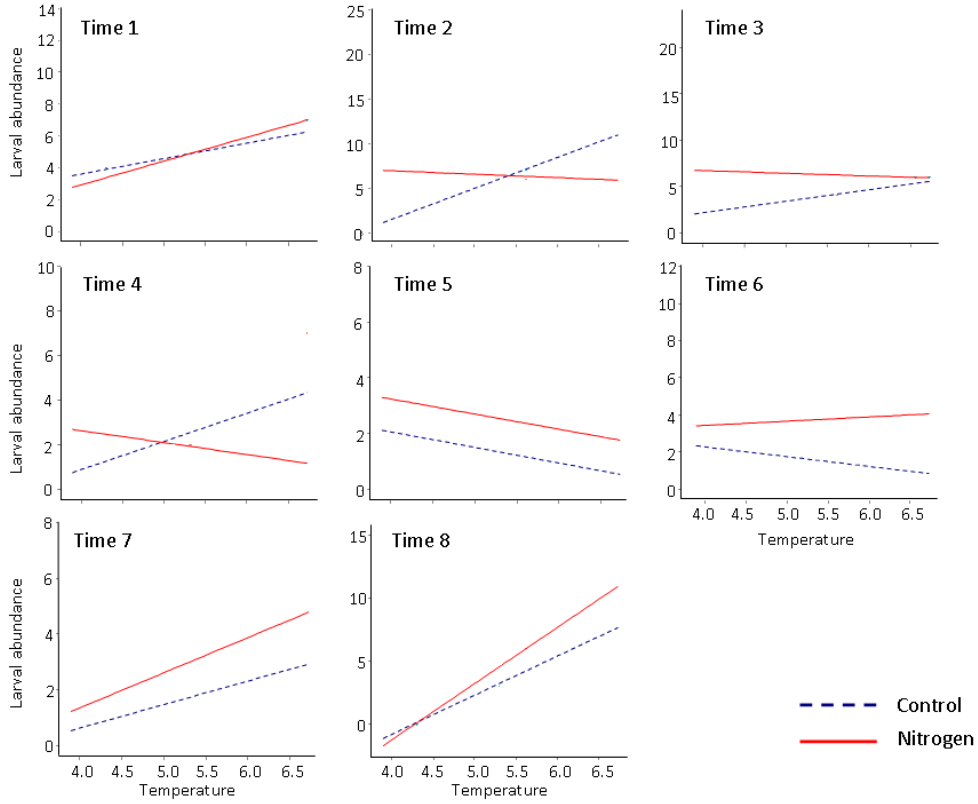


Figure S4: Multiple plots representing the larval abundance in control and nitrogen plots through time. The sequence of plots shows the three-way interaction between temperature, nitrogen and time, i.e. nitrogen and temperature show a time-dependent interactive effect on larval abundance. It interesting to note that control plots behave similarly to the nitrogen, but with a time delay: from Time 1 through to Time 5, the effect of temperature in nitrogen plots transitions from positive to negative, returning to a strong positive effect at time 8. In control plots, the temperature effect remains more constant from Time 1 to Time 4, and then equals the negative effect as in nitrogen Time 5. From here, the effect of temperature becomes more positive, but is slower and less steep than the effect of temperature in the nitrogen plots. Note that Time 1 to Time 5 corresponds to October 2008 to February 2009 (Spring and Summer), Time 6 and 7 correspond to April and May 2009 (Fall) and Time 8 corresponds to September 2009 (early Spring), therefore representing a full year cycle excluding the months of snow cover in Winter.

Appendix 1.7: Changes in abundance of the three most common herbivore species through time and in response to temperature and nitrogen addition

Table S9: Results of mixed effects models (with Poisson errors) testing how the abundance of A) *Persectania aversa*, B) *Orocrambus ramosellus* and C) *Orocrambus simplex* changed through time and in response to temperature and nitrogen. Models are minimum adequate models, after removing non-significant parameters from the maximal model (see main text) to provide the best fit (lowest Akaike Information Criterion, AIC score). Each fixed effect was tested by removing it from the appropriate model for each specific term and testing for a change in unexplained deviance using a likelihood ratio test.

All three species responded positively to both drivers in isolation, though with varying magnitude. Similarly, all three species showed a positive interaction between temperature and time, indicating that phenological changes in abundance depended on temperature. However, these three species showed different coefficients for their temperature x nitrogen interactions, which ranged from negative to positive. Additionally, they showed different responses to the treatments in their change in abundance through time (i.e. phenology). While *Persectania aversa* showed no response, the two crambid species each responded in contrasting ways: the phenology of *Orocrambus ramosellus* showed a positive interaction coefficient between time and the drivers, whereas *Orocrambus simplex* showed a negative 3-way interaction.

