Benefits from below: Silicon supplementation maintains legume productivity under predicted climate change scenarios

Scott N. Johnson[1*], James M. Ryalls[1], Andrew Gherlenda[1], Adam Frew[1], Sue E. Hartley[2]

[1]Hawkesbury Institute for the Environment, Western Sydney University, Australia, [2]York Environment and Sustainability Institute, University of York, United Kingdom

Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest

Author contribution statement

Keywords

alfalfa, Aphids, Atmospheric change, Climate Change, Global Warming, silica, Silicon

Abstract

Word count: 293

Many studies demonstrate that elevated atmospheric carbon dioxide concentrations (eCO2) can promote root nodulation and biological nitrogen fixation (BNF) in legumes such as lucerne (Medicago sativa). But when elevated temperature (eT) conditions are applied in tandem with eCO¬2, a more realistic scenario for future climate change, the positive effects of eCO2 on nodulation and BNF in M. sativa are often much reduced. Silicon (Si) supplementation of M. sativa has also been reported to promote root nodulation and BNF, so could potentially restore the positive effects of eCO2 under eT. Increased nitrogen availability, however, could also increase host suitability for aphid pests, potentially negating any benefit. We applied eCO2 (+240ppm) and eT (+4ºC), separately and in combination, to M. sativa growing in Si supplemented (Si+) and un-supplemented soil (Si-) to determine whether Si moderated the effects of eCO2 and eT. Plants were either inoculated with the aphid Acyrthosiphon pisum or insect-free. In Si-soils, eCO2 stimulated plant growth by 67% and nodulation by 42%, respectively, whereas eT reduced these parameters by 26% and 48%, respectively. Aphids broadly mirrored these effects on Si- plants, increasing colonization rates under eCO2 and performing much worse (reduced abundance and colonization) under eT when compared to ambient conditions, confirming our hypothesized link between root nodulation, plant growth and pest performance. Examined across all CO2 and temperature regimes, Si supplementation promoted plant growth (+93%), and root nodulation (+50%). Acyrthosiphon pisum abundance declined sharply under eT conditions and was largely unaffected by Si supplementation. In conclusion, supplementing M. sativa with Si had consistent positive effects on plant growth and nodulation under different CO2 and temperature scenarios. These findings offer potential for using Si supplementation to maintain legume productivity under predicted climate change scenarios without making legumes more susceptible to insect pests.

Ethics statements

(Authors are required to state the ethical considerations of their study in the manuscript, including for cases where the study was exempt from ethical approval procedures)

Does the study presented in the manuscript involve human or animal subjects: No

Benefits from below: Silicon supplementation maintains legume productivity under predicted climate change scenarios

Scott N. Johnson[1*], James M.W. Ryalls[1], Andrew N. Gherlenda[1], Adam Frew[1,2] and Susan E. Hartley[3]

[1]Hawkesbury Institute for the Environment, Western Sydney University, Penrith, NSW, Australia

[2]Current address: School of Agricultural and Wine Sciences, Charles Sturt University, Wagga Wagga, NSW, Australia

[3] York Environment and Sustainability Institute, Department of Biology, University of York, York, United Kingdom

*Correspondence: Scott Johnson, Scott.Johnson@westernsydney.edu.au

Running title: Silicon modifies legume responses to climate change

Abstract

Many studies demonstrate that elevated atmospheric carbon dioxide concentrations (eCO_2) can promote root nodulation and biological nitrogen fixation (BNF) in legumes such as lucerne (*Medicago sativa*). But when elevated temperature (eT) conditions are applied in tandem with eCO_2, a more realistic scenario for future climate change, the positive effects of eCO_2 on nodulation and BNF in *M. sativa* are often much reduced. Silicon (Si) supplementation of *M. sativa* has also been reported to promote root nodulation and BNF, so could potentially restore the positive effects of eCO_2 under eT. Increased nitrogen availability, however, could also increase host suitability for aphid pests, potentially negating any benefit. We applied eCO_2 (+240ppm) and eT (+4ºC), separately and in combination, to *M. sativa* growing in Si supplemented (Si+) and un-supplemented soil (Si-) to determine whether Si moderated the effects of eCO_2 and eT. Plants were either inoculated with the aphid *Acyrthosiphon pisum* or insect-free. In Si- soils, eCO_2 stimulated plant growth by 67% and nodulation by 42%, respectively, whereas eT reduced these parameters by 26% and 48%, respectively. Aphids broadly mirrored these effects on Si- plants, increasing colonization rates under eCO_2 and performing much worse (reduced abundance and colonization) under eT when compared to ambient conditions, confirming our hypothesized link between root nodulation, plant growth and pest performance. Examined across all CO_2 and temperature regimes, Si supplementation promoted plant growth (+93%), and root nodulation (+50%). *Acyrthosiphon pisum* abundance declined sharply under eT conditions and was largely unaffected by Si supplementation. In conclusion, supplementing *M. sativa* with Si had consistent positive effects on plant growth and nodulation under different CO_2 and temperature scenarios. These findings offer potential for using Si supplementation to maintain legume productivity under predicted climate change scenarios without making legumes more susceptible to insect pests.

