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

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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 the direct effect of rising temperatures on insects. 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 globally increasing 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.

Introduction

Global environmental changes triggered by human activities are affecting all ecosystems on Earth, and understanding their consequences for communities of
organisms 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). 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, Nuffio 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 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. Understanding the generality of herbivore phenological responses to climate will be critical for predicting pest outbreaks. 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. 1997, M.E.A 2005), has a vast array of effects on plants, generally promoting higher biomass, affecting competition (Reich et al. 2006), 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?
Material and Methods

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). 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.

**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) 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 in Appendix 1). The total temperature gradient across all plots (the average temperature in each plot over the entire period of data recording ranged from 3.89 to 6.72 °C) amounted to 2.83 °C. This temperature gradient falls within the range of temperature increases predicted for the region 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 such as oxygen availability and radiation; Hodkinson 2005). Local topography may create significant microclimatic variation, which could modify insect performance over short vertical distances that override the more general altitudinal trends (Weiss et al. 1988). 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. 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). The N-fertilisation treatment comprised a total application of 50 Kg ha$^{-1}$ yr$^{-1}$, (see Nitrogen treatment application in Appendix 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).

Vegetation survey

In December 2009, we carried out a vegetation survey of each 12 x 12m subplot, using the percent cover (PC) method described (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).

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 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 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 tested all models entering temperature first followed by elevation. However, we also ran all the models with inverted order 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, 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 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 transect 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 alongside the drivers to find the best-fitting model.

In these models, we used a Poisson error for abundance and species richness data

and a Gaussian error 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 2).

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 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 ($Z = -5.11$, $P < 0.0001$) 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 ($t = 4.86$, $P_{\text{MCMC}} = 0.0001$). Shifts in composition were not uniform across sites, which prevented homogenization of the plant community (test for homogeneity of multivariate dispersion: Temperature: $F_{2,12} = 0.07$, $P = 0.925$; Nitrogen: $F_{1,28} = 0.35$, $P = 0.586$).

Nitrogen fertilization did not significantly affect the 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 ($N: 30.34% \pm 3.21, C: 23.67 \pm 3.9$, $t = 3.34$, $P_{\text{MCMC}} = 0.02$). Additionally, nitrogen increased the proportion of green leaf relative to dead-standing brown leaf ($t = 5.12$, $P_{\text{MCMC}} = 0.0001$), 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 < 0.0001), confirming that the fertilization treatment affected plant nitrogen content, and could therefore potentially affect herbivores.

**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 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: $Z = 5.11$, $P < 0.0001$).

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 3 and Figure S3 in Appendix 4). Nitrogen addition and the temperature by nitrogen interaction did not significantly affect community dispersion ($P > 0.05$ in both cases).

**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 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: $|Z| > 2.1$, $P < 0.05$ in all cases), and increased plant quality (leaf nitrogen: $Z = 5.44$, $P < 0.0001$) caused by nitrogen addition.

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; coefficients Tables S4, S5 in Appendix 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) and biomass (Tables S6-S9 in Appendix 5). Nitrogen addition was also associated with higher larval abundances, and this effect became stronger through time.
The effect of the two drivers in combination was less than additive (negative N x temperature interaction; Appendix 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 5 and Appendix 6).

For the three most common species, 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 in isolation, though with varying magnitude (Appendix 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 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 8).

**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 cold plots; $t = 5.98$, $P_{MCMC} = 0.0001$) 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 ($|t| > 2.7$, $P_{MCMC} < 0.05$ 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 ($t = 5.14$, $P_{MCMC} = 0.0001$).

**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 comprised solely 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 to positive 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 response of individual species to the interacting drivers likely blurred the trend 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
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 organisation, can we fully understand how current and future environmental changes are likely to affect biodiversity, ecosystem functioning and community stability.

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**Figure. 1:** Flow diagram of the study system, showing the pathways through which temperature and nitrogen deposition can 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. Note that these symbols represent the driver x time interaction effect in the “Herbivore phenology” compartment. 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 (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.
Figure 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.
Plant composition
- Species composition:
  - Native plant richness
  - Invasive plant richness
  - Total plant richness

Plant quantity
- Tussock availability (green tussock)
- Non-native grass (relative abundance)

Plant quality
- Leaf nitrogen

Herbivore assemblage
- Biotic homogenisation
  - Species relative abundance
  - Species richness
  - Total abundance
  - Total biomass

Herbivore phenology
- (drivers x time interactions)
  - Assemblage phenology
  - Species development
  - Abundance through time
  - Biomass through time

Temperature

Nitrogen

Key:
- ■ = positive effect
- □ = negative effect
- ▲ = multivariate effect
- △ = Temperature x Nitrogen interaction

1. Temperature
2. Nitrogen
3. Plant quantity
4. Plant quality
5. Plant composition
6. Herbivore assemblage
7. Herbivore phenology
8. Plant composition
9. Plant quality
10. Plant quantity
11. Herbivore assemblage
Mean total counts +/- SE

- Warm
- Moderate
- Cold