A)	Df	AIC	ΔAIC	Chisq	P-value	
Full model	10	963.35		11.31	0.334	
Temperature x nitrogen	1	967.98	4.63	6.64	0.010	*
Nitrogen x time	10	978.00	14.65	34.65	0.0001	***
Temperature x time	10	1113.23	149.88	169.88	<0.0001	***
Nitrogen	1	995.89	32.54	18.39	0.0001	***
Temperature	1	1124.2	160.85	11.41	0.003	**
Time	10	2189.8	1226.45	1087.11	<0.0001	***

B)	Df	AIC	ΔAIC	Chisq	P-value	
Full model	10	799.78				
3-way interaction	10	804.57	4.79	24.7	0.006	**
Temperature x nitrogen	1	803.07	3.29	0.506	0.477	
Nitrogen x time	10	838.01	38.23	53.44	<0.0001	***
Temperature x time	10	887.56	87.78	102.99	<0.0001	***
Nitrogen	1	844.45	44.67	12.16	0.002	**
Temperature	1	889.81	90.03	7.97	0.019	*
Time	10	2405.57	1605.79	1512.10	<0.0001	***

C)	Df	AIC	ΔAIC	Chisq	P	
Full model	10	468.54				
3-way interaction	10	474.67	6.13	26.12	0.004	**
Temperature x nitrogen	1	474.78	6.24	2.11	0.146	
Nitrogen x time	10	490.92	22.38	36.25	<0.0001	***
Temperature x time	10	551.76	83.22	97.08	<0.0001	***
Nitrogen	1	504.58	36.04	18.37	0.0001	***
Temperature	1	556.61	88.07	9.56	0.008	**
Time	10	1484.47	1015.93	941.25	<0.0001	***

Appendix 1.8: Changes in herbivore individual body mass through time in response to temperature and nitrogen addition

Table S10: Results of linear mixed effects model with Gaussian errors and body mass of individual larvae in the whole assemblage as the response, with species identity included as a random effect. We found effects of the drivers on larval phenology/development. Overall, mean body mass of each larva across the whole assemblage (after removing between-species variance by including species as a random effect), showed an interaction effect between each global change driver and time: temperature was responsible for a higher and earlier biomass peak, whereas the positive effect of nitrogen on biomass became stronger through the season. However, the effect of both drivers in combination was sub-additive (3-way temperature x N x time interaction was negative and only marginally significant)

Each fixed effect was tested by removing it from the best-fitting model, selected by minimizing the Akaike Information Criterion (AIC), and testing for a change in unexplained deviance using a likelihood ratio test. Individual coefficients and tests of their significance are shown below. Asterisks indicate level of significance (.≤ 0.1, * ≤ 0.05, ** ≤ 0.01, *** ≤ 0.001).

A)

	Df	AIC	ΔAIC	Chisq Chi	P	
Fullmodel	10	35666				
3 way interaction	10	35668	2	7.57	0.056	.
Temperature x nitrogen	1	35666	0	0.38	0.538	
Temperature x time	10	35675	9	13.49	0.004	**
Nitrogen x time	10	35678	10	16.45	0.0009	***
Temperature	1	35674	8	15.46	0.0004	***
Nitrogen	1	35675	9	2.23	0.329	
Time	10	36353	687	673.72	<0.0001	***

B) Full coefficient table of model for changes in body mass of individual larvae in the whole assemblage with Gaussian error distribution.

	Estimate	Std. Error	t value	pMCMC	
(Intercept)	19.46	89.46	0.21	0.715	
Temperature	31.26	10.94	2.86	0.004	**
Nitrogen	-58.30	75.24	-0.78	0.450	
time9	35.55	84.97	0.42	0.660	
time10	15.73	89.76	0.18	0.851	
time11	163.95	114.86	1.43	0.050	*
temp:nitrogen	11.58	12.45	0.93	0.401	
temp:time9	-1.88	14.25	-0.13	0.706	
temp:time10	24.77	15.08	1.64	0.261	
temp:time11	11.84	19.89	0.60	0.976	
nitrogenN:time9	35.72	103.57	0.35	0.968	
nitrogenN:time10	116.17	108.98	1.07	0.521	
nitrogenN:time11	301.49	134.41	2.24	0.146	
temp:nitrogenN:time9	-5.74	17.54	-0.33	0.946	
temp:nitrogenN:time10	-27.19	18.42	-1.48	0.287	
temp:nitrogenN:time11	-58.30	23.25	-2.51	0.078	.

Literature cited in Appendix 1:

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Appendix 2

Appendix 2.1: Artificial warming experiment: study location and experimental temperature control

Figure S5: Satellite imagery of the upper half of the South Island, New Zealand (Source: Google Earth), showing the location of the artificial warming experiment at Cass, the altidtudinal gradient experiment, near Lewis Pass, in relation to the city of Christchurch.