Keywords: alfalfa, aphids, atmospheric change, climate change, global warming, silica, silicon

Introduction

Projected increases in atmospheric carbon dioxide (CO_2) have been shown experimentally to stimulate biological nitrogen fixation (BNF) in legumes (Soussana and Hartwig, 1996; Zanetti et al., 1996; Hungate et al., 1999; Edwards et al., 2006; Lam et al., 2012). These effects are strongest immediately after exposure to elevated CO_2 (eCO_2) (Hungate et al., 2004) and when other nutrients (especially phosphorus) are not limiting (Rogers et al., 2009). Elevated CO_2 (eCO_2) can promote BNF via several mechanisms, including larger numbers of N_2 fixing symbiotic bacteria in the rhizosphere (Schortemeyer et al., 1996), increased numbers of nodules which house N_2 fixing rhizobia bacteria (Ryle and Powell, 1992) and enhanced nitrogenase activity (Norby, 1987). Broadly speaking, eCO_2 allows legumes to increase rates of photosynthesis and allocate more carbon belowground to support increased root nodulation and therefore BNF (Aranjuelo et al., 2014).

Researchers are becoming increasingly aware of the importance of testing multiple environmental change factors because they are predicted to occur concurrently and often have either synergistic or antagonistic impacts on one another (Robinson et al., 2012; Johnson and Jones, 2017). Climate models predict, for instance, that air temperatures will increase in tandem with increases in atmospheric CO_2 and warmer temperature may negate any positive impacts of eCO_2 on plant growth (Newman et al., 2011). This may be particularly true in legume systems because higher temperatures can have inhibitory effects on BNF due to the relatively low tolerance of N_2-fixing bacteria to higher temperatures (Zahran, 1999; Whittington et al., 2013; Aranjuelo et al., 2014). The optimal temperature for root nodule symbiosis for temperate legumes is thought to be around $15\text{-}25^0C$, above which detrimental effects can become evident (Aranjuelo et al., 2014). Elevated temperature (eT) can directly hinder the development and functionality of root nodulation and accelerate nodule senescence (Piha and Munns, 1987; Aranjuelo et al., 2006). In addition, eT can inhibit nodulation via plant-mediated mechanisms, including reduced root hair formation, fewer nodulation sites and poorer adherence of bacteria to root hairs (Hungria and Franco, 1993; Hungria and Vargas, 2000; Aranjuelo et al., 2014).

Soil conditions play an important role in determining the extent to which eCO_2 and eT affect root nodulation in legumes (Aranjuelo et al., 2014). Several studies report that supplementation of soil silicon (Si) levels promotes growth in legumes (Horst and Marschner, 1978; Miyake and Takahashi, 1985; Guo et al., 2006; Johnson et al., 2017), though we know less about the functional role of Si in legumes compared to other plant families such as the Poaceae (Epstein, 1999; Cooke and Leishman, 2011). Moreover, Si supplementation can increase rates of root nodulation and symbiosis with nitrogen fixing bacteria (Nelwamondo and Dakora, 1999; Mali and Aery, 2008). However, how these positive effects of Si on nodulation are affected by eCO_2 or eT, alone or in combination, have not yet been addressed. If Si could maintain nodulation rates under future climate change scenarios, such as eT, which usually decrease it, then such supplementation could be important in the mitigation of climate change impacts on agriculture.

