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

3  
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12  
13 **Abstract**

14  
15 Warmer temperatures can alter the phenology and distribution of individual species.  
16 However, differences across species may blur community-level phenological responses to  
17 climate or cause biotic homogenization by consistently favoring certain taxa.  
18 Additionally, the response of insect communities to climate will be subject to plant-  
19 mediated effects, which may or may not overshadow the direct effect of rising  
20 temperatures on insects. Finally, recent evidence for the importance of interaction effects  
21 between global change drivers suggests that phenological responses of communities to  
22 climate may be altered by other drivers. We used a natural temperature gradient  
23 (generated by elevation and topology), combined with experimental nitrogen fertilization,

24 to investigate the effects of elevated temperature and globally increasing anthropogenic  
 25 nitrogen deposition on the structure and phenology of a semi-natural grassland herbivore  
 26 assemblage (lepidopteran insects).

27 We found that both drivers, alone and in combination, severely altered how the  
 28 relative abundance and composition of species changed through time. Importantly,  
 29 warmer temperatures were associated with biotic homogenization, such that herbivore  
 30 assemblages in the warmest plots had more similar species composition than those in  
 31 intermediate or cool plots. Changes in herbivore composition and abundance were largely  
 32 mediated by changes in the plant community, with increased non-native grass cover  
 33 under high treatment levels being the strongest determinant of herbivore abundance. In  
 34 addition to compositional changes, total herbivore biomass more than doubled under  
 35 elevated nitrogen and increased more than four-fold with temperature, bearing important  
 36 functional implications for herbivores as consumers and as a prey resource. The crucial  
 37 role of non-native plant dominance in mediating responses of herbivores to change,  
 38 combined with the frequent non-additive (positive and negative) effects of the two  
 39 drivers, and the differential responses of species, highlights that understanding complex  
 40 ecosystem responses will benefit from multi-factor, multi-trophic experiments at  
 41 community scales or larger.

42

43 **Introduction**

44

45 Global environmental changes triggered by human activities are affecting all  
 46 ecosystems on Earth, and understanding their consequences for communities of

47 organisms is a major challenge. Numerous studies have revealed effects of climate  
 48 change on the distribution of different taxa (Parmesan et al. 1999, Walther et al. 2002,  
 49 Hickling et al. 2006), often underpinned by range shifts (Wilson et al. 2005). Different  
 50 rates of range expansion and/or contraction by different species, coupled with differential  
 51 performance of species, can alter the organization of communities (Parmesan and Yohe  
 52 2003, Yang et al. 2011). Consequently, a subset of species (those with a wide thermal  
 53 tolerance or an ability to exploit temperature-driven resource shifts) are likely to become  
 54 more dominant within their native communities, and also to expand their ranges. If this  
 55 subset is consistent across locations, their increasing range and competitive ability could  
 56 drive biotic homogenization (increasing similarity of communities from different  
 57 locations; (Olden et al. 2004)), with important consequences for ecosystem stability and  
 58 functioning (Loreau et al. 2003). Recent studies have revealed the effects of warmer  
 59 temperatures on temporal distributions of species, though species within the same  
 60 community may show variable phenological responses to climate change (Primack et al.  
 61 2009, Nufio et al. 2010).

62 Different phenological responses to climate change across functional groups and  
 63 trophic levels may disrupt crucial biotic interactions, and thereby percolate widely  
 64 through ecological communities (Harrington et al. 1999, Tylianakis et al. 2008, Both et  
 65 al. 2009). In the case of insect herbivores, it is well documented that changes in plant  
 66 quality and composition can significantly alter herbivore life history, performance and  
 67 host-plant choice (Awmack and Leather 2002, Morrison and Hay 2011). However,  
 68 consumer-resource synchrony has a major impact on the population densities of  
 69 herbivores such as leaf-feeding Lepidoptera, and years with high plant-herbivore

70 synchrony may result in outbreaks of herbivorous insects (van Asch and Visser 2007). On  
 71 the other hand, asynchrony of insect activity with plant resources can determine the  
 72 magnitude of impact of herbivores on their host plant populations (Russell and Louda  
 73 2004), and alter insect population dynamics (Wallisdevries and Van Swaay 2006) to  
 74 cause shifts in dominance of species and higher taxonomic groups (Richardson et al.  
 75 2002, Tylianakis et al. 2008). Therefore, the response of consumers to global change  
 76 drivers is a complex combination of their direct response and the indirect bottom-up  
 77 effect of drivers on resources, such that the net outcome can be difficult to estimate with  
 78 single-trophic-level studies. Understanding the generality of herbivore phenological  
 79 responses to climate will be critical for predicting pest outbreaks. Thus, there is a need to  
 80 address biotic responses to global change drivers such as climate within a community  
 81 context and at multiple trophic levels.