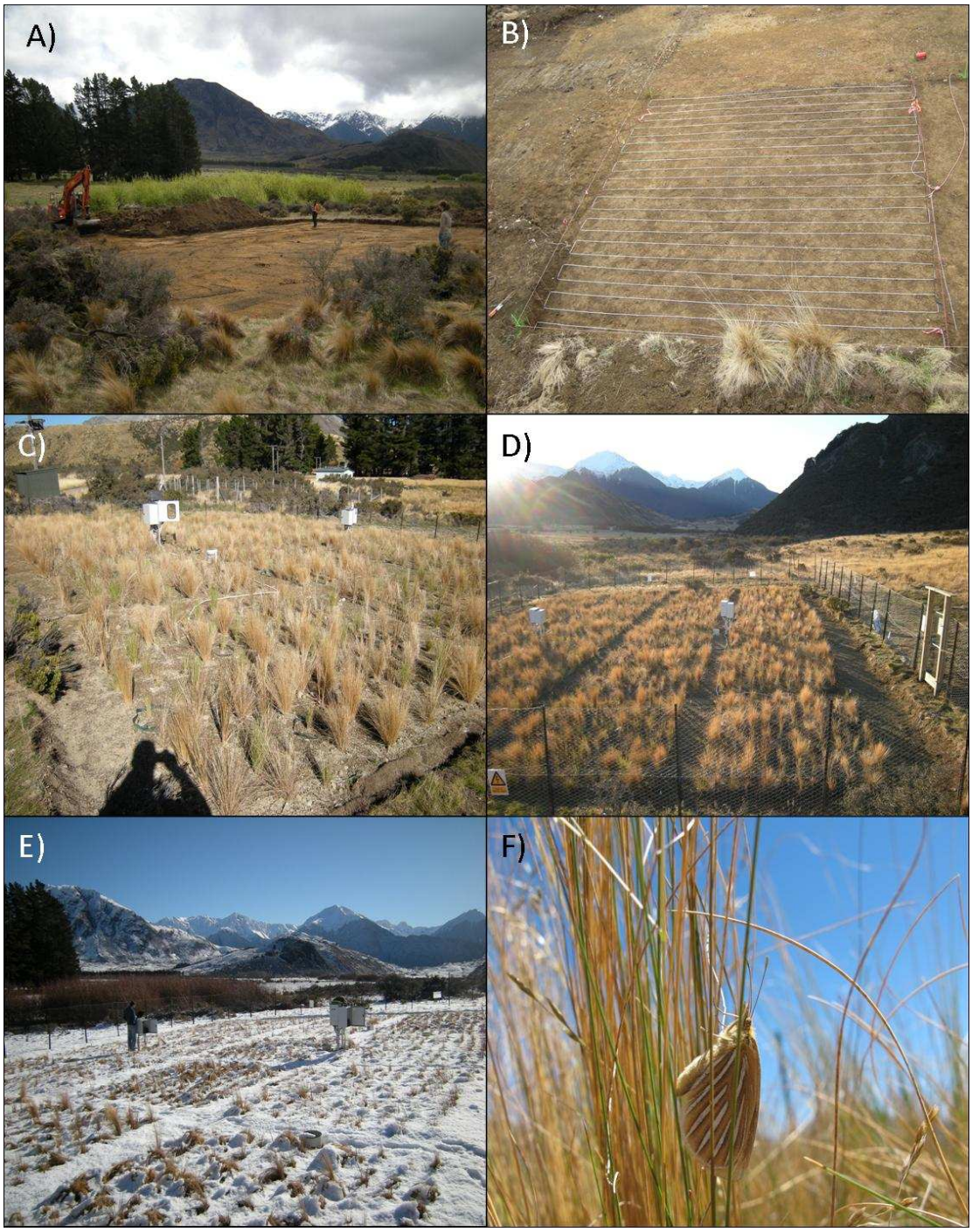

Figure S6: Photo sequence of the establishment of the Cass warming experiment. A) We dug the experimental area in October 2008, B) installed the heating cables in each plot with standardized layout, buried them in the ground and C) planted 2880 tussocks, 144 per plot with replicated composition. D) The experimental warming was first activated in April 2009 and the experiment was left to run E) through winter and spring, before F) we began sampling Lepidoptera larvae in January 2010 (midsummer).

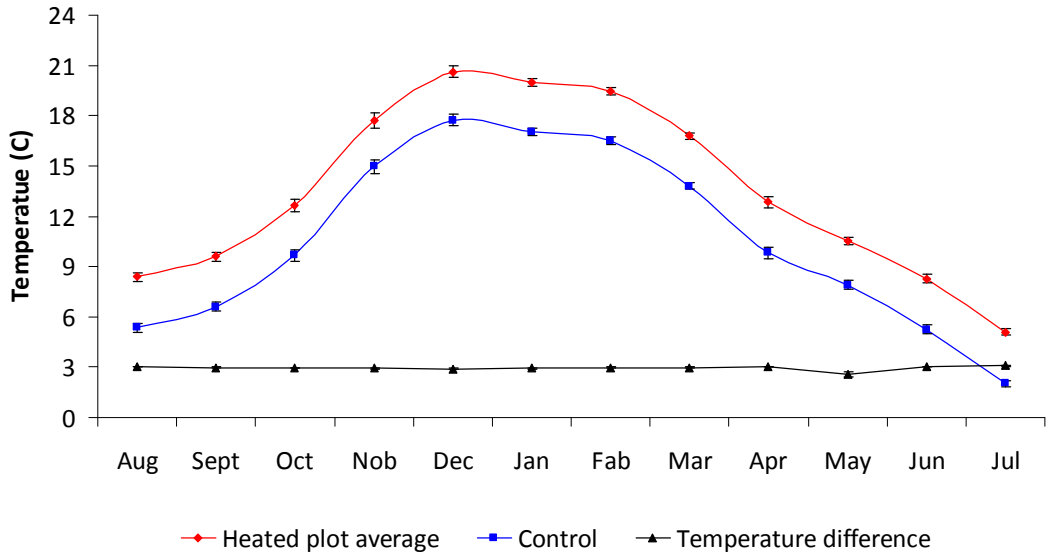

Figure S7 Average monthly temperature (±SE) for Aug 2009-July 2010 in a heated plot against the control plot and differential temperature between the treatments. Monthly averages are calculated as the average daily temperature for that month. The experimental warming maintained the temperature difference between heated and control plots very close to the three degree target throughout the year.

