Warming affects different components of plant–herbivore interaction in a simplified community but not net interaction strength

Helena Van De Velde, Ivan Nijs and Dries Bonte

Global warming impacts natural communities through effects on performance of individual species and through changes in the strength of interactions between them. While there is a body of evidence of the former, we lack experimental evidence on potential changes in interaction strengths. Knowledge about multispecies interactions is fundamental to understand the regulation of biodiversity and the impact of climate change on communities. This study investigated the effect of warming on a simplified community consisting of three species: rosy apple aphid *Dysaphis plantaginea* feeding on plantain, *Plantago lanceolata*, and a heterospecific neighbouring plant species, perennial ryegrass, *Lolium perenne*. The aphid does not feed on *L. perenne*. The experimental design consisted of monocultures and mixtures of *L. perenne* and *P. lanceolata* at three temperature levels. We did not find indication for indirect temperature effects on *D. plantaginea* through changes in leaf nitrogen or relative water content. However, experimental warming affected the life history traits of the aphid directly, in a non-linear manner. Aphids performed best at moderate warming, where they grew faster and had a shorter generation time. In spite of the increased population growth of the aphids under warming, the herbivory rates were not changed and consequently the plant-herbivore interaction was not altered under warming. This suggests reduced consumption rates at higher temperature. Also plant competition affected the aphids but through an interaction with temperature. We provide proof-of-concept that net interactions between plants and herbivores should not change under warming despite direct effects of warming on herbivores when plant-plant interaction are considered. Our study stresses the importance of indirect non-trophic interactions as an additional layer of complexity to improve our understanding of how trophic interactions will alter under climate change.

The global mean air temperature is expected to increase as a result of rising levels of atmospheric CO₂ and other greenhouse gases (IPCC 2014). Numerous studies provide evidence for effects of anthropogenic warming on biota but most of them have concentrated on the level of individuals and species. For example, temperature is the dominant abiotic factor for poikilothermic animals, such as insects, which do not have physiological mechanisms for regulating their internal temperature. Therefore, warming has the potential to affect most life history parameters of terrestrial insects. Studies have revealed that warming shortens development time (Bale et al. 2002) and increases fecundity (Meisner et al. 2014) of insect herbivores until some threshold. Temperature also regulates plant productivity but in a non-linear fashion; warming can stimulate plant biomass production via higher photosynthesis and/or mineralization rates (Rustad et al. 2001, Wu et al. 2011), but retards it via associated drought and heat stress (De Boeck et al. 2008, Sherry et al. 2008). While such influences of warming at the single species level are fairly well understood, in nature species are connected in complex networks, therefore interactions such as competition and herbivory need to be considered.

The effect of temperature on life history processes (e.g. development, growth, reproduction, mortality) can be described by the thermal response curve, usually an asymmetric parabola (Huey and Kingsolver 1989, Logan et al. 1976). The curves may differ between species due to different levels of performance of the response, different rates of response or different peak or optimal temperatures (Dell et al. 2014). Such asymmetries in the thermal responses of interacting species can subsequently induce qualitative and quantitative changes in consumer–resource dynamics, with important consequences for the dynamics and persistence of populations and communities (Dell et al. 2014). For instance, the growth rate of insect herbivores responds more strongly to temperature than the growth rate of plants (Bale et al. 2002, Berg et al. 2010). Therefore, theory predicts that both herbivore consumption rates and fitness increase exponentially with increased temperature (O’Connor et al. 2011). However, experimental studies have reported that
rising temperatures may have highly variable effects on insect herbivory; for example: increased herbivory in warmed plots in the field (de Sassi and Tylianakis 2012, Liu et al. 2011, Roy et al. 2004) and in the lab (Kukal and Dawson 1989, O’Connor 2009), neutral effect of warming on herbivory (Richardson et al. 2002) and even decreased herbivory with warming (Burt et al. 2014). Over short timescales, warming may therefore destabilize community dynamics by increasing or decreasing feeding rates.