82 In addition to this growing emphasis on the need for community-scale data, there  
 83 has been increasing concern that the effect of individual global change drivers may not  
 84 reflect their synergistic effects in the real world. Recent evidence of complex interactions  
 85 among co-occurring drivers (Didham et al. 2007, Tylianakis et al. 2008, Forister et al.  
 86 2010) calls for the integration of multiple drivers in global change research. For instance,  
 87 nitrogen deposition, which is increasing rapidly worldwide (Vitousek et al. 1997, M.E.A  
 88 2005), has a vast array of effects on plants, generally promoting higher biomass, affecting  
 89 competition (Reich et al. 2006), and reducing biodiversity (Stevens et al. 2004). Changes  
 90 in basal plant resources are known to affect herbivore performance, which usually  
 91 responds positively to elevated nitrogen (Throop and Lerdau 2004). However, such  
 92 effects may need to be re-examined in the context of their interplay with temperature. For

93 example, Wallisdevries and van Swaay (2006) showed that excess nitrogen advanced  
 94 plant growth in the spring, thereby forcing herbivores to develop under colder conditions  
 95 and offsetting the thermal benefit of warming via a sub-additive warming by nitrogen  
 96 interaction. This and other studies (reviewed in Tylianakis *et al.* 2008) suggest that the  
 97 ability of species to respond phenologically to warming may be altered in the context of  
 98 other global change drivers acting simultaneously.

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

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

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

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

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

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

115

116 **Material and Methods**

117

118 *Study site*

119 We established our experiment in tussock grasslands of the Hope River Valley,  
 120 North Canterbury, New Zealand, which is located at the foothills of the Southern Alps,  
 121 and ranges from 600 to 1,700 m elevation (see Study Site in Appendix 1). Large amounts  
 122 of forest were cleared by early European settlers in the mid 1800's and later over sown  
 123 for pasture. These grasslands are now characterized by a mixture of native and non-native  
 124 flora (Barratt et al. 2005), with the native component largely comprising tussock grasses  
 125 that previously inhabited open areas (usually above the treeline) and the exotic  
 126 component being mainly pasture plants. A large proportion of New Zealand's insect  
 127 fauna is endemic (Myers et al. 2000, Barratt et al. 2005). In particular, the lepidopteran  
 128 fauna shows very high levels of endemism (White 1991); all 39 species identified in this  
 129 experiment were endemic, and were historically limited to the alpine grasslands above  
 130 tree line prior to forest clearing. Thus, their down-slope migration reflects each species'  
 131 ability to follow the range expansion of their habitat and persist under altered conditions,  
 132 rather than a historical preference of certain taxa for the climate below tree line. Of these  
 133 species, 37 are generalist grass (Poaceae) feeders, and are therefore not limited in their  
 134 range expansion by a specialist association with exclusively alpine plants. Similarly, host  
 135 plants of the two specialist species were also found below the tree line. Our experimental  
 136 plots are all situated below the natural tree line, thereby offering a comparison between  
 137 newly-generated communities that differ in climate, rather than between original alpine

138 vs. newly-created communities. Therefore, this represents an ideal system for climate-  
 139 change research.