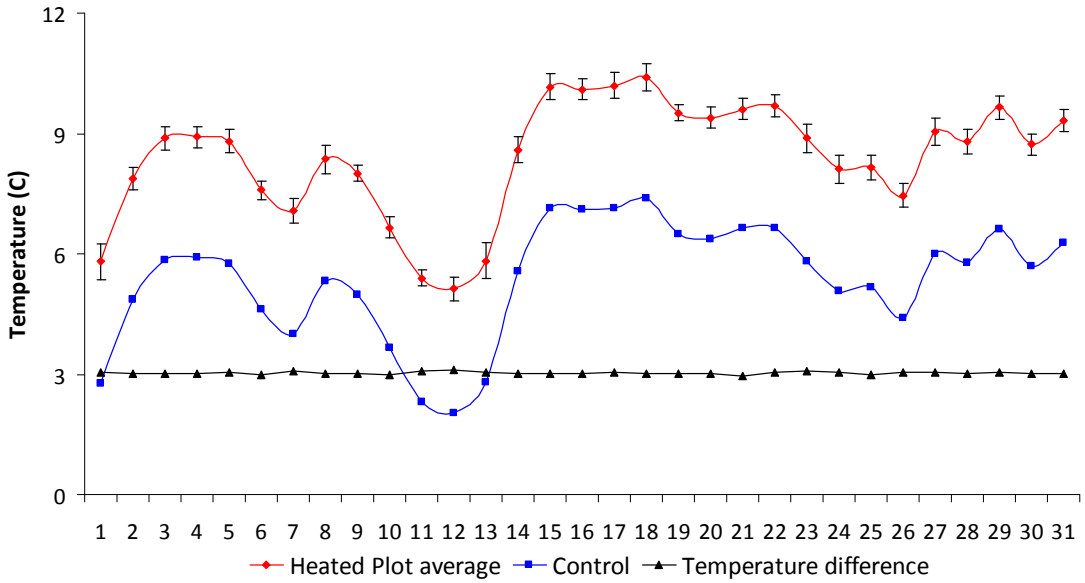

Figure S8: Average daily temperature for October 2010 (the month of overall peak larval abundance) in a heated plot against the control plot and differential temperature between the treatments. For the heating plot, an average (±SE) is calculated from the three thermocouples in the plot. For the control plot, values are the daily average of the one thermocouple installed. The experimental warming maintained the temperature difference between heated and control plots very close to the three degree target, and accurately followed daily temperature fluctuations.

Appendix 3

Appendix 3.1: List of all parasitoid and Lepidoptera species

Table S12: List of species. All herbivore species are known to be endemic to New Zealand, and historically associated with alpine grasslands (J. Dugdale, personal communication). All tachinid fly species (Diptera) are also endemic to New Zealand (J.Dudgale, personal communication). All hymenoptera species are believed to be endemic but, due to the species-level resolution for some taxa, we cannot exclude the presence of exotic species, although this seems unlikely (J. Berry, personal communication).

	Code	Order	Family	Species
PARASITOIDS	aclbra	Hymenoptera	Ichneumonidae	*Aclosmation* sp.1
	alegre	Hymenoptera	Braconidae	*Aleoides gressitti*
	alesp3	Hymenoptera	Braconidae	*Aleoides* sp.3
	aucsp1	Hymenoptera	Ichneumonidae	*Aucklandella* sp.1
	aucspp	Hymenoptera	Ichneumonidae	*Aucklandella* sp.2
	brarog	Hymenoptera	Braconidae	*indet rogadine*
	calape	Diptera	Tachinidae	*Calcager apertum* (Hutton)
	caltri	Diptera	Tachinidae	*Calotachina tricolor* (Malloch)
	camnud	Diptera	Tachinidae	*Campylia nudarum* (Malloch)
	camsp1	Hymenoptera	Ichneumonidae	*Campoletis* sp.1
	camsp2	Hymenoptera	Ichneumonidae	*Campoletis* sp.2
	camspx	Hymenoptera	Ichneumonidae	*Campoplex* sp.1
	camter	Diptera	Tachinidae	*Campylia temerarium* (Hutton)
	casina	Hymenoptera	Ichneumonidae	*Casinaria* sp.1
	cotsp1	Hymenoptera	Braconidae	*Cotesia* sp.1
	cotsp2	Hymenoptera	Braconidae	*Cotesia* sp.2
	degsp1	Hymenoptera	Ichneumonidae	*Degithina* sp.1
	diasp2	Hymenoptera	Ichneumonidae	*Diadegma* sp.2
	diaspx	Hymenoptera	Ichneumonidae	*Diadegma* sp.1
	dolsp1	Hymenoptera	Braconidae	*Dolichogenidea* sp.1
	eupsp1	Hymenoptera	Braconidae	*Euphorine* sp.1
	eutlic	Hymenoptera	Ichneumonidae	*Eutanyacra licitatoria* (Erichson)
	glysp5	Hymenoptera	Braconidae	*Glyptapanteles*
	gramon	Diptera	Tachinidae	*Gracilicera monticola* (Malloch)
	grapol	Diptera	Tachinidae	*Gracilicera politiventris* (Malloch)
	hetext	Diptera	Tachinidae	*Heteria extensa* (Malloch)
	hetple	Diptera	Tachinidae	*Heteria plebia* (Malloch)
	hetpun	Diptera	Tachinidae	*Heteria punctigera* (Malloch)
	ichind	Hymenoptera	Ichneumonidae	*Indet*
	levans	Hymenoptera	Ichneumonidae	*Levansa* sp.1
	macsp1	Hymenoptera	Braconidae	*Macrocentrus sp* sp.1
	metcin	Hymenoptera	Braconidae	*Meteorus cinctellus*
	metcob	Hymenoptera	Braconidae	*Meteorus cobbus*
	metspx	Hymenoptera	Braconidae	*Meteorus* sp.1
	palatr	Diptera	Tachinidae	*Pales atrox* (Hutton)
	paleff	Diptera	Tachinidae	*Pales efferata* (Hutton)
	palnyc	Diptera	Tachinidae	*Pales nyctemeriana* (Hudson)
	plalon	Diptera	Tachinidae	*Plagiomyia longicornis* (Malloch)
	trispx	Hymenoptera	Pteromalidae	*Trichomalopsis* sp.1
	zelvar	Diptera	Tachinidae	*Zealandotachina varipes* (Malloch)