It is well-known that neighbouring plants affect the herbivore damage to a focal plant (Barbosa et al. 2009, Root 1973, Underwood et al. 2014). Neighbours can either increase (associational susceptibility) or decrease (associative resistance) herbivore attraction (Tahvanainen and Root 1972). Also the relative frequency of plant species in the neighbourhood and plant density can affect the plant–herbivore interaction. The density of conspecific neighbours, for example, can both increase or decrease herbivore load and feeding behaviour; these are referred to as resource concentration effects (Root 1973) or dilution effects (Otway et al. 2005), respectively. Hence, warming can indirectly influence plant–herbivore interactions via effects on neighbouring plants and these indirect effects of warming may enhance or counteract the direct effects.

While the impact of climate change at the single species level is clear, the impact at the community level requires further investigation because results from single-species experiments have to be scaled up to understand the effects of climate change on community composition and ecosystem functioning. Therefore, community-scale experiments are needed, preferably with multiple trophic levels. This study investigated the effect of warming on a simple community consisting of three species: rosy apple aphid Dysaphis plantaginea (Hemiptera: Aphididae) feeding on plantain, Plantago lanceolata, and a heterospecific neighbour plant species, perennial rye grass, Lolium perenne. The aphid does not feed on L. perenne. The experimental design consisted of monocultures and mixtures of L. perenne and P. lanceolata at three temperatures levels. Plantago lanceolata plants were subjected to herbivory by the aphid D. plantaginea. Our goals were to investigate the effects of warming on each of the species and on the interactions between them.

**Material and methods**

**Study species**

The rosy apple aphid *Dysaphis plantaginea* is an important apple pest in Europe and North America. *Dysaphis plantaginea* overwinters as eggs on apple trees, the primary host plant, and migrates in spring to the obligate alternate host, *Plantago lanceolata* (Alford 2014). On *Plantago spp.*, they give birth to apterous (wingless) morphs that reproduce by parthenogenesis (Blommers et al. 2004). Laboratory cultures of *D. plantaginea* were established for several years from individuals originating from a wild population in Avignon, France. The aphids were reared in small cages on *P. lanceolata* under laboratory conditions of 22 ± 1°C.

We used two common grassland species, *L. perenne*, a perennial hemicyryptophyte that grows in dense tussocks (Beddows 1967), and *P. lanceolata*, a rosette-forming perennial forb (Sagar and Harper 1964). Both species originate from a wild population in England. *L. perenne* is not a host plant for *D. plantaginea*.

**Experimental setup**

*Plantago lanceolata* and *L. perenne* were grown from seed on greenhouse benches under controlled laboratory conditions (16 h daylight : 8 h darkness and 22 ± 1°C) and isolated from aphid infestations. The species were sown with a time lag of one week to prevent differences in size at the start of the experiment (Cortufo and Gorissen 1997) due to differences in germination rate. Two or three week-old seedlings were transplanted into 1.5 l pots, filled with sandy soil (93.2% sand, 4.6% silt, 2.2% clay; field capacity 0.13 m³ m⁻³; pH 7.6; Kjeldahl-N 0.42 g kg⁻¹; 1% C in humus). The pots were randomly placed in environmentally controlled growth chambers, with three chambers for each of the three temperature treatments: 17°C, 20°C, and 23°C. Temperatures were chosen to reflect the range of potential increase in the next century, with the lowest temperature corresponding to the average temperature of a summer day in Belgium. Each temperature treatment consisted of 25 plant communities (pots) with three different plant compositions: 1) 5 monocultures of *L. perenne*, 2) 10 monocultures of *P. lanceolata*, and 3) 10 mixtures of both plant species in a 50:50 ratio. Each community contained four individuals because we chose a replacement design to study the effect of interspecific competition on plant–herbivore interaction under warming. The plants were watered every two days according to the 10-year average of 14–15 raining days per month during the growing season. The quantities of water supplied to the pots (65 ± 5 ml) were calculated from the amount of rainfall during the summer months in Ghent. All pots received the same amounts of water so that any enhanced consumption of water would result in soil drought. All communities were fertilized with 10 g m⁻² NH₄NO₃, 5 g m⁻² P₂O₅, 10 g m⁻² K₂O and micro-elements (Fe, Mn, Zn, Cu, B, Mo). The fertilizer was given dissolved in water in four equal amounts.

