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

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Abstract

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.

Keywords:

Climate change; *Dysaphis plantaginea* Hemiptera; life-history traits; plant-insect interaction; plant-plant interaction

Introduction

The global mean air temperature is expected to increase as a result of rising levels of atmospheric CO_2 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

79 Tylianakis 2012, Liu et al. 2011, Roy et al. 2004) and in the lab (Kukal and Dawson 1989, O'Connor
80 2009), neutral effect of warming on herbivory (Richardson et al. 2002) and even decreased herbivory
81 with warming (Burt et al. 2014). Over short timescales, warming may therefore destabilize community
82 dynamics by increasing or decreasing feeding rates.

83 It is well-known that neighbouring plants affect the herbivore damage to a focal plant (Barbosa et al.
84 2009, Root 1973, Underwood et al. 2014). Neighbours can either increase (associational susceptibility)
85 or decrease (associational resistance) herbivore attraction (Tahvanainen and Root 1972). Also the
86 relative frequency of plant species in the neighbourhood and plant density can affect the plant-
87 herbivore interaction. The density of conspecific neighbours, for example, can both increase or
88 decrease herbivore load and feeding behaviour; these are referred to as resource concentration effects
89 (Root 1973) or dilution effects (Otway et al. 2005), respectively. Hence, warming can indirectly
90 influence plant-herbivore interactions via effects on neighbouring plants and these indirect effects of
91 warming may enhance or counteract the direct effects.

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

103

104 # Material and methods

105 ## *Study species*

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

113 We used two common grassland species, *L. perenne*, a perennial hemicryptophyte that grows in dense
114 tussocks (Beddows 1967), and *P. lanceolata,* a rosette-forming perennial forb (Sagar and Harper
115 1964). Both species originate from a wild population in England. *L. perenne* is not a host plant for *D.*
116 *plantaginea*.

117 ## *Experimental setup*

118 *P. lanceolata* and *L. perenne* were grown from seed on greenhouse benches under controlled
119 laboratory conditions (16 h daylight : 8 h darkness and 22 ± 1 °C) and isolated from aphid infestations.
120 The species were sown with a time lag of one week to prevent differences in size at the start of the
121 experiment (Cotrufo and Gorissen 1997) due to differences in germination rate. Two or three week-old
122 seedlings were transplanted into 1.5 L pots, filled with sandy soil (93.2% sand, 4.6% silt, 2.2% clay;
123 field capacity 0.13 $m^3 m^{-3}$; pH 7.6; Kjeldahl-N 0.42 g kg^{-1}; 1% C in humus). The pots were randomly
124 placed in environmentally controlled growth chambers, with three chambers for each of the three
125 temperature treatments: 17 °C, 20 °C and 23 °C. Temperatures were chosen to reflect the range of
126 potential increase in the next century, with the lowest temperature corresponding to the average
127 temperature of a summer day in Belgium. Each temperature treatment consisted of 25 plant
128 communities (pots) with three different plant compositions: (1) 5 monocultures of *L. perenne*; (2) 10

129 monocultures of *P. lanceolata*; and (3) 10 mixtures of both plant species in a 50:50 ratio. Each
130 community contained four individuals because we chose a replacement design to study the effect of
131 interspecific competition on plant-herbivore interaction under warming. The plants were watered
132 every two days according to the 10-year average of 14-15 raining days per month during the growing
133 season. The quantities of water supplied to the pots (65 ± 5 ml) were calculated from the amount of
134 rainfall during the summer months in Ghent. All pots received the same amounts of water so that any
135 enhanced consumption of water would result in soil drought. All communities were fertilized with 10
136 g m^{-2} NH_4NO_3, 5 g m^{-2} P_2O_5, 10 g m^{-2} K_2O and micro-elements (Fe, Mn, Zn, Cu, B, Mo). The fertilizer
137 was given dissolved in water in four equal amounts.

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

144 We controlled for temperature effects on the initial biomass production of both plant species by
145 exposing the monocultures and mixtures of the three temperature treatments to the same number of
146 growing degree days before the start of the infestation. We preferred to simulate synchrony between
147 the phenology of the herbivore and its host rather than an ecologically mismatched interaction, i.e. an
148 induced asymmetry between plant and aphid biomass at the onset of the experiment. The aphids were
149 introduced on *P. lanceolata* plants 1508 growing degree days from the start of the experiment in each
150 temperature treatment. Growing degree days were calculated from the temperature of the chambers
151 using the Baskerville and Emin (1969) method, applying a base growth temperature (the threshold
152 temperature below which the rate of development is considered to be insignificant) of 4 °C (Grant
153 1968). In each temperature treatment, five monocultures of *P. lanceolata* and five mixtures were
154 randomly chosen for aphid infestation. At the start of the infestation, three adult, apterous aphids were
155 placed with a dry paintbrush on the apex of each *P. lanceolata* plant in monocultures and mixtures.

156 Consequently, at the start of the infestation each pot contained 12 (monocultures) or 6 (mixtures)
157 aphids. Pots that did not receive aphids acted as control pots.