140

141 *Experimental design*

142 As a climatic gradient, we used an elevation gradient as a ‘space for time  
 143 substitution’ (Pickett 1989, Hodkinson 2005). We established five vertical transects  
 144 (Figure S1 in Appendix 1) of three plots, each at 150 m intervals of elevation, such that  
 145 there was a total of 300 m difference in altitude between the lowest and the highest plot in  
 146 each transect (see Site locations and details in Appendix 1). The total temperature  
 147 gradient across all plots (the average temperature in each plot over the entire period of  
 148 data recording ranged from 3.89 to 6.72 °C) amounted to 2.83 °C. This temperature  
 149 gradient falls within the range of temperature increases predicted for the region within the  
 150 next 100 years (IPCC 2007). The topography of the area meant that temperature did not  
 151 vary consistently with elevation (i.e. some sites were slightly warmer or colder than  
 152 expected for their elevation). This allowed us to test the effects of temperature alongside  
 153 elevation (to account for other environmental variables that co-vary with such as oxygen  
 154 availability and radiation; Hodkinson 2005). Local topography may create significant  
 155 microclimatic variation, which could modify insect performance over short vertical  
 156 distances that override the more general altitudinal trends (Weiss et al. 1988). We used  
 157 the overall mean site temperature for the period February to December 2009 (during  
 158 which consistent data were available for all sites) as a covariate to elevation in the  
 159 analysis. Note, however, that analyses incorporated transect as a random (blocking)

160 factor, so any environmental differences among transects would not confound treatment  
 161 effects.

162 At each elevation, we established a 24 x 12 m sampling plot. We further subdivided  
 163 each plot into two 12 x 12 m subplots, and randomly assigned one of these to a nitrogen  
 164 addition treatment (addition or control with no added N). This resulted in a split-plot  
 165 design, with temperature varying at the scale of plots ( $n = 15$ ), blocked by transects ( $n =$   
 166  $5$ ), and N treatments applied to subplots ( $n = 30$ ) nested within plots (see Site locations  
 167 and details and Table S1 in Appendix 1). The N-fertilisation treatment comprised a total  
 168 application of  $50 \text{ Kg ha}^{-1} \text{ yr}^{-1}$ , (see Nitrogen treatment application in Appendix 1), which  
 169 falls within the current range of globally-observed rates of atmospheric deposition  
 170 (M.E.A. 2005).

171 Sampling of insects began in October 2008, and continued at monthly intervals  
 172 until December 2009. Sampling was interrupted over the winter period June-August 2009  
 173 when snow cover made the sites inaccessible. In April 2009, adverse weather also  
 174 prevented access to some sites due to river flooding. We completed a total of 11 sampling  
 175 rounds successfully.

176

177 To minimize disturbance and depletion of caterpillars in the experimental area, we  
 178 subdivided each 12 x 12 m subplot into 4 strips of 3 x 12 m each, and sequentially  
 179 sampled one strip only during each sampling round. This ensured a time window of at  
 180 least 4 months before re-sampling of the same section. This timeframe is substantially  
 181 longer than the average larval life stage of Lepidoptera, and therefore prevented bias in  
 182 the abundance of any sample caused by depletion from previous sampling rounds. Plant

183 searches for larvae involved thorough teasing apart of denser vegetation to locate any  
 184 hidden larvae. Morphospecies were validated as true species through identification of  
 185 reared adults or larval characteristics, so that 6143 caterpillars were identified  
 186 successfully. The adult identities were confirmed by lepidopteran taxonomist J.S.  
 187 Dugdale, who also provided support in developing diagnostic features for larval  
 188 identification (see Experimental sampling and rearing in Appendix 1).

189

190 *Vegetation survey*

191 In December 2009, we carried out a vegetation survey of each 12 x 12m subplot,  
 192 using the percent cover (PC) method described (Mueller-Dombois and Ellenberg 2003),  
 193 which provides an accurate estimation of plant cover and species composition. For each  
 194 subplot separately, percent cover data were transformed to relative abundances by  
 195 dividing the percent cover of each species by the sum of percent cover values for all  
 196 species present. As tussocks were the primary food plant for Lepidoptera larvae, we  
 197 determined tussock biomass by estimating their average size and abundance in each plot  
 198 (see Vegetation survey in Appendix 1).