LEPIDOPTERA	agradm	Lepidoptera	Noctuidae	*Agrotis admirationis* (Guenee)
	alecuc	Lepidoptera	Noctuidae	*Aletia cucullina* (Guenee)
	alesis	Lepidoptera	Noctuidae	*Aletia sistens* (Guenee)
	argant	Lepidoptera	Nymphalidae	*Argyrophenga antipodum* (Doubleday)
	asaabr	Lepidoptera	Geometridae	*Asaphodes abrogata* (Walker)
	asaaeg	Lepidoptera	Geometridae	*Asaphodes aegrota* (Butler)
	asacla	Lepidoptera	Geometridae	*Asaphodes clarata* (Walker)
	daspar	Lepidoptera	Geometridae	*Dasyuris partheniata* (Guenée)
	epieri	Lepidoptera	Tortrcidae	*Epichorista eribola* (Meyrick)
	episir	Lepidoptera	Tortrcidae	*Epichorista siriana* (Meyrick)
	eudsab	Lepidoptera	Pyralidae	*Eudonia sabulosella* (Walker)
	eudsub	Lepidoptera	Pyralidae	*Eudonia submarginalis* (Walker)
	graago	Lepidoptera	Noctuidae	*Graphania agorastis* (Meyrick)
	gralig	Lepidoptera	Noctuidae	*Graphania lignana* (Walker)
	gramut	Lepidoptera	Noctuidae	*Graphania mutans* (Walker)
	granul	Lepidoptera	Noctuidae	*Graphania nullifera* (Walker)
	graphr	Lepidoptera	Noctuidae	*Graphania phricias* (Meyrick)
	graseq	Lepidoptera	Noctuidae	*Graphania sequens* (Howes)
	helcor	Lepidoptera	Geometridae	*Helastia corcularia* (Guenee)
	hyddel	Lepidoptera	Geometridae	*Hydriomena deltoidata* (Walker)
	ichmar	Lepidoptera	Noctuidae	*Ichneutica marmorata* (Walker)
	merleu	Lepidoptera	Tortrcidae	*Merophyas leucaniana* (Walker)
	methut	Lepidoptera	Arctiidae	*Metacrias huttoni* (Butler)
	metstra	Lepidoptera	Noctuidae	*Meterana* sp.1
	noc	Lepidoptera	Noctuidae	*Noctuid* sp.1
	opoomo	Lepidoptera	Tineidae	*Opogona omoscopa* (Meyrick)
	oroaet	Lepidoptera	Crambidae	*Orocrambus aethonellus* (Meyrick)
	orofle	Lepidoptera	Crambidae	*Orocrambus flexuosellus* (Doubleday)
	ororam	Lepidoptera	Crambidae	*Orocrambus ramosellus* (Doubleday)
	orosim	Lepidoptera	Crambidae	*Orocrambus simplex* (Butler)
	orotri	Lepidoptera	Crambidae	*Orocrambus tritonellus* (Meyrick)
	orosp1	Lepidoptera	Crambidae	*Orocrambus* sp.1
	parbre	Lepidoptera	Geometridae	*Paranotorius brephosata* (Meyrick)
	perave	Lepidoptera	Noctuidae	*Persectania aversa* (Walker)
	procom	Lepidoptera	Noctuidae	*Proteuxoa comma* (Walker)
	tme	Lepidoptera	Noctuidae	*Tmetolophota* sp.1
	tmeaco	Lepidoptera	Noctuidae	*Tmetolophota acontistis* (Meyrick)
	tmearo	Lepidoptera	Noctuidae	*Tmetolophota arotis* (Meyrick)
	tmeatr	Lepidoptera	Noctuidae	*Tmetolophota atristriga* (Walker)
	tmelis	Lepidoptera	Noctuidae	*Tmetolophota lissoxyla* (Meyrick)
	tmepro	Lepidoptera	Noctuidae	*Tmetolophota propria* (Walker)
	tmesem	Lepidoptera	Noctuidae	*Tmetolophota semivittata* (Walker)
	tmesp1	Lepidoptera	Noctuidae	*Tmetolophota* sp.1
	tmeste	Lepidoptera	Noctuidae	*Tmetolophota steropastis* (Meyrick)
	tmetem	Lepidoptera	Noctuidae	*Tmetolophota temenaula* (Meyrick)
	tmetor	Lepidoptera	Noctuidae	*Tmetolophota toroneura* (Meyrick)
	tmeuna	Lepidoptera	Noctuidae	*Tmetolophota unicolor* (Walker)
	tmeuni	Lepidoptera	Noctuidae	*Tmetolophota unica* (Meyrick)
	tmewsd	Lepidoptera	Noctuidae	*Tmetolophota* sp.2
	xanocc	Lepidoptera	Geometridae	*Xanthorrhoe occulta* (Philpott)