Plants received artificial light, with 16 h daylight : 8 h darkness photoperiod regime. In order to compensate for potential light differences within and between chambers, plants were rotated weekly between all chambers and plant positions within chambers were simultaneously randomized. During infestation, all pots were individually enclosed with a 40 cm-tall transparent plastic cylinder covered with a lightweight netting to ensure aphids did not migrate between pots. This infrastructure did not appear to physically limit plant growth.

We controlled for temperature effects on the initial biomass production of both plant species by exposing the monocultures and mixtures of the three temperature treatments to the same number of growing degree days before the start of the infestation. We preferred to simulate synchrony between the phenology of the herbivore and its host rather than an ecologically mismatched interaction, i.e. an induced asymmetry between plant and aphid biomass at the onset of
the experiment. The aphids were introduced on _P. lanceolata_ plants 1508 growing degree days from the start of the experiment in each temperature treatment. Growing degree days were calculated from the temperature of the chambers using the Baskerville and Emin (1969) method, applying a base growth temperature (the threshold temperature below which the rate of development is considered to be insignificant) of 4°C (Grant 1968). In each temperature treatment, five monocultures of _P. lanceolata_ and five mixtures were randomly chosen for aphid infestation. At the start of the infestation, three adult, apterous aphids were placed with a dry paintbrush on the apex of each _P. lanceolata_ plant in monocultures and mixtures. Consequently, at the start of the infestation each pot contained 12 (monocultures) or 6 (mixtures) aphids. Pots that did not receive aphids acted as control pots.

### Data collection

Aphid populations were counted daily. When the population on the monocultures had reached 300 aphids on average, the aphids were collected three days later. All remaining aphids were transferred to 70% ethanol and counted under a stereomicroscope to determine the final numbers. After counting, the aphid population from each pot was dried at 70°C for 48 h and weighed. The critical number of aphids in monocultures matched the threshold value for dispersal of aphids when plant conditions are sub-optimal (Dixon 1998). We thus terminated the experiment before the aphid populations would crash, to avoid compromising the measurement of plant responses.

We wanted to examine whether the recovery from an aphid infestation differed as a function of temperature using chlorophyll a fluorescence measurements. Therefore, all plants were harvested after a recovery period of 10 days. During the harvest, aboveground parts were separated from belowground parts and live from dead biomass by species. Root and shoot were weighted fresh. We could not separate the roots of _L. perenne_ and _P. lanceolata_, therefore only the belowground biomass of the monocultures was measured. All plant material was dried at 70°C for 48 h, and weighed again. The relative water content of the shoots was calculated as the difference between fresh and dry weight divided by the fresh weight. For statistical analysis, the sum of aboveground biomass per species was divided by the number of plants of that species in each pot. Total leaf area of _P. lanceolata_ in control pots was determined with a portable area meter. _Plantago lanceolata_ plants in control pots were ground in a mill, and three subsamples of each pot were analyzed for nitrogen content using an organic element analyser.

Chlorophyll a fluorescence, which can detect photosynthetic stress effects prior to visible leaf damage (Lichtenhaler and Miehe 1997), was measured on the youngest fully expanded leaf of each plant species per pot. These measurements were performed prior to and after the infestation and at the end of the experiment. Readings were taken at the start of the daylight regime on 30-min dark-acclimated leaves with a plant efficiency analyzer, on the same day for all treatments. Maximum quantum yield of photosystem II was calculated as $F_v/F_m = (F_m - F_o)/F_m$, where $F_v$ = variable fluorescence, $F_m$ = maximum fluorescence and $F_o$ = steady state fluorescence.