199

200 *Data analysis*

201 We performed all analyses on plant and herbivore community composition and  
 202 phenology using permutational distance multivariate ANOVA, carried out with the  
 203 PRIMER V6 software and the PERMANOVA package (Clarke and Gorley 2006,  
 204 Anderson et al. 2008). We conducted two sets of analyses using two different  
 205 dissimilarity measures, one accounting for species composition and abundance (Modified

206 Gower base 10) and one focusing on species presence/absence (Jaccard dissimilarity, see  
 207 Dissimilarity measures in Appendix 2). For both plant and herbivore analyses, we  
 208 included nitrogen (control vs. elevated) and plant composition (see Plant composition in  
 209 the herbivore community composition analyses in Appendix 2) as fixed effects. We  
 210 included temperature as a covariate to elevation (low, mid, or high within each transect)  
 211 using Type I, sequential sums of squares, to test if there were any elevation effects (e.g.,  
 212 due to solar radiation) beyond those explained by temperature. We tested all models  
 213 entering temperature first followed by elevation. However, we also ran all the models  
 214 with inverted order and found no significant effect of elevation, which indicates that any  
 215 temperature effects were not confounded by other factors correlated with elevation.  
 216 Nevertheless, we retained elevation as a fixed factor, to be conservative when attributing  
 217 variance to the temperature covariate.

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

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

229 acting as the error term for testing the nitrogen effect, and finally subplots acting as the  
 230 error for the repeated sampling through time.

231 We tested for biotic homogenization of both plant and herbivore composition using  
 232 a permutational distance-based test for homogeneity of multivariate dispersions, based on  
 233 a modified Gower dissimilarity to account for both relative abundance and presence of  
 234 species (Anderson et al. 2006). This test compares community similarity within different  
 235 levels of a factor, in our case, among replicates of temperature and nitrogen treatments  
 236 (see Test for biotic homogenization in Appendix 2). Increasing similarity of replicates of  
 237 a given treatment would therefore indicate that the treatment selects consistently for the  
 238 same community composition.

239 To account for our split-plot design, we used generalized linear mixed effects  
 240 models for all remaining univariate analyses (Bolker et al. 2009), which were conducted  
 241 using the lme4 package (Bates and Maechler 2010) in R version 2.10.1 (R Development  
 242 Core Team 2009). These included plots nested in transect as random effects, and also  
 243 subplots nested in plots where repeated measures through time were being tested. To  
 244 ascertain the main determinants of change in plant community composition, we tested the  
 245 effect of the drivers and elevation on the proportion cover of exotic grasses (which are  
 246 known to be food plants for caterpillars), nitrogen leaf content, plant richness (native,  
 247 exotic and total) and tussock biomass.

248 To test for changes in herbivore phenology, we analysed larval abundance, biomass  
 249 and individual larval bodyweight through time, with elevation, nitrogen treatment and  
 250 time as fixed factors and temperature as a covariate to elevation. For analysis of  
 251 individual larval bodyweight, we also included species identity as a random factor,

252 crossed with the nested random factors (transect, plot, and subplot), to test how  
 253 bodyweight changed within each species in response to the drivers.  
 254 Overall herbivore species richness, total (summed) larval biomass and herbivore  
 255 abundance were tested with elevation, temperature and nitrogen as predictors, to test the  
 256 net effects of the drivers. In addition, to compare the direct vs. indirect effects of the  
 257 drivers, we then included plant composition, proportion of exotic grasses and tussock  
 258 biomass alongside the drivers to find the best-fitting model.

259 In these models, we used a Poisson error for abundance and species richness data  
 260 and a Gaussian error for biomass, individual bodyweight, and proportion cover data.  
 261 Proportion cover was arcsine square root transformed to meet the assumptions of  
 262 normality and homoscedasticity. We included all interactions between temperature,  
 263 nitrogen and time (where applicable) in the initial (maximal) model. Final simplified  
 264 models were then fitted using restricted maximum likelihood (REML), as recommended  
 265 by Bolker *et al.* (2009), and tested for overdispersion. Elevation was not significant in  
 266 any model (tested alongside temperature), and provided an inferior fit when models with  
 267 temperature were directly compared with models that included elevation instead.  
 268 Therefore, we removed elevation from the final models (see Mixed effects models and  
 269 Table S3 in Appendix 2).

270

271

272 **Results**

273

274 We found a suite of direct and plant-mediated effects of the drivers on the herbivore  
 275 assemblage and evidence of non-additive, interactive effects of the drivers on phenology  
 276 (Figure 1).