158 ***Data collection***

159 Aphid populations were counted daily. When the population on the monocultures had reached 300
160 aphids on average, the aphids were collected three days later. All remaining aphids were transferred to
161 70% ethanol and counted under a stereomicroscope to determine the final numbers. After counting, the
162 aphid population from each pot was dried at 70 °C for 48 h and weighed. The critical number of
163 aphids in monocultures matched the threshold value for dispersal of aphids when plant conditions are
164 sub-optimal (Dixon 1998). We thus terminated the experiment before the aphid populations would
165 crash, to avoid compromising the measurement of plant responses.

166 We wanted to examine whether the recovery from an aphid infestation differed as a function of
167 temperature using chlorophyll a fluorescence measurements (see below). Therefore, all plants were
168 harvested after a recovery period of 10 days. During the harvest, aboveground parts were separated
169 from belowground parts and live from dead biomass by species. Root and shoot were weighted fresh.
170 We could not separate the roots of *L. perenne* and *P. lanceolata*, therefore only the belowground
171 biomass of the monocultures was measured. All plant material was dried at 70 °C for 48 h, and
172 weighed again. The relative water content of the shoots was calculated as the difference between fresh
173 and dry weight divided by the fresh weight. For statistical analysis, the sum of aboveground biomass
174 per species was divided by the number of plants of that species in each pot. Total leaf area of *P.*
175 *lanceolata* in control pots was determined with a portable area meter (LI-3000A, LI-COR, NE, USA).
176 *P. lanceolata* plants in control pots were ground in a mill, and three subsamples of each pot were
177 analyzed for nitrogen content using a Flash 2000 Organic element analyser (Thermo Scientific,
178 Bremen, Germany).
179 Chlorophyll a fluorescence, which can detect photosynthetic stress effects prior to visible leaf damage
180 (Lichtenthaler and Miehe 1997), was measured on the youngest fully expanded leaf of each plant
181 species per pot. These measurements were performed prior to and after the infestation and at the end
182 of the experiment. Readings were taken at the start of the daylight regime on 30-min dark-acclimated

183 leaves with a Hansatech Plant Efficiency Analyzer (King's Lynn, Norfolk, UK), on the same day for
184 all treatments. Maximum quantum yield of photosystem II was calculated as $F_v/F_m = (F_m-F_0)/F_m$ where
185 F_v = variable fluorescence, F_m = maximum fluorescence and F_0 = steady state fluorescence.

186 ***Data analysis***

187 To investigate the effect of a neighbouring plant species and warming on the plant-herbivore
188 interaction, we fitted a structural equation model (SEM) (Grace 2006, Lamb et al. 2011) using the
189 lavaan library in R (Rosseel 2012, Team 2014). The response of individual aphids was measured as
190 the generation time and the response of the population as the number of aphids at the population peak
191 (see below). We hypothesized that (Fig. 1A):

192 • warming shortens the individual generation time of aphids. Shortening of generation time with
193 increasing temperature is expected to enhance the growth rate of the population.

194 • warming decreases the leaf nitrogen and water content (An et al. 2005, Flynn et al. 2006,
195 Jamieson et al. 2012) and thus indirectly reduces the host plant quality for insect herbivores.

196 • interspecific competition in mixtures reduces the biomass of *P. lanceolata*. Therefore, in
197 mixtures, *P. lanceolata* would experience more stress and be more vulnerable for aphids
198 attack.

199 Because we control for the initial biomass at the start of the infestation (see experimental setup), we
200 expect that warming would only slightly increase the biomass of *L. perenne* and *P. lanceolata* at the
201 end of the experiment. Prior to fitting the SEM, we checked that relationships were linear using
202 general linear models. We standardized live aboveground biomass of *L. perenne*, live aboveground
203 biomass of *P. lanceolata* (control), leaf nitrogen, generation time, maximum number of aphids and
204 live aboveground biomass of *P. lanceolata* (with aphids) by dividing raw values by the standard
205 deviation in order to equalize variances. We used the χ^2 goodness of fit statistic to test whether the
206 covariance matrix generated by the model differed significantly from the data (a P-value > 0.05
207 indicates that the observed and expected covariance matrices are not significantly different, suggesting
208 adequate model fit).

All data, except for leaf nitrogen, were also analyzed with ANOVA. Analyses were performed in SAS (version 9.4, SAS Institute Inc., Cary, NC) 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. Average aphid weight was determined by dividing population weight by population number.

The plant responses were tested separately for *L. perenne* and *P. lanceolata*. Relative herbivory effects were calculated as (live aboveground biomass of *P. lanceolata* with herbivores – live aboveground biomass of *P. lanceolata* without herbivores)/(live aboveground biomass of *P. lanceolata* without herbivores) and the relative biomass of *P. lanceolata* as (live aboveground biomass of *P. lanceolata* – live aboveground biomass of *L. perenne*)/(live aboveground biomass of *L. perenne)*. Live aboveground biomass and relative water content of the shoots were log-10 transformed, dead aboveground biomass was square root transformed and 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.