Appendix 3.2: Basis set of independence claims for the Altitudinal Gradient Experiment path model.

Each independence claim (in brackets) expresses the independence between the two variables, after controlling for the effect of the conditioning variable(s). When an indirect pathway is presented in the path model (e.g. A->B->C), it assumes that A and C are independent (independence claim), holding B (the conditioning variable) constant. All independence claims for the entire model must be tested, and their P-values are used to calculate a *C*-statistic = $-2\sum^{k}_{i=1} \ln(p_i)$, which is then compared to a chi-square (χ^2) distribution with $2k$ degrees of freedom to assess the overall fit of the model (Shipley 2009).

# Independence claim (variable 1, variable 2)	**{conditioning variables}, P-value for the independence claim**
#1 (temperature, Plant quality)	{nitrogen}, P = 0.656
#2 (herbivore abundance, nitrogen)	{resource availability, plant quality, temperature}, P = 0.887
#3 (herbivore richness, resource availability)	{herbivore abundance, temperature, nitrogen}, P = 0.553
#4 (herbivore richness, Plant quality)	{herbivore abundance, nitrogen}, P = 0.725
#5 (herbivore richness, temperature)	{herbivore abundance}, P = 0.624
#6 (herbivore richness, nitrogen)	{herbivore abundance}, P = 0.114
#7 (herbivore composition, herbivore abundance)	{temp., plant quality, resource avail., herbivore rich.}, P = 0.27
#8 (herbivore composition, resource availability)	{temperature, herbivore richness, nitrogen}, P = 0.085
#9 (herbivore composition, Plant quality)	{temperature, herbivore richness, nitrogen}, P = 0.708
#10 (herbivore composition, nitrogen)	{temperature, herbivore richness}, P = 0.877
#11 (parasitoid abundance, Plant quality)	{herbivore richness, herbivore abundance, nitrogen}, P = 0.025
#12 (parasitoid abundance, nitrogen)	{herbivore richness, herbivore abund,, resource avail.}, P = 0.330
#13 (parasitoid abundance, resource availability)	{herbivore richness, herbivore abund., resource avail.}, P = 0.044
#14 (parasitoid abundance, herbivore composition)	{herbivore richness,herbivore abundance, temperature}, P = 0.379