277

278 *Plant community response to the environmental change drivers:*

279 The multivariate analysis showed a strong effect of temperature and a more subtle  
 280 effect of nitrogen on the plant community. Temperature affected both species  
 281 composition and relative abundance ( $F_{1,13} = 3.40$ ,  $P = 0.002$ ) within the plant community.  
 282 Temperature was correlated with a reduction of native species richness ( $Z = -5.11$ ,  $P <$   
 283  $0.0001$ ) and an increase in exotic species ( $Z = 2.21$ ,  $P = 0.030$ ), which resulted in an  
 284 overall decrease in plant species richness ( $Z = -2.91$ ,  $P = 0.004$ ). This result was  
 285 supported by a strong positive effect of temperature on the relative proportion cover of  
 286 exotic grasses in the vegetation ( $t = 4.86$ ,  $P_{MCMC} = 0.0001$ ). Shifts in composition were not  
 287 uniform across sites, which prevented homogenization of the plant community (test for  
 288 homogeneity of multivariate dispersion: Temperature:  $F_{2,12} = 0.07$ ,  $P = 0.925$ ; Nitrogen:  
 289  $F_{1,28} = 0.35$ ,  $P = 0.586$ ).

290 Nitrogen fertilization did not significantly affect the overall plant composition or  
 291 species richness, but rather favored an increase in exotic grasses, which had a higher  
 292 proportion cover in the fertilized plots than in the controls (N:  $30.34\% \pm 3.21$ , C:  $23.67$   
 293  $\pm 3.9$ ,  $t = 3.34$ ,  $P_{MCMC} = 0.02$ ). Additionally, nitrogen increased the proportion of green leaf  
 294 relative to dead-standing brown leaf ( $t = 5.12$ ,  $P_{MCMC} = 0.0001$ ), thereby increasing the  
 295 biomass of live tussock available as a food source for herbivores. The nitrogen content of  
 296 tussock leaves was significantly higher in the nitrogen-addition plots (on average 20.7%

297 ( $\pm 4.2$  SE) higher,  $P < 0.0001$ ), confirming that the fertilization treatment affected plant  
 298 nitrogen content, and could therefore potentially affect herbivores.

299

300 *Herbivore assemblage response to global change drivers:*

301 We found effects of both temperature and nitrogen addition on herbivore  
 302 community structure. In particular, both drivers caused a shift in community composition,  
 303 altering the relative abundance and presence/absence (Jaccard dissimilarity) of larvae  
 304 from different species (Figure S2 and Tables S4 and S5 in Appendix 3). Total herbivore  
 305 species richness varied under the different treatments, but differences in species richness  
 306 were driven by the effect of the treatments on total abundance (sample size), which  
 307 affected richness, rather than a treatment effect on richness *per se* (sample size effect on  
 308 richness:  $Z = 5.11$ ,  $P < 0.0001$ ).

309 Warmer temperatures homogenised herbivore assemblages, such that they were  
 310 most similar to each other in the warmest plots from the different transects ( $F_{2,12} = 6.08$ ,  $P$   
 311  $= 0.015$ ), despite being further apart spatially than plots within each transect. However,  
 312 dispersion did not differ significantly between sites at moderate and coldest temperatures  
 313 (Figure S2 in Appendix 3 and Figure S3 in Appendix 4). Nitrogen addition and the  
 314 temperature by nitrogen interaction did not significantly affect community dispersion ( $P$   
 315  $> 0.05$  in both cases).

316

317 *Relative importance of direct vs. plant mediated effects*

318 We found strong collinearity between the effects of the global change drivers and  
 319 plant composition on herbivore community structure (see Appendix 3). Although this

320 strongly suggests that the effects of temperature and nitrogen on the herbivore  
 321 community may have been mediated via plant community shifts, we cannot objectively  
 322 attribute this shared variance to either predictor with certainty. Nevertheless, a significant  
 323 temperature by nitrogen interaction term present in all models after controlling for plant-  
 324 mediated effects indicated that temperature retained a direct effect on herbivore  
 325 community structure that was independent from its effect on plants, but was dependent on  
 326 nitrogen availability ( $F_{1,28} = 2.13$ ,  $P = 0.033$ ).