235 # Results

236 ## *Overview by SEM*

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

247 ## *Detailed analyses of the separate paths by linear models*

248 Temperature, but not plant composition or leaf nitrogen, altered the generation time of aphids (Fig.
249 1B; Table 1). It was shorter at 20 °C compared to 17 °C but increased again at 23 °C (Fig. 2A). As
250 expected, a shorter generation time increased the population, measured as Nmax (Fig 1B;
251 Supplementary material Appendix 1 Table A1). Furthermore, Nmax differed significantly according to
252 an interaction between temperature and plant composition (Fig. 2B; Table 1). Temperature did not
253 alter Nmax of monocultures because we artificially defined it. However, Nmax of monocultures act as
254 controls for mixtures. Pairwise comparisons revealed that competition between *L. perenne* and *P.*
255 *lanceolata* at 17 °C significantly decreased Nmax but increased it at 20 °C and did not alter it at 23 °C.
256 In line with Nmax, also the aphids' population growth constant differed significantly according to an
257 interaction between temperature and plant composition (Fig. 2C; Table 1). The aphids' population
258 growth in monocultures was significantly higher at 23 °C compared to 17 °C and 20 °C. However, in
259 mixtures, the growth increased significantly at 20 °C and remained higher at 23 °C. Again, pairwise
260 comparisons revealed that competition between *L. perenne* and *P. lanceolata* decreased the aphids'

261 population growth at 17 °C but increased it at 20 °C and did not alter the growth at 23 °C. In addition,
262 the average aphid weight peaked at 20 °C but remained unaffected by plant composition (Fig. 3; Table
263 1). We conclude that aphid populations on *P. lanceolata* at 20 °C were characterised by stronger
264 exponential growth, short generation times, larger aphids and larger maximum population size than at
265 17 °C. This pattern was most obvious in mixtures.

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

275 Temperature and plant composition did not alter the live aboveground biomass and, relative water
276 content of the shoots of *P. lanceolata* (Fig. 4A; Table 2), nor the relative herbivory effects ($F_{2,27}$ =
277 0.48, P = 0.6252, Fig. 5A) at the end of the experiment. However, at 23 °C there was more *P.*
278 *lanceolata* biomass with respect to *L. perenne* in mixtures (aphid treatment and controls combined),
279 whereas the opposite was true at 17 °C ($F_{2,24}$ = 6.13, P = 0.0071, Fig. 5B). Furthermore, in controls,
280 the live aboveground biomass of *P. lanceolata* was significantly higher in mixtures compared to
281 monocultures irrespective of the temperature (Fig. 1B, Fig. 4A). The dead aboveground biomass of *P.*
282 *lanceolata*, on the other hand, differed significantly according to an interaction between temperature
283 and plant composition (Fig. 4B; Table 2). This was mainly due to a significant increase in dead
284 biomass in monocultures compared to mixtures at 17 °C. In general, dead aboveground and
285 belowground biomass increased at 20 °C, but decreased again at 23 °C to similar levels as 17 °C (Fig.
286 4B; Table 2; Supplementary material Appendix 1 Fig. A1). In contrast, leaf nitrogen of *P. lanceolata*
287 decreased slightly at 20 °C (Fig. 6; Table 2). The specific leaf area was significantly higher at 23 °C

compared to the other temperature treatments (Table 2; Supplementary material Appendix 1 Fig. A3). Before infestation, F_v/F_m of *P. 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, Fig. 4D; 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, F_v/F_m was higher at 20 and 23 °C compared to 17 °C ($F_{2,36}$ = 16.13, P <0.0001, Supplementary material Appendix 1 Fig. A5). However, after infestation ($F_{2,36}$ = 7.82, P = 0.0015, Supplementary material Appendix 1 Fig. A5) and at the end of the experiment ($F_{2,36}$ = 4.29, P = 0.0201, Supplementary material Appendix 1 Fig. A5), F_v/F_m 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 neighbouring 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 warm²ing.

Direct effect of warming on aphid performance

As expected, experimental warming directly affected the life history traits of the aphid *D. 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 *P. 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 (Holling 1959, Holling 1965, Solomon 1949). 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

339 altered leaf nitrogen status either, since nitrogen content was slightly lower at 20 °C compared to 17
340 °C, increasing again to the control value at 23 °C. Warming has been shown to decrease leaf nitrogen
341 content in earlier studies (An et al. 2005, Flynn et al. 2006), thus reducing host plant quality for insect
342 herbivores, but our structural equation model showed that leaf nitrogen did not affect the aphids.
343 Therefore, the increased exponential growth of aphids at higher temperatures must be due to direct
344 temperature effect.

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

354 ***Effect of plant species composition***

355 Interspecific competition decreased the live aboveground biomass of *L. perenne* but did not alter the
356 live aboveground biomass of *P. lanceolata*. In mixtures, *L. perenne* may have absorbed fewer
357 nutrients compared to monocultures which may have resulted in more available nutrients for *P.*
358 *lanceolata*. In addition, interspecific competition affected maximum population size and exponential
359 growth rate of the aphids through an interaction with temperature. Interspecific competition at 17 °C
360 negatively affected the performance of the aphids by reducing their population growth rate and
361 maximum population size compared to 20 °C. By contrast, the opposite was observed at 20 °C but not
362 at 23 °C. We expected a higher aphid performance under interspecific competition irrespective of
363 temperature as the growth-differentiation balance hypothesis predicts reduced defence against
364 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 of 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.