While rhizobial colonization promotes legume growth and vigour, this improved host quality can also increase susceptibility to belowground (Quinn and Hower, 1986; Gerard, 2001; Johnson and McNicol, 2010) and aboveground insect herbivores (Dean et al., 2009; Kempel et al., 2009; Katayama et al., 2010; Dean et al., 2014; Whitaker et al., 2014). Beneficial effects of rhizobia on herbivores most likely arise through increased provision of nitrogen, which is frequently limiting in insect herbivore diets (Mattson, 1980). Increased provision of nitrogen may, however, allow plants to invest in plant defenses with negative impacts on

95 herbivores (Pineda et al., 2010; Brunner et al., 2015). While Si supplementation usually
96 increases plant resistance to herbivores (mainly reported in the Poaecae; Reynolds et al.,
97 2009), it may also indirectly increase susceptibility to herbivores via increases in legume
98 growth and nutritional quality (Johnson et al., 2017).
99
100 The objective of this study was to determine how eCO_2 and eT, acting alone and in
101 combination, affected root nodulation and plant growth in *M. sativa* in untreated (Si-) and Si
102 supplemented (Si+) soil. We additionally aimed to establish whether these factors affected
103 the abundance and colonization success of an insect herbivore (the aphid *Acyrthosiphon*
104 *pisum*). We hypothesised that eCO_2 increases growth and root nodulation in *M. sativa* but eT
105 negates these effects. Si supplementation increases nodulation, even under eT, and therefore
106 maximises plant growth regardless of CO_2 and temperature conditions. We hypothesized that
107 aphid abundance would be positively linked to plant growth and nodulation, whether driven
108 by Si supplementation or changes in CO_2 and temperature conditions.
109
110 **Methods and Materials**
111 *Insect cultures and plant material*
112 Four *Acyrthosiphon pisum* cultures were established from a single parthenogenetic adult
113 female collected from a pasture containing grasses and legumes, including lucerne, at the
114 Hawkesbury Campus of the Western Sydney University, NSW (latitude −33.608847,
115 longitude 150.747016). Cultures were maintained on propagated lucerne (*M. sativa* L.) plants
116 (Sequel cultivar) in each of the four CO_2 and temperature combinations (conditions below)
117 for at least six generations (c. 7 weeks) prior to the experiment. For the experiment, *M. sativa*
118 (Sequel) were grown from seed (Seedmark, Adelaide, South Australia) in glasshouse rooms
119 receiving supplemental light (15:9 light:dark) under the same conditions. Plants were grown
120 in 70 mm diameter pots containing c. 700g of soil excavated from the Hawkesbury campus of
121 Western Sydney University (location as above). The soil is typified as low-fertility sandy
122 loam in the Clarendon Formation (Chromosol) (Barton et al., 2010), which has low
123 bioavailable Si content of 10-17 mg kg^{-1} (Johnson et al., 2017).
124
125 *Growth conditions and experimental procedures*
126 Eighty lucerne plants were grown in each of four CO_2 and temperature-controlled glasshouse
127 chambers (320 plants in total) using a fully factorial design of ambient CO_2 (aCO_2; 400 µmol
128 mol^{-1}) and eCO_2 (640 µmol mol^{-1}) at ambient (aT) and elevated temperature (ambient + 4°C;
129 eT). aT was set at 26/18°C day/night representing the average daily temperature (November
130 to May) over the past 30 years for Richmond, NSW (Australian Bureau of Meteorology). eT
131 (30/22 °C day/ night) replicated the maximum predicted temperature increase for this region
132 within this century (CSIRO, 2007-2016). Environmental conditions were monitored
133 continuously throughout the experiment and temperature readings were verified with portable
134 temperature loggers. To minimize 'chamber effects' associated with using four chambers,
135 plants were circulated within each chamber every five days (apart from when plants were
136 inoculated with aphids to avoid dislodgement of the insects) and chambers were swapped
137 every c. 10 days by transferring plants between chambers and adjusting the environmental
138 conditions accordingly. While this does not eliminate pseudoreplication, using this approach
139 in these chambers has provided matching empirical results to fully replicated experiments,
140 whether using multiple chamber replicates or multiple experimental runs (Johnson et al.,
141 2016b).
142
143 Plants were irrigated with c. 70 ml of tap water (Si 3ppm) three times a week. After growing
144 for a further two weeks, half (40) of the plants continued to receive tap water (Si- plants or