### Data analysis

To investigate the effect of a neighbouring plant species and warming on the plant-herbivore interaction, we fitted a structural equation model (SEM) (Grace 2006, Lamb et al. 2011) using the lavaan library in R (Rosseel 2012, <www.r-project.org>). The response of individual aphids was measured as the generation time and the response of the population as the number of aphids at the population peak. We hypothesized that (Fig. 1A):

- warming shortens the individual generation time of aphids. Shortening of generation time with increasing temperature is expected to enhance the growth rate of the population.
- warming decreases the leaf nitrogen and water content (An et al. 2005, Flynn et al. 2006, Jamieson et al. 2012) and thus indirectly reduces the host plant quality for insect herbivores.
- interspecific competition in mixtures reduces the biomass of _P. lanceolata_. Therefore, in mixtures, _P. lanceolata_ would experience more stress and be more vulnerable for aphids attack.

Because we control for the initial biomass at the start of the infestation, we expect that warming would only slightly increase the biomass of _L. perenne_ and _P. lanceolata_ at the end of the experiment. Prior to fitting the SEM, we checked that relationships were linear using general linear models. We standardized live aboveground biomass of _L. perenne_, live aboveground biomass of _P. lanceolata_ (control), leaf nitrogen, generation time, maximum number of aphids and live aboveground biomass of _P. lanceolata_ (with aphids) by dividing raw values by the standard deviation in order to equalize variances. We used the $\chi^2$ goodness of fit statistic to test whether the covariance matrix generated by the model differed significantly from the data (a p-value $>0.05$ indicates that the observed and expected covariance matrices are not significantly different, suggesting adequate model fit).

All data, except for leaf nitrogen, were also analyzed with ANOVA. Analyses were performed in SAS (ver. 9.4, SAS Inst.) using general linear models (GLM). Several aphid population parameters were tested as a function of temperature and plant composition and plant responses as a function of temperature, plant composition and aphid infestation. Non-significant factors were always backwards excluded from the model. In case of significant effects, a posteriori means comparisons using Tukey test corrected for multiple comparisons were made. Effects were considered significant at $p \leq 0.05$.

The number of days between introduction of the adult aphid and the appearance of the first offspring was used as an approximation of generation time. The maximum number of aphids (Nmax) equates the herbivory rate at a certain time point. For each population, an exponential growth curve was fitted through the aphid abundances from day one until the day of population peak. The growth constant k of the curve $N = N_0 e^{kt}$ served as a measure of population growth speed.
Aboveground biomass was square root transformed and \( \frac{F_v}{F_m} \) was arcsine transformed to meet the data distribution assumptions. Leaf nitrogen was analysed with general linear mixed models in SAS with temperature and plant composition as fixed factors and pot as a random factor because we had three subsamples of each pot.

Data deposition

Data available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.d5h06> (Van De Velde et al. 2016).
Table 1. Summary of ANOVA results for effects of temperature and plant composition on aphid performance growing on Plantago lanceolata. Plant communities consist of monocultures of *P. lanceolata* and mixtures of *Lolium perenne* and *P. lanceolata*. *p*-values are presented in bold when significant (<0.05).