391 ***Conclusion***

392 We found warming and aphid herbivory to alter plant community composition but not net interaction
393 strength between plants and herbivores within the simplified experimental community. This is in
394 contrast to theoretical predictions (Gilbert et al. 2014) that consider consumer-prey models and not
395 plant-plant interactions. The stability of net interaction strength, suggests that the response of a
396 simplified community to warming may scale up to understand the effect of warming on more complex
397 community and ecological networks.
398 Our controlled laboratory experiment allowed us to precisely measure the effect of interspecific
399 competition on plant-herbivore interaction under warming. Such single-factor climate experiments can
400 improve mechanistic understanding because the low complexity makes isolating specific processes
401 easier (De Boeck et al. 2015). However, in the field, plant communities are subjected to multiple
402 climate change drivers including also elevated CO_2 and altered water conditions. These factors can
403 also interact and multifactor climate experiments have shown that combined responses can be smaller
404 than that expected from additive, single-factor effects (Wu et al. 2011). Moreover, in natural grassland
405 usually more species are present. Therefore, future studies need to validate whether net interactions
406 strengths also remain stable with multiple climate change drivers in more complex communities in
407 natural ecosystems. To conclude, this proof-of-concept study provides evidence that when taking
408 plant-plant interaction into account, the net interactions with herbivores should not change under
409 warming despite direct effects of warming on herbivores. Therefore, our study stresses the importance
410 of indirect non –trophic interactions as an additional layer of complexity to improve our understanding
411 of how trophic interactions will alter under climate change.
412

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417 *plantaginea*.

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532 Supplementary material (available online as Appendix oik.XXX at <
533 www.oikosjournal.org/readers/appendix >). Appendix 1: Fig. A1. Effect of temperature and aphid
534 infestation on belowground biomass of *Plantago lanceolata*. Fig. A2. Effect of plant composition and
535 aphid infestation on F_v/F_m of *Plantago lanceolata*. Fig. A3. Effect of temperature on specific leaf area
536 of *Plantago lanceolata* plants. Fig. A4. Effect of temperature on belowground biomass of *Lolium*
537 *perenne*. Fig. A5. Effect of temperature on F_v/F_m of *Lolium perenne*. Table A1. Partial slopes of the
538 structural equation model presented in Figure 1B.

539

Table and figure legends

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).

546 **Table 2** Summary of ANOVA results for effects of temperature, plant composition and aphid
547 infestation on plant performance. Plant communities consist of monocultures and mixtures of *Lolium*
548 *perenne* and *Plantago lanceolata*. P values are presented in bold when significant (<0.05).

549

550 **Fig. 1** (A) Specific predictions and (B) structural equation model showing how temperature and plant
551 composition affect aphid population and the final live aboveground biomass of *Plantago lanceolata*.
552 Solid arrows represent significant relationships ($P < 0.05$), dashed lines are nonsignificant. Black
553 arrows are positive relationships, grey lines negative. Standardized path coefficients are shown next to
554 pathways. For the effect of temperature, the average path coefficients are shown. The individual path
555 coefficients of high and moderate warming can be seen in Table A1. Significant effects of both high
556 and moderate warming are indicated with an asterisk ($P < 0.05$). Live aboveground biomass of *Lolium*
557 *perenne*, live aboveground biomass of *P. lanceolata* (control), leaf nitrogen, generation time,
558 maximum number of aphids and live aboveground biomass of *P. lanceolata* (with aphids) were scaled
559 before analysis.

560

561 **Fig. 2** Effect of temperature and plant composition on aphid population dynamics. A) Effect of
562 temperature on the generation time of aphids (all plant compositions combined). B) Effect of
563 temperature and plant composition on the maximum number of aphids. C) Effect of temperature and
564 plant composition on the growth constant k of the exponential growth curve. Bars represent means ±
565 SE. Plant communities consist of monocultures of *Plantago lanceolata* (black bars) and mixtures of
566 *Lolium perenne* and *P. lanceolata* (grey bars). Significant pairwise differences are indicated by
567 different letters above the bars ($P < 0.05$). Significant differences between monocultures and mixtures
568 at a given temperature are indicated with an asterisk ($P < 0.05$).

569

570 **Fig. 3** Effect of temperature on average aphid weight (mean ± SE, all plant compositions combined).

571 Significant pairwise differences are indicated by different letters above the bars ($P < 0.05$).

572

573 **Fig. 4** Effect of temperature, plant composition and aphid infestation on A) the live aboveground
574 biomass of *Plantago lanceolata,* B) the dead aboveground biomass of *P. lanceolata,* C) the live
575 aboveground biomass of *Lolium perenne* and D) dead aboveground biomass of *L. perenne*. Bars
576 represent means ± SE. Plants were grown in monocultures of *L. perenne* or *P. lanceolata* (black bars),
577 mixtures of *L. perenne* and *P. lanceolata* (dark grey bars), monocultures with aphids (light grey bars)
578 or mixtures with aphids (white bars).

579

580 **Fig. 5** Relative change in plant biomass of *Plantago lanceolata* due to warming and aphid herbivory.
581 A) Aboveground biomass effects of herbivory on *P. lanceolata* exposed to different temperatures
582 relative to controls. The relative herbivory effect was calculated as (live aboveground biomass of *P.*
583 *lanceolata* with herbivores – live aboveground biomass of *P. lanceolata* without herbivores)/(live
584 aboveground biomass of *P. lanceolata* without herbivores). Plant communities consisted of
585 monocultures of *P. lanceolata* (black bars) and mixtures of *Lolium perenne* and *P. lanceolata* (grey
586 bars). B) Effect of temperature and C) effect of aphid infestation on aboveground biomass of *P.*
587 *lanceolata* relative to aboveground biomass of *L. perenne*. The relative aboveground biomass of *P.*
588 *lanceolata* was calculated as (live aboveground biomass of *P. lanceolata* – live aboveground biomass
589 of *L. perenne*)/(live aboveground biomass of *L. perenne*). Bars represent means ± SE. Significant
590 pairwise differences are indicated by different letters above the bars ($P < 0.05$).