327 Changes in total herbivore abundance were largely associated with temperature-  
 328 correlated changes in plant composition, in particular an increase in cover of non-native  
 329 grasses (effects of plant composition, proportion exotic grasses and tussock biomass:  $|Z|$   
 330  $> 2.1$ ,  $P < 0.05$  in all cases), and increased plant quality (leaf nitrogen:  $Z = 5.44$ ,  $P <$   
 331  $0.0001$ ) caused by nitrogen addition.

332  
 333 *Phenology of herbivore assemblage and common species*

334 We found strong evidence for phenological effects of the drivers on herbivores at  
 335 the community scale. Temperature influenced herbivore community-compositional  
 336 change through time (positive temperature x time interaction; coefficients Tables S4, S5  
 337 in Appendix 3), such that temporal changes in community composition (i.e. community-  
 338 level phenological changes) were greater at higher temperatures, producing a temporally  
 339 more-variable community. Higher temperatures caused an earlier peak of larval  
 340 abundance by one month, and were associated with higher overall larval abundance  
 341 (Figure 2) and biomass (Tables S6-S9 in Appendix 5). Nitrogen addition was also  
 342 associated with higher larval abundances, and this effect became stronger through time

343 (positive N x time interaction; Tables S6 and S7 in Appendix 5). The effect of the two  
 344 drivers in combination was less than additive (negative N x temperature interaction;  
 345 Appendix 5), such that the effect of nitrogen was strongest in colder sites and weakened  
 346 with increasing temperature. Finally, the effect of temperature on the change in larval  
 347 abundance through time depended on nitrogen availability, indicating an interactive effect  
 348 of the two drivers on phenology (significant temperature x nitrogen x time interaction,  
 349 Tables S6 and S7 in Appendix 5 and Appendix 6).

350

351 For the three most common species, which were present at all sites, we were able to  
 352 test how abundance changed through time in response to the treatments. All three species  
 353 responded positively to both drivers in isolation, though with varying magnitude  
 354 (Appendix 7). Similarly, all three species showed a positive interaction between  
 355 temperature and time, indicating that phenological changes in abundance depended on  
 356 temperature. However, these three species showed different responses to the interaction  
 357 of the drivers (temperature x nitrogen), which ranged from negative to positive.

358 Consequently, their phenological response (i.e. change in abundance through time) to the  
 359 drivers in combination also ranged from negative to non significant or positive (Appendix  
 360 7). We found virtually identical results when analyzing the mean body mass of each larva  
 361 across the whole assemblage as an estimate of larval development rate (see Appendix 8).

362

### 363 *Total herbivore biomass*

364 Changes in abundance were reflected in the total biomass of the herbivore  
 365 assemblage. Total lepidopteran herbivore biomass responded positively to warmer

366 temperatures (414% increase in total biomass compared with cold plots;  $t = 5.98$ ,  $P_{\text{MCMC}} = 0.0001$ ) and nitrogen-richer conditions (267% increase in total biomass compared with  
 367 control plots;  $t = 2.38$ ,  $P_{\text{MCMC}} = 0.02$ ). After testing the direct effect of the drivers, we  
 368 included plant parameters to identify plant-mediated effects. Plant composition had a  
 369 significant influence on herbivore biomass ( $|t| > 2.7$ ,  $P_{\text{MCMC}} < 0.05$  for the first two PCA  
 370 axes); in contrast with the results on abundance, plant composition did not overshadow  
 371 the significance of temperature, but absorbed the effect of nitrogen. In particular, the  
 372 availability of green tussock biomass ( $t = 4.18$ ,  $P_{\text{MCMC}} = 0.0016$ ) and the proportion of  
 373 non-native grasses to other plants ( $t = 2.81$ ,  $P_{\text{MCMC}} = 0.02$ ) best explained herbivore  
 374 biomass, alongside a strong direct effect of temperature ( $t = 5.14$ ,  $P_{\text{MCMC}} = 0.0001$ ).

376

377 **Discussion**

378

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

389 replacement or altered dominance within the herbivore assemblage, rather than changes  
 390 in the number of species *per se*.