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Treatment</th>
<th>DF</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Generation time</td>
<td>Temperature</td>
<td>2,24</td>
<td>27.17</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Plant composition</td>
<td>1,24</td>
<td>0.77</td>
<td>0.389</td>
</tr>
<tr>
<td></td>
<td>Temperature × Plant composition</td>
<td>2,24</td>
<td>2.43</td>
<td>0.109</td>
</tr>
<tr>
<td>Maximum number of aphids</td>
<td>Temperature</td>
<td>2,24</td>
<td>3.66</td>
<td>0.041</td>
</tr>
<tr>
<td></td>
<td>Plant composition</td>
<td>1,24</td>
<td>0.02</td>
<td>0.892</td>
</tr>
<tr>
<td></td>
<td>Temperature × Plant composition</td>
<td>2,24</td>
<td>6.57</td>
<td>0.005</td>
</tr>
<tr>
<td>Exponential growth constant</td>
<td>Temperature</td>
<td>2,24</td>
<td>28.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Plant composition</td>
<td>1,24</td>
<td>0.07</td>
<td>0.796</td>
</tr>
<tr>
<td></td>
<td>Temperature × Plant composition</td>
<td>2,24</td>
<td>7.19</td>
<td>0.004</td>
</tr>
<tr>
<td>Average aphid weight</td>
<td>Temperature</td>
<td>2,24</td>
<td>8.27</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>Plant composition</td>
<td>1,24</td>
<td>0.49</td>
<td>0.490</td>
</tr>
<tr>
<td></td>
<td>Temperature × Plant composition</td>
<td>2,24</td>
<td>1.22</td>
<td>0.280</td>
</tr>
</tbody>
</table>

Results
Overview by SEM

The hypothesized structural relationship adequately fits the data ($\chi^2 = 17.044$, DF = 11 and $p = 0.073$). Fig. 1B and Supplementary material Appendix 1 Table A1 show that the following pathways were supported: 1) indirect paths from temperature via aphid individuals to aphid population, 2) direct paths from plant composition to live aboveground biomass of *Lolium perenne*, 3) direct path from plant composition to live aboveground biomass of *Plantago lanceolata* in control pots, and 4) direct path from temperature to leaf nitrogen. However, live aboveground biomass of *P. lanceolata* (in control pots) and leaf nitrogen did not affect aphid populations. Finally, neither temperature, plant composition or leaf nitrogen, nor aphid population had an influence on live aboveground biomass of *Plantago lanceolata* (with aphids). Summarizing these results, we conclude that temperature directly affected aphids by shortening the generation time. Shorter generation time in turn, increased the aphid population.

Detailed analyses of the separate paths by linear models

Temperature, but not plant composition or leaf nitrogen, altered the generation time of aphids (Fig. 1B, Table 1). It was shorter at 20°C compared to 17°C but increased again at 23°C (Fig. 2A). As expected, a shorter generation time increased the population, measured as Nmax (Fig 1B, Supplementary material Appendix 1 Table A1). Furthermore, Nmax differed significantly according to an interaction between temperature and plant composition (Fig. 2B, Table 1). Temperature did not alter Nmax of monocultures because we artificially defined it. However, Nmax of monocultures act as controls for mixtures. Pairwise comparisons revealed that competition between *L. perenne* and *P. lanceolata* at 17°C significantly decreased Nmax but increased it at 20°C and did not alter it at 23°C. In line with Nmax, also the aphids’ population growth constant differed significantly according to an interaction between temperature and plant composition (Fig. 2C, Table 1). The aphids’ population growth in monocultures was significantly higher at 23°C compared to 17°C and 20°C. However, in mixtures, the growth increased significantly at 20°C and remained higher at 23°C. Again, pairwise comparisons revealed that competition between *L. perenne* and *P. lanceolata* decreased the aphids’ population growth at 17°C but increased it at 20°C and did not alter the growth at 23°C. In addition, the average aphid weight peaked at 20°C but remained unaffected by plant composition (Fig. 3, Table 1). We conclude that aphid populations on *P. lanceolata* at 20°C were characterised by stronger exponential growth, short generation times, larger aphids and larger maximum population size than at 17°C. This pattern was most obvious in mixtures.