591

592 **Fig. 6** Effect of temperature on percentage of nitrogen in leaves of *Plantago lanceolata* that did not
593 receive aphids. Bars represent means ± SE are indicated (all plant compositions combined). Significant
594 pairwise differences are indicated by different letters above the bars ($P < 0.05$).

595

596 **Table 1**

Measurement	Treatment	df	F	P
Generation time	Temperature	2,24	27.17	**<0.001**
	Plant composition	1,24	0.77	0.389
	Temperature × Plant composition	2,24	2.43	0.109
Maximum number of aphids	Temperature	2,24	3.66	**0.041**
	Plant composition	1,24	0.02	0.892
	Temperature × Plant composition	2,24	6.57	**0.005**
Exponential growth constant	Temperature	2,24	28.5	**<0.001**
	Plant composition	1,24	0.07	0.796
	Temperature × Plant composition	2,24	7.19	**0.004**
Average aphid weight	Temperature	2,24	8.27	**0.002**
	Plant composition	1,24	0.49	0.490
	Temperature × Plant composition	2,24	1.22	0.280

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599

600 **Table 2**

		Plantago lanceolata			*Lolium perenne*		
Measurement	Treatment	df	F	P	df	F	P
Live aboveground biomass	Temperature	2,48	1.09	0.345	2,36	22.23	**<0.001**
	Plant composition	1,48	2.66	0.120	1,36	14.18	**0.006**
	Infestation	1,48	118.4	**<0.001**	1,36	10.97	**0.002**
	Temperature × infestation	2,48	0.05	0.952	2,36	0.2	0.822
	Temperature × plant composition	2,48	0.12	0.892	2,36	1.68	0.201
	Plant composition × infestation	1,48	1.11	0.298	-	-	-
	Temperature × infestation × plant composition	2,48	1.37	0.264	-	-	-
Dead aboveground biomass	Temperature	2,48	7.08	**0.002**	2,36	23.45	**<0.001**
	Plant composition	1,48	1.13	0.292	1,36	6.88	**0.013**
	Infestation	1,48	302.78	**<0.001**	1,36	2.18	0.148
	Temperature × infestation	2,48	0.77	0.468	2,36	0.74	0.484
	Temperature × plant composition	2,48	7.01	**0.002**	2,36	5.29	**0.010**
	Plant composition × infestation	1,48	0.24	0.630	-	-	-
	Temperature × infestation × plant composition	2,48	0.81	0.450	-	-	-
Belowground biomass	Temperature	2,24	39.34	**<0.001**	2,12	47.35	**<0.001**
	Infestation	1,24	43.67	**<0.001**	-	-	-
	Temperature × infestation	2,24	1.82	0.183	-	-	-
Relative water content of the shoots	Temperature	2,44	0.36	0.702	2,36	4.4	**0.020**
	Plant composition	1,44	1.61	0.211	1,36	0.69	0.410
	Infestation	1,44	27.79	**<0.001**	1,36	0.35	0.558
	Temperature × infestation	2,44	0.45	0.641	2,36	0.18	0.836

	Temperature × plant composition	2,44	1.81	0.175	2,36	0.25	0.780
	Plant composition × infestation	1,44	2.82	0.100	-	-	-
	Temperature × infestation × plant composition	2,44	1.81	0.176	-	-	-
Leaf nitrogen	Temperature	2,24	4.93	**0.016**	-	-	-
	Plant composition	1,26	0.04	0.834	-	-	-
	Temperature × plant composition	2,27	0.71	0.501	-	-	-
Specific leaf area	Temperature	2,24	12.2	**0.001**	-	-	-
	Plant composition	1,24	1.26	0.272	-	-	-
	Temperature × plant composition	2,24	0.98	0.391	-	-	-