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

410 We found that temperature significantly altered phenology at the community scale,  
 411 advancing the time of peak abundance for individual species, increasing their peak

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

433         In the face of rising temperatures, a major concern is how changes to the timing of  
 434 biological events will affect overall ecosystem functioning and resilience (Edwards and

435 Richardson 2004). Abundance and biomass changes through time were affected by  
 436 temperature and nitrogen, as a consequence of increased dominance by a few species and  
 437 earlier development of the whole community with warmer temperatures. These results  
 438 carry important implications for herbivores as both consumers and prey, as several  
 439 studies have revealed decoupling of consumer-resource dynamics following climate  
 440 change (Memmott et al. 2007, Tylianakis et al. 2008, Both et al. 2009). Trophic mismatch  
 441 between herbivores and their natural enemies could lead to important cascading effects  
 442 on herbivory (Stireman et al. 2005), and studies of such mismatch at a community level  
 443 are needed.

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

454 Plant-mediated effects on herbivore communities could arise through a number of  
 455 pathways. Changes in plant availability and quality are known to be exploited differently  
 456 by different herbivore species (Awmack and Leather 2002), potentially leading to shifts  
 457 in herbivore dominance and abundance as we observed. Beyond the simple increase in

458 resource availability, herbivores may also benefit from access to the different nutritional  
 459 content of different plants. Additionally, naïve non-native plants may lack appropriate  
 460 defense mechanisms against local herbivores (Parker et al. 2006, Morrison and Hay  
 461 2011). Alternatively, altered community-wide plant phenology could extend the overall  
 462 availability of plants as a food resource through time, favoring particular species that  
 463 develop at the extremes of the growing season, and therefore contributing to changes in  
 464 herbivore assemblage and its temporal dynamics.

465         With this study, we showed that warming and nitrogen directly affected the  
 466 organization of herbivore communities and their phenology, and promoted the  
 467 establishment of simplified, more homogenous communities even without affecting alpha  
 468 diversity. These results highlight the importance of empirical studies at the community  
 469 level, rather than a species-by-species approach, since individual species can respond in  
 470 idiosyncratic ways that do not reflect average community-wide responses. Furthermore,  
 471 we demonstrated that plant-mediated effects can strongly contribute to overall changes in  
 472 herbivore abundance, species dominance and biomass, in addition to the direct effects of  
 473 the drivers. Understanding the relative importance of different effect pathways is crucial  
 474 to global change research, with particular relevance to predicting herbivore outbreaks.  
 475 Furthermore, the combination of two drivers (temperature and nitrogen) caused frequent,  
 476 non-additive interactions that affected the response of community structure and  
 477 phenology to either driver on its own. This contributes rare empirical evidence of real-  
 478 world responses of natural systems to interacting global environmental changes, which  
 479 has been highlighted as a necessary challenge for ecology (Didham *et al.* 2007;  
 480 Tylianakis *et al.* 2008). Studies of single drivers would not have generated an adequate

481 understanding of the community responses we observed, nor could these have been  
 482 predicted from the known effects of temperature (Bale et al. 2002) and nitrogen (Throop  
 483 and Lerdau 2004) in isolation on herbivore performance. Only by scaling up our  
 484 understanding of changes from species to higher levels of organisation, can we fully  
 485 understand how current and future environmental changes are likely to affect  
 486 biodiversity, ecosystem functioning and community stability.