Aphid infestation reduced considerably the relative aboveground biomass (F$^{1,24} = 14.35$, p = 0.0009), the live aboveground biomass, the belowground biomass and the relative water content of the shoots of *P. lanceolata* (Fig. 4A, Fig. 5C, Table 2, Supplementary material Appendix 1 Fig. A1). Concurrently, *P. lanceolata* infested with aphids showed reduced F/Fm at the end of the infestation (F$^{1,48} = 8.58$, p = 0.0051, Supplementary material Appendix 1 Fig. A2) and F/Fm of infested plants dropped further at the end of the experiment (F$^{1,48} = 48.16$, p < 0.0001, Supplementary material Appendix 1 Fig. A2). This indicated that the plants did not recover from the aphid infestation. In addition, aphid infestation increased the dead aboveground biomass of *P. lanceolata* (Fig. 4B; Table 2) and the live aboveground biomass of *L. perenne* in mixtures (Fig. 4C, Table 2).

Temperature and plant composition did not alter the live aboveground biomass and, relative water content of the shoots of *P. lanceolata* (Fig. 4A, Table 2), nor the relative herbivory effects (F$^{2,24} = 0.48$, p = 0.6252, Fig. 5A) at the end of the experiment. However, at 23°C there was not more *P. lanceolata* biomass with respect to *L. perenne* in mixtures (aphid treatment and controls combined), whereas the opposite was true at 17°C (F$^{2,24} = 6.13$, p = 0.0071, Fig. 5B). Furthermore, in controls, the live aboveground biomass of *P. lanceolata* was significantly higher in mixtures compared to monocultures irrespective of the temperature (Fig. 1B, 4A). The dead aboveground biomass of *P. lanceolata*, on the other hand, differed significantly according to an interaction between temperature and plant composition.
lanceolata was slightly lower at 17°C compared to the other temperature treatments (F$_{2,48}$ = 7.40, p = 0.0014), but temperature did not alter F$_{v}$/F$_{m}$ after infestation and at the end of the experiment. We conclude that aphid infestation and temperature had more effect on P. lanceolata compared to plant composition.
Temperature affected all measured plant responses of *L. perenne* (Table 2). Notably, its live aboveground biomass, the shoot relative water content and surprisingly the dead aboveground biomass decreased with increasing temperature (Fig. 4C–D, Table 2). In line with *P. lanceolata* responses, the belowground biomass of *L. perenne* peaked at 20°C and decreased again at 23°C to reach similar levels as at 17°C (Table 2, Supplementary material Appendix 1 Fig. A4). Before infestation, Fv/Fm was higher at 20 and 23°C compared to 17°C (F2,36 = 16.13, p < 0.0001, Supplementary material Appendix 1 Fig. A5). However, after infestation (F2,36 = 7.82, p = 0.0015, Supplementary material Appendix 1 Fig. A5) and at the end of the experiment (F2,36 = 4.29, p = 0.0201, Supplementary material Appendix 1 Fig. A5), Fv/Fm dropped slightly at 20°C compared to 17°C and 23°C. Competition with *P. lanceolata* reduced the live and dead aboveground biomass of *L. perenne* irrespective of the temperature (Fig. 4C, Table 2).

**Discussion**

To understand the impact of climate warming on the complex networks of species in communities, species interactions need to be considered. We investigated the effect of warming on a model community consisting of an aphid feeding on *P. lanceolata* and a heterospecific neighboring plant species *L. perenne*. Warming affected the aphid’s performance directly, but not indirectly through changes in host plant quality. Aphids performed best at moderate warming.

**Direct effect of warming on aphid performance**

As expected, experimental warming directly affected the life history traits of the aphid *Dysaphis plantaginea*, though in a non-linear manner. Aphid populations at 20°C were characterised by shorter generation times, stronger exponential growth, larger aphids and tended to have larger maximum population sizes compared to 17°C. Therefore, 20°C may be the upper thermal threshold for the aphid *D. plantaginea*. Generally, above the upper temperature threshold, activity costs are higher, inducing behavioural and physiological changes. Indeed, at 23°C the observed generation time was longer and Nmax and aphid weight were lower. Yet, this level of warming still accelerated the exponential growth of the population by means of higher fecundity (Meisner et al. 2014, Ramalho et al. 2015). Probably, higher mortality caused by exposure to stressful temperatures underlies the observed lower Nmax despite of the faster exponential growth at 23°C. This would be in line with the theory that mortality increases when temperature exceeds the optimal range (Amarasekare and Savage 2012).