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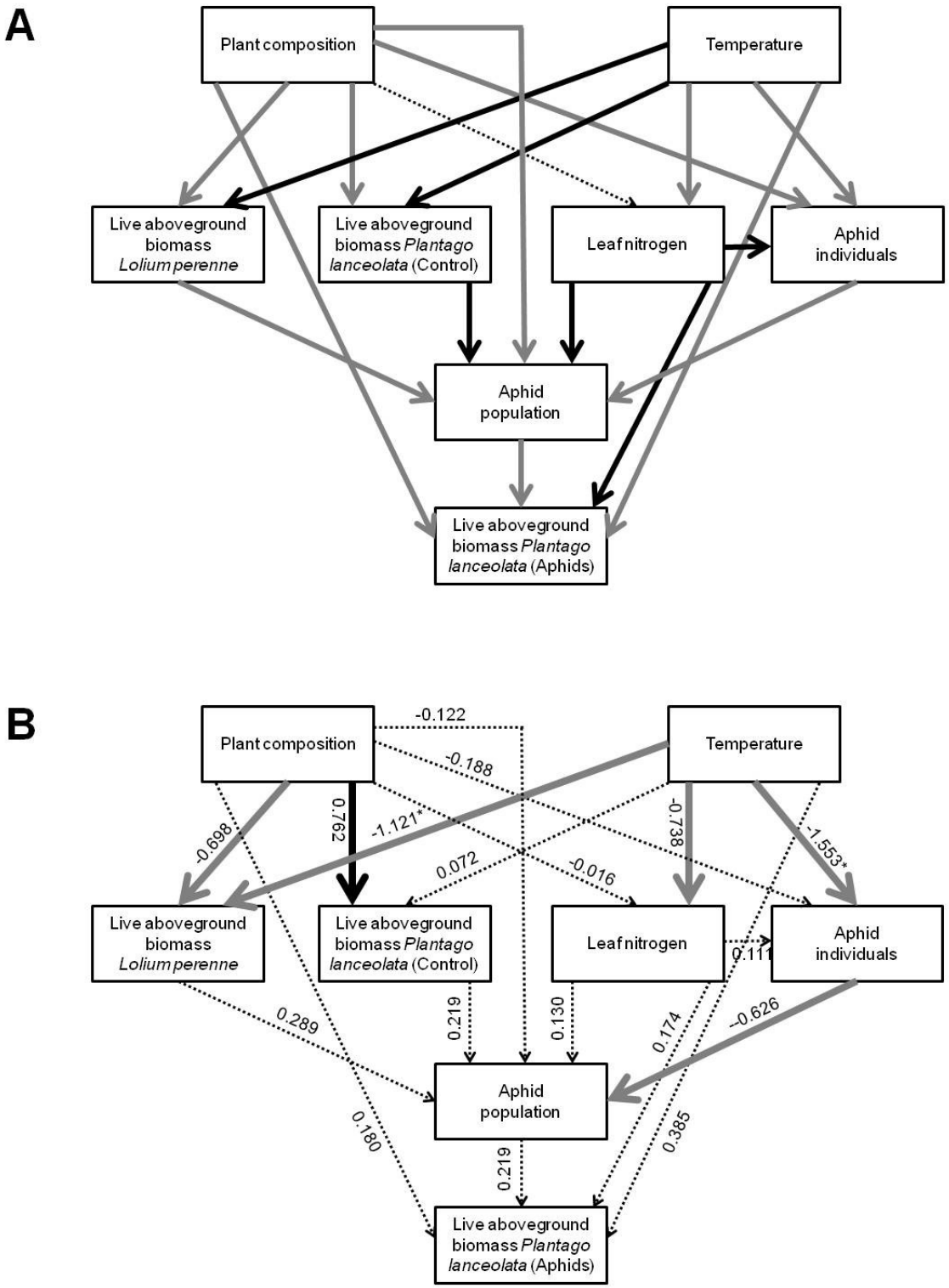
602

603 **Figure 1**



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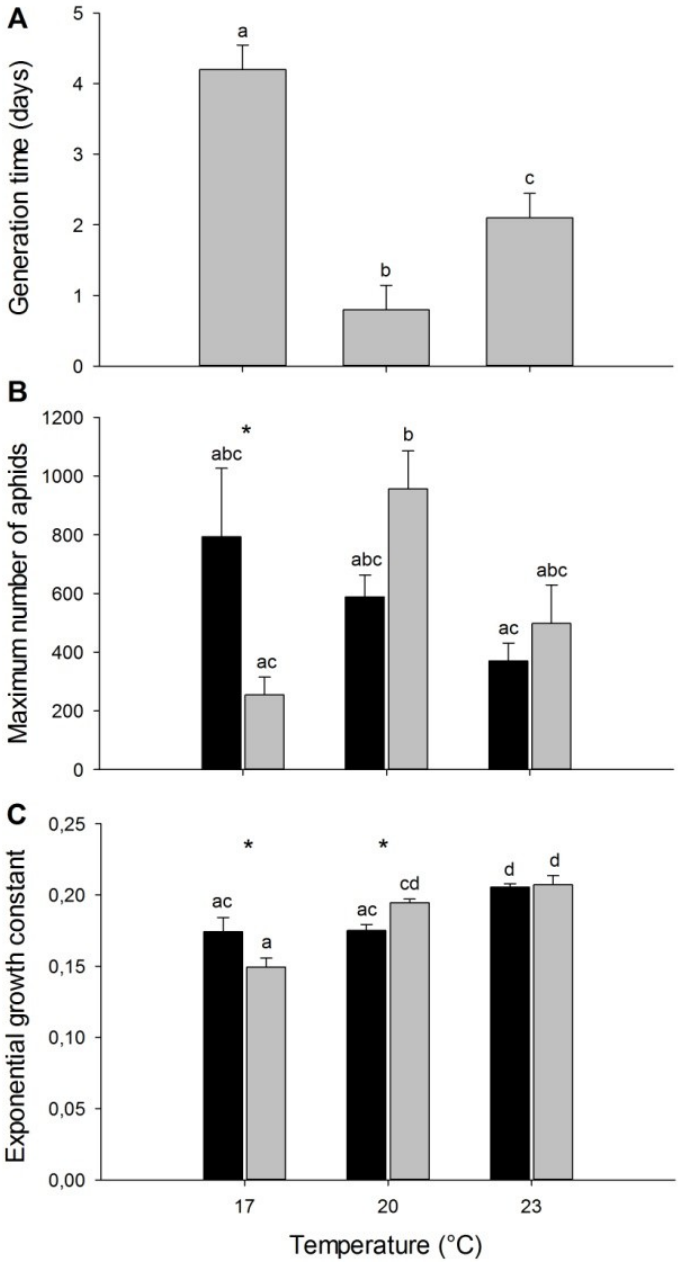
606 **Figure 2**