487

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489

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498

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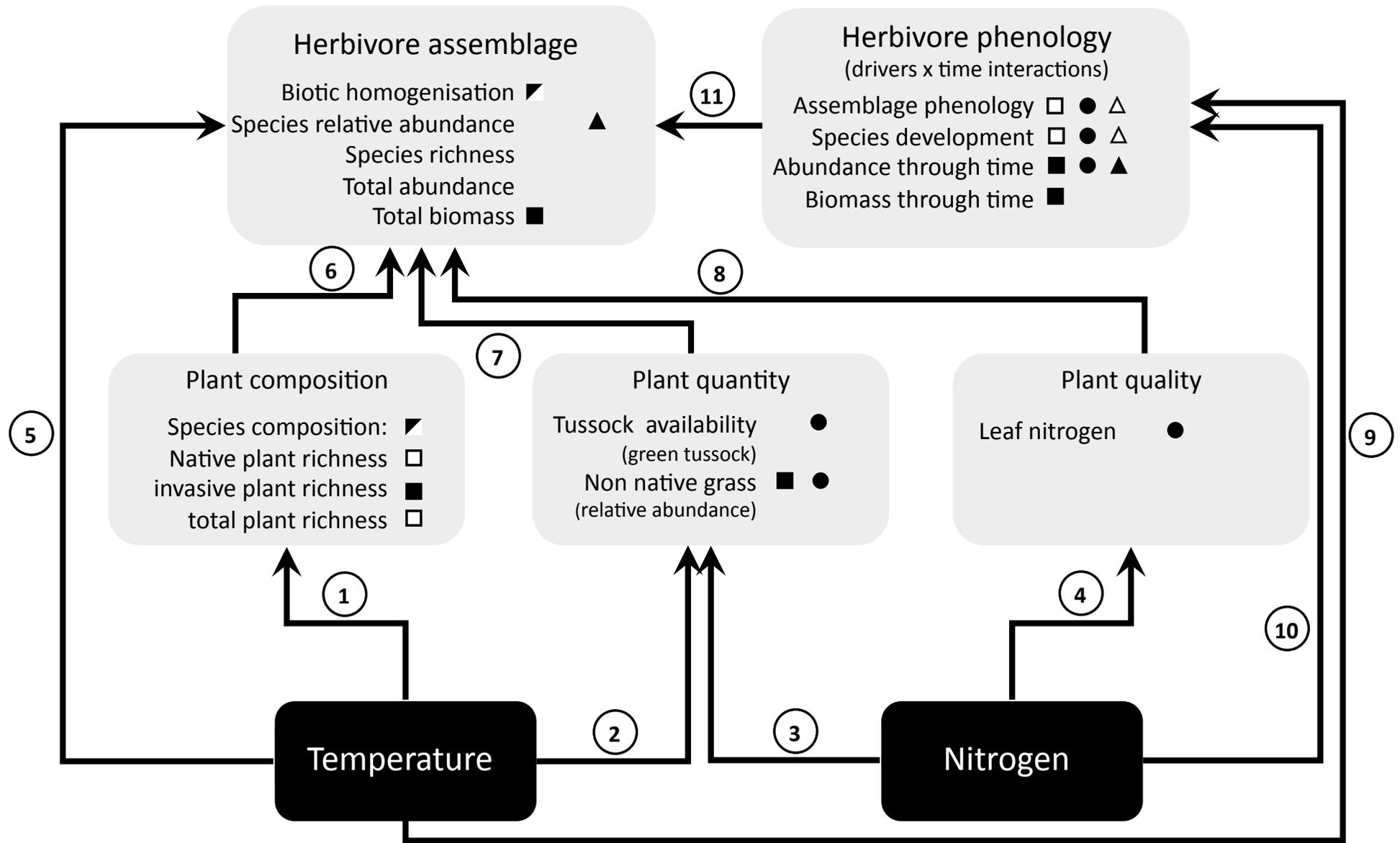
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1 **Figure. 1:** Flow diagram of the study system, showing the pathways through which  
 2 temperature and nitrogen deposition can affect the herbivore community. Arrows indicate  
 3 direct effects on different community variables (shaded areas).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

22  
 23

24 **Figure 2:** Phenological response of the community to temperature, shown as mean (+/-  
25 SE) total abundance (counts) of caterpillars through time (in months). For visual clarity,  
26 plots are grouped into three temperature categories, though analyses treated temperature  
27 as a continuous predictor.

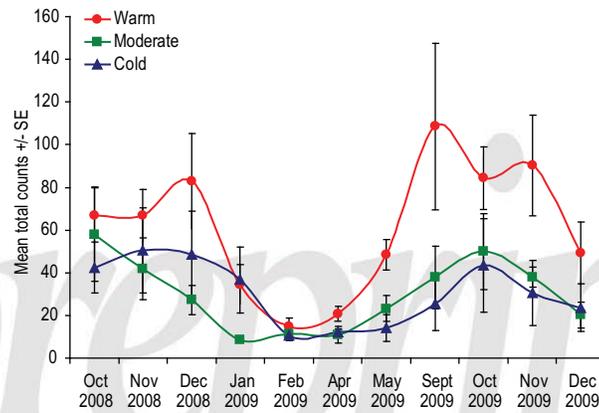
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■ = positive effect  
 □ = negative effect  
 ▴ = multivariate effect

□ = Temperature  
 ○ = Nitrogen  
 △ = Temperature x Nitrogen interaction

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