The relative biomass losses of *Plantago lanceolata* due to insect herbivores were not altered with warming. Therefore, the higher dry weight of aphids at 20°C points towards a functional instead of numerical response of the aphids with moderate warming (Solomon 1949, Holling 1959, 1965). At that temperature, aphids grew faster probably due to a higher efficiency in converting food into body matter.
Indirect effect of warming on aphid performance

Insect herbivores are influenced by the food quality of the plant material they consume (Awmack and Leather 2002, Mattson Jr 1980). In aphids, reproduction depends on the nutritional status and availability of the host plant (Awmack and Leather 2002, Dixon 1998). Therefore, warming might alter aphid performance also indirectly through bottom–up effects, by changing host plant availability and quality. In the current study, however, we have controlled for temperature effects on the initial biomass production in order to exclude a different carrying capacity. Warming did not alter the biomass production of *P. lanceolata* at the end of the experiment; hence the faster exponential growth of the aphids at higher temperature cannot originate from more available food. It cannot arise from an altered leaf nitrogen status either, since nitrogen content was slightly lower at 20 °C compared to 17 °C, increasing again to the control value at 23 °C. Warming has been shown to decrease leaf nitrogen content in earlier studies (An et al. 2005, Flynn et al. 2006), thus reducing host plant quality for insect herbivores, but our structural equation model showed that leaf nitrogen did not affect the aphids. Therefore, the increased exponential growth of aphids at higher temperatures must be due to direct temperature effect.

On the other hand, leaf nitrogen content could be a poor index of nutritional value since aphids depend more on the soluble amino acids in the phloem (Schoonhoven et al. 2005). We can therefore not exclude that changes in the quality rather than the quantity of nitrogen-based compounds in the phloem, or changes in other plant nutrients than the one
we measured such as phosphorus or potassium (Jansson and Ekbom 2002), may have contributed to the observed faster growth rates at higher temperature. In addition, the water content of foliage can also have an effect on the growth of aphids (Schoonhoven et al. 2005). Yet, we found no effect of warming on the relative water content of \( P. \) lanceolata shoots. All in all, we found fewer indications for indirect than direct effects of warming on aphid performance.

**Effect of plant species composition**

Interspecific competition decreased the live aboveground biomass of \( L. \) perenne but did not alter the live aboveground biomass of \( P. \) lanceolata. In mixtures, \( L. \) perenne may have absorbed fewer nutrients compared to monocultures which may have resulted in more available nutrients for \( P. \) lanceolata. In addition, interspecific competition affected maximum population size and exponential growth rate of the aphids through an interaction with temperature. Interspecific competition at 17°C negatively affected the performance of the aphids by reducing their population growth rate and maximum population size compared to 20°C. By contrast, the opposite was observed at 20°C but not at 23°C. We expected a higher aphid performance under interspecific competition irrespective of temperature as the growth-differentiation balance hypothesis predicts reduced defence against herbivores under interspecific competition, owing to greater investment of energy in "defence" against competitors (Herms and Mattson 1992). Pellissier et al. (2014) demonstrated that temperature affects secondary metabolite production in \( P. \) lanceolata, which are well-known for their role in plant defence against insect herbivory. In \( P. \) lanceolata the secondary plant compound iridoid glycosides increased in response to herbivory which negatively influenced both its specialist and generalist insect herbivores (Bowers et al. 1992). Low temperatures can constrain the induction of iridoid glycosides and therefore reduce the resistance against herbivory. Today it is not clear how interspecific competition and temperature interact to affect plant defence. Further experimentation is necessary to untangle these factors and their ultimate influence on herbivores. In conclusion, we showed that plant composition and temperature interacted to affect aphid performance but the mechanism at the basis of the observed patterns requires elucidation.