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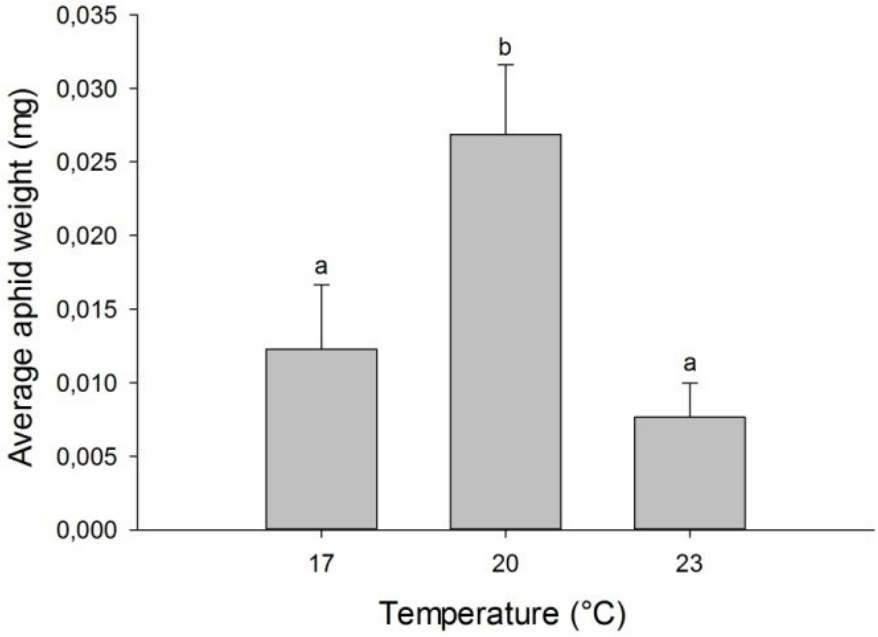
612 **Figure 3**



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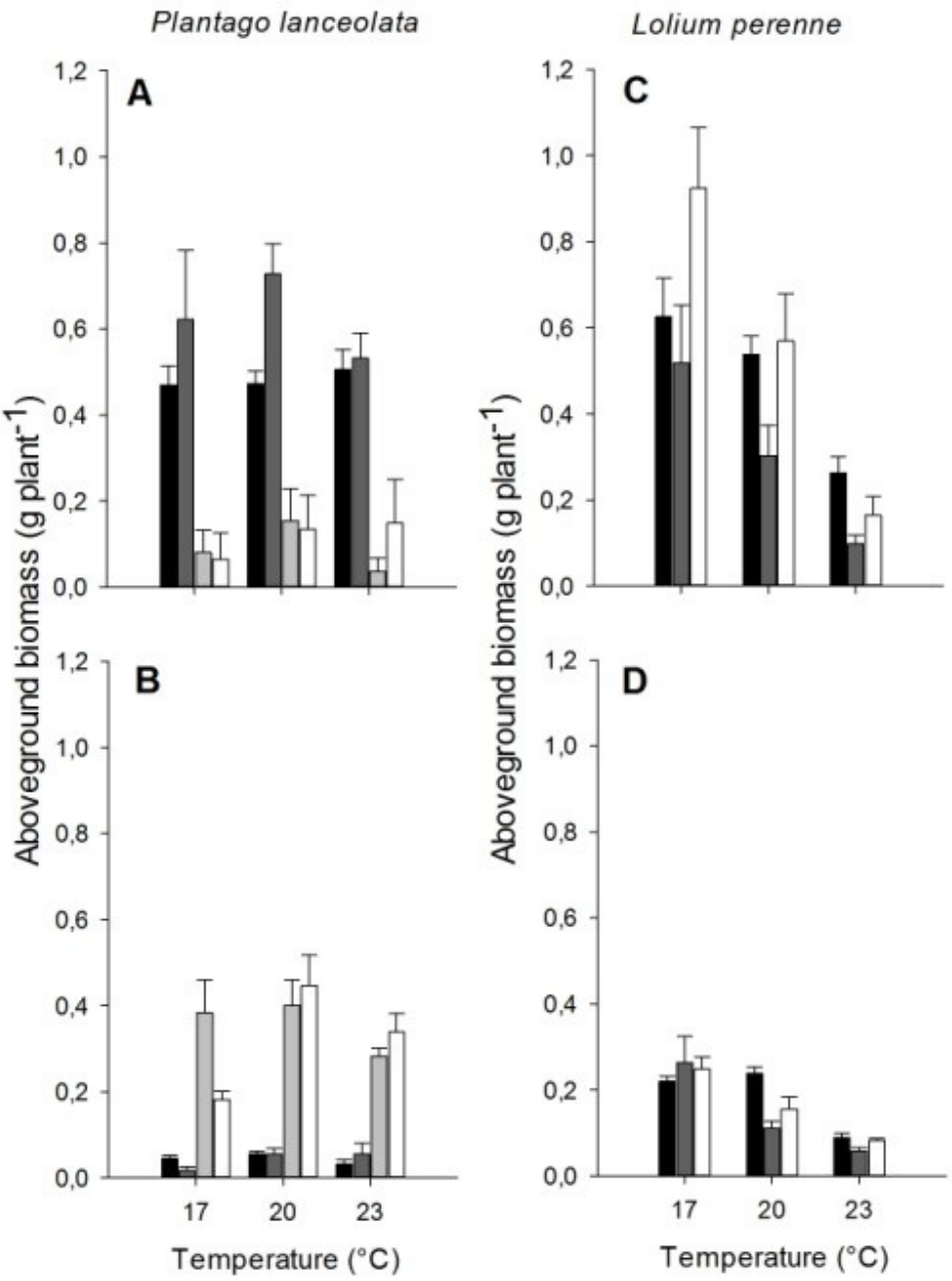
625 **Figure 4**