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#15 (parasitoid richness, herbivore abundance)       {parasitoid abund., herb. rich., resource avail.,temp.}, P = 0.111
#16 (parasitoid richness, nitrogen)                  {parasitoid abundance, herbivore richness, nitrogen}, P = 0.714
#17 (parasitoid richness, Plant quality)             {parasitoid abundance, herbivore richness, nitrogen}, P = 0.746
#18 (parasitoid richness, temperature)               {parasitoid abundance, herbivore richness}, P = 0.266
#19 (parasitoid richness, resource availability)     {parasitoid abundance, herbivore richness}, P = 0.859
# 20 (parasitoid composition, parasitoid richness)   {para. abund., herb. rich., herb. abund., herb. comp.}, P = 0.294
# 21 (parasitoid composition, herbivore richness)    {parasitoid abund, herbivore abund, herbivore comp}, P = 0.055
# 22 (parasitoid composition, Plant quality)         {herb comp, herb abund, parasitoid abunde, nitrogen}, P = 0.483
# 23 (parasitoid composition, resource availability) {herb comp, herb abund, para abund, nitrogen, temp}, P = 0.450
# 24 (parasitoid composition, nitrogen)              {herb comp, herbivore abundance, parasitoid abundance}, P = 0.483
# 25 (parasitoid composition, temperature)           {herbivore composition, herbivore abundance, para abund) P = 0.623
# 26 (Vulnerability, Generality)                     {parasitoid composition, temperature}, P = 0.252
# 27 (Vulnerability, herbivore composition)          {parasitoid composition, temp, herbivore richness}, P = 0.076
# 28 (Vulnerability, parasitoid richness)            {parasitoid comp, parasitoid rich, herbivore richness}, P = 0.079
# 29 (Vulnerability, parasitoid abundance)           {parasitoid comp, temp, herb abund, herbi richness}, P = 259
# 30 (Vulnerability, herbivore richness)             {parasitoid composition, herbivore abundance}, P = 0.476
# 31 (Vulnerability, herbivore abundance)            {parasitoid comp=, temp, plant qual, resource avail}, P = 0.323
# 32 (Vulnerability, resource availability)          {parasitoid composition, temperature, nitrogen}, P = 0.035
# 33 (Vulnerability, nitrogen)                       {parasitoid composition}, P = 0.735
# 34 (Vulnerability, temperature)                    {parasitoid composition}, P = 0.361
# 35 (Generality, Connectance)                       {temperature, herbivore composition}, P = 0.661
# 36 (Generality, parasitoid composition)            {temp, herb abund, parasitoid