**Effect of warming on herbivory rates**

The herbivory rates on \( P. \) lanceolata were quantified as relative changes in plant biomass due to insect herbivores. In this study, the aphids performed best at moderate warming, where they grew faster and had a shorter generation time. Despite this, the relative biomass losses of \( P. \) lanceolata did not alter under warming and consequently the net interaction strength between plants and herbivores was not changed under warming. This finding points to reduced consumption rates at higher temperature which may result from metabolic demand exceeding energetic supply, such that energy available for tasks beyond cellular maintenance, such as digestion, feeding and movement, decreases sharply at high temperatures (Somero 2011). However, our finding that warming did not affect the herbivory rates is in contrast to theoretical studies which predict that ectothermic herbivores must increase food intake at higher temperatures to offset increased metabolic or nutritional demand (O’Connor et al. 2001). As a result, herbivory rates should increase exponentially with rising temperature, more than primary production, reducing plant biomass at higher temperatures (Gillooly et al. 2001, O’Connor 2009, O’Connor et al. 2011). Lemoine et al. (2014) concluded that the effect of temperature on herbivory rates are highly variable, depending on the identity of the herbivore–plant combination.

**Conclusion**

We found warming and aphid herbivory to alter plant community composition but not net interaction strength between plants and herbivores within the simplified experimental community. This is in contrast to theoretical predictions (Gilbert et al. 2014) that consider consumer–prey models and not plant–plant interactions. The stability of net interaction strength, suggests that the response of a simplified community to warming may scale up to understand the effect of warming on more complex community and ecological networks.

Our controlled laboratory experiment allowed us to precisely measure the effect of interspecific competition on plant–herbivore interaction under warming. Such single-factor climate experiments can improve mechanistic understanding because the low complexity makes isolating specific processes easier (De Boeck et al. 2015). However, in the field, plant communities are subjected to multiple climate change drivers including also elevated CO₂ and altered water conditions. These factors can also interact and multifactor climate experiments have shown that combined responses can be smaller than that expected from additive, single-factor effects (Wu et al. 2011). Moreover, in natural grassland usually more species are present. Therefore, future studies need to validate whether net interactions strengths also remain stable with multiple climate change drivers in more complex communities in natural ecosystems. To conclude, this proof-of-concept study provides evidence that when taking plant–plant interaction into account, the net interactions with herbivores should not change under warming despite direct effects of warming on herbivores. Therefore, our study stresses the importance of indirect non-trophic interactions as an additional layer of complexity to improve our understanding of how trophic interactions will alter under climate change.

**Acknowledgements** – H. Van De Velde is a Research Assistant of the Fund for Scientific Research-Flanders (FWO). Dries Bonte was funded by the FWO research network ‘An eco-evolutionary network of biotic interactions’. We thank the Earth and Life Institute (Université Catholique de Louvain) for providing \( D. \) plantaginea.

**References**


de Sassi, C. and Tylianakis, J. M. 2012. Climate change disproportionately increases herbivore over plant or parasitoid biomass. – PLoS ONE 7: e40557.


Grace, J. B. 2006. Structural equation modeling and natural systems. – Cambridge Univ. Press.


Supplementary material (available online as Appendix oik-03415 at <www.oikosjournal.org/appendix/oik-03415>).
Appendix 1: Figure A1. Effect of temperature and aphid infestation on belowground biomass of *Plantago lanceolata*. Figure A2. Effect of plant composition and aphid infestation on *Fv/Fm* of *Plantago lanceolata*. Figure A3. Effect of temperature on specific leaf area of *Plantago lanceolata* plants. Figure A4. Effect of temperature on belowground biomass of *Lolium perenne*. Figure A5. Effect of temperature on *Fv/Fm* of *Lolium perenne*. Table A1. Partial slopes of the structural equation model presented in Fig. 1B.