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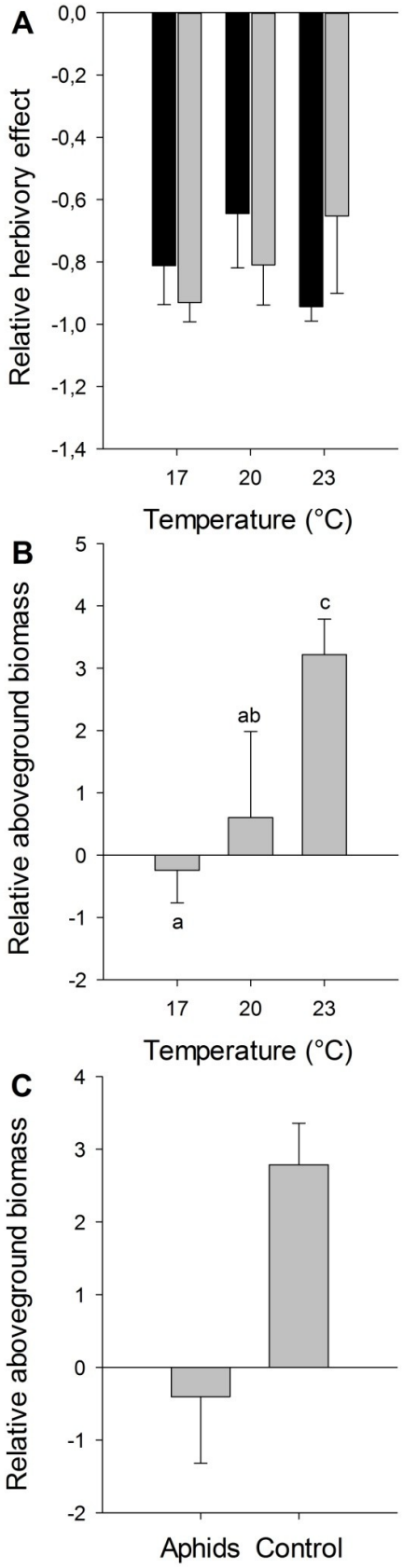
631 **Figure 5**



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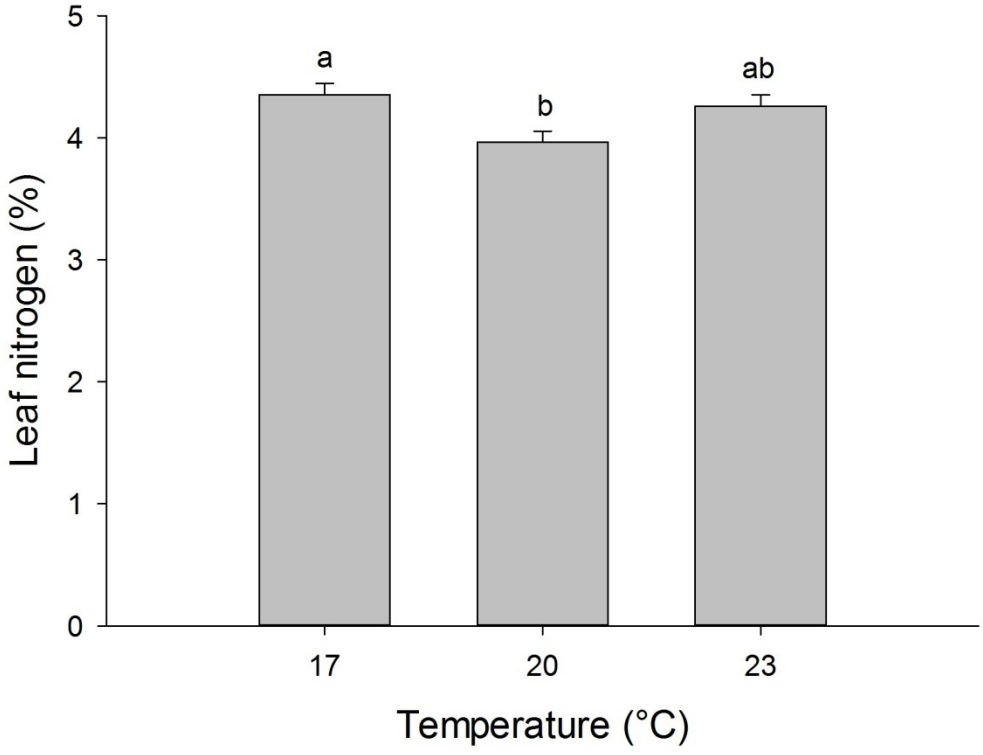
633 **Figure 6**



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