abund, herbivore comp}, P = 0.739
# 37 (Generality, parasitoid abundance)              {temp, herb richs, herb abund, parasitoid abundance}, P = 0.505
# 38 (Generality, parasitoid richness)               {temp, herb rich, para abund, herbivore composition}, P = 0.526
# 39 (Generality, herbivore richness)                {temperature, herbivore abundance}, P = 0.573
# 40 (Generality, herbivore abundance)               {temperature, resource availability, Plant quality}, P = 0.987
# 41 (Generality, Plant quality)                     {temperature, nitrogen}, P = 0.546
# 42 (Generality, nitrogen)                          {temperature}, P = 0.612
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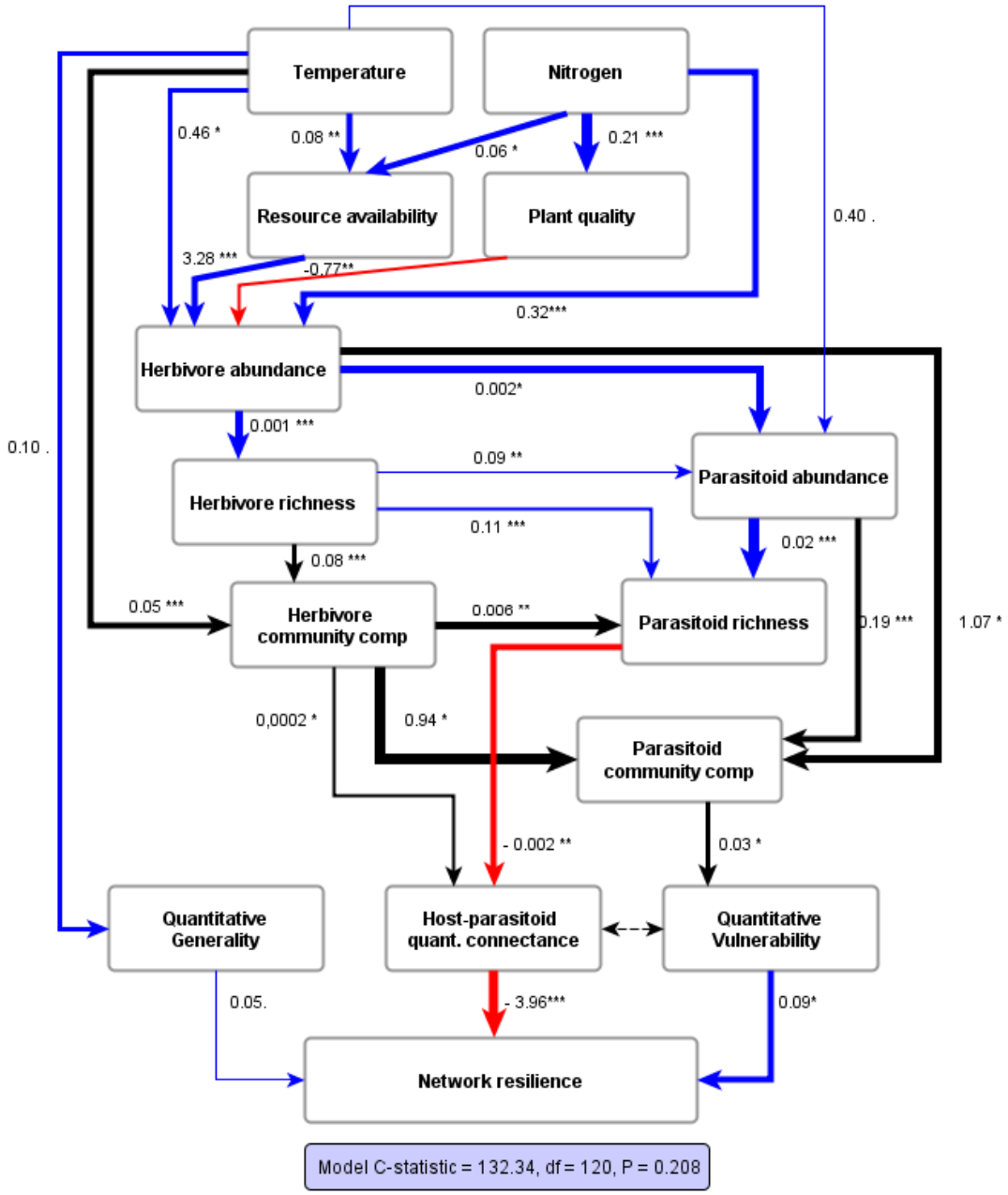
# 43 (Connectance, parasitoid composition)          {para rich, herb comp, para abund, herb abundance}, P = 0.276
# 44 (Connectance, parasitoid abundance)            {para rich, herbe comp, temp, herb abund, herb rich}, P = 0.195
# 45 (Connectance, herbivore richness)              {para rich, herb comp, herbivore abundance}, P = 0.465
# 46 (Connectance, herbivore abundance)             {para rich, herb comp, res avail, plant qual, temp}, P = 0.904
# 47 (Connectance, Plant quality)                   {parasitoid richness, herbivore composition, nitrogen}, P = 0.090
# 48 (Connectance, resource availability)           {parasitoid richness, herbivore composition, nitrogen}, P = 0.144
# 49 (Connectance, nitrogen)                        {parasitoid richness, herbivore composition}, P = 0.758
# 50 (Connectance, temperature)                     {parasitoid richness, herbivore composition}, P = 0.528
# 51 (Connectance, Vulnerability)                   {parasitoid richness, herbivore composition}, P = 0.017
```

Appendix 3.3: Path analysis diagrams

A)



B)

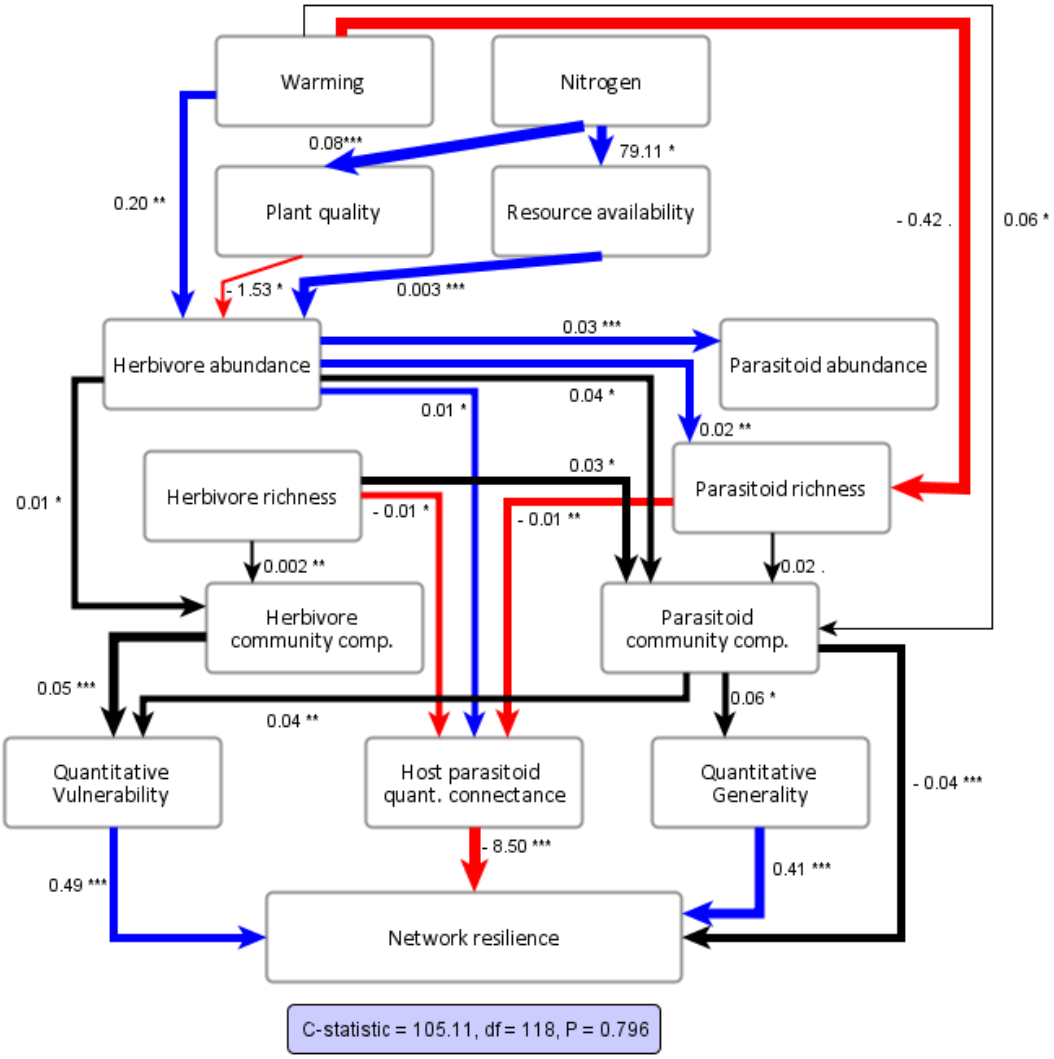


Figure S9: Multilevel path analysis (Shipley 2009) diagram for the A) altitudinal gradient experiment and b) artificial warming experiment. Arrows indicate significant causal effect, blue indicates a positive effect, red a negative effect and black a multivariate effect. Numbers represent unstandardised path coefficients, whilst arrow width is scaled to the standardized path coefficient. Asterisks indicate levels of significance: *P.* < 0.1, *P < 0.05, **P < 0.001, ***P < 0.0001

Literature cited in Appendix 3

Shipley, B. 2009. Confirmatory path analysis in a generalized multilevel context. Ecology **90**:363–368.