145 Si- soil hereafter) at the same intervals while the other half (selected at random) received 70
146 ml of 500 mg l^{-1} soluble silica in the form of $NaSiO_3.9H_2O$ three times a week (Si+ plants or
147 Si+ soil hereafter). When plants were six weeks old, 20 of the plants receiving the Si
148 supplementation and 20 of the plants receiving tap water (selected at random) were
149 inoculated with two teneral adult *A. pisum.* White mesh (organza) bags (125 x 170 mm) were
150 applied tightly around the rim of all pots confining aphids to their allocated plants. After two
151 weeks, bags were removed aphids counted (including colonization success; at least one aphid
152 being present). Plants were cleaned free of soil with water before and the number of active
153 (pink) root nodules quantified. Maximum rooting depth was also quantified to provide a
154 rudimentary measure of nodule density in order to give an indication as whether changes in
155 nodule abundance were a function of root growth or nodule density on the roots (i.e. nodules
156 per unit of root growth). Plants were freeze dried for 48 hr and weighed. Leaves were
157 separated from the stems and ball-milled to a fine power prior to analysis for Si
158 concentrations.
159
160 *Foliar Si analysis*
161 It was necessary to pool foliar samples (2-3 plants per sample), giving nine replicates of each
162 treatment combination (CO_2, temperature, Si application and aphid inoculation). Foliar Si
163 concentrations were analyzed with X-ray fluorescence spectrometry using the method
164 described by Reidinger, Ramsey & Hartley (2012). In summary, plant material was ground to
165 a fine powder and pressed into 13mm-diameter pellets. Foliar Si concentration was
166 determined using a Niton XL3t XRF analyzer (Thermo Fisher Scientific, Inc., MA, USA), for
167 a measurement time of 30 seconds. Results we expressed as foliar Si concentration (as % of
168 dry mass), calibrated against plant-certified reference material of known Si content
169 (Garbuzov et al., 2011).
170
171 *Statistical analysis*
172 Goodness-of-fit tests, using the 'goodfit' function in the vcd package (Friendly, 2000), were
173 employed to determine which distributions best described the data. Plant dry mass and nodule
174 density were transformed (logarithm and square-root, respectively) prior to analysis to meet
175 model assumptions and give residual diagnostic plots which fitted a normal distribution and
176 showed least heteroscedasticity. Plant dry mass and nodule density were analyzed using
177 analysis of variance with CO_2, temperature, aphid presence and Si supplementation included
178 as fixed effects individually and in interaction with one another. Root nodule counts and
179 aphid abundance were analyzed with generalized linear models with negative binomial error
180 structures and log-link function using the same configuration of fixed effects as above. Aphid
181 colonization success was analyzed in the same way but with binomial error structure and logit
182 link function. Statistical tests of plant mass and nodulation were conducted on data
183 collectively, before repeating the tests separately for Si- and Si+ plants since there were
184 significant interactions between Si treatment and environmental treatments. Where non-
185 significant effects were observed in full models (i.e. all factors included), non-significant
186 factors were removed to determine whether this affected model inferences with more
187 parsimonious models (e.g. fewer multi-way interaction terms were included in the model) –
188 see Table S1 All analysis was conducted in the R statistical package.
189
190 **Results**
191 Plant growth was stimulated by eCO_2 and Si supplementation by 41% and 93%, respectively,
192 (Fig. 1; Table 1). In contrast, eT and aphid presence depressed plant growth by 13% and
193 17%, respectively (Fig. 1; Table 1). Temperature depressed plant growth in Si- soil (Fig. 1A),

194 but not in Si+ soil (Fig. 1B), though there was an interactive effect of CO_2 and temperature in
195 the latter, with eCO_2 promoting plant growth more at eT than under aT conditions (Fig. 1B).
196
197 Root nodulation increased when plants grew under eCO_2 (+27%) and Si+ conditions (+50%)
198 (Fig. 2, Table 1), but eT caused significant declines in nodulation (-32%). In Si- soil, root
199 nodulation patterns generally mirrored changes in plant growth (Fig. 1A and 2A,
200 respectively). Levels of root nodulation were universally high in plants growing in Si+ soil
201 and other factors (CO_2, temperature and aphid presence) no longer had significant impacts
202 (Fig. 2B). This was particularly true for the negative impacts of eT, which was reversed under
203 Si+ conditions, reflected by the significant interaction of these treatments (Table 1, Fig 2).
204 Our rudimentary estimate of nodule density (nodules per unit of root depth) suggested this
205 was not affected by CO_2 (other than the weak interaction described below) but declined by
206 25% under eT (Table S1, Fig. S1). Nodule density increased (c. +45%) under Si+ conditions
207 and, like nodule abundance, there was a significant interaction between Si treatment and
208 temperature, whereby negative effects of eT were revered under Si+ conditions (Table S1,
209 Fig. S1). There was a very weak interaction between Si, aphids and CO_2.
210
211 Si concentration in the foliage was unaffected by CO_2, temperature and aphid presence,
212 though unexpectedly there was a small but significant decline in foliar Si concentrations
213 when growing in Si+ soil (Fig. 3, Table 1).
214
215 Aphid abundance was not significantly affected by eCO_2 (Fig. 4), although colonization
216 success increased by 14% under eCO_2 (Table 2). In contrast, eT caused substantial declines (-
217 65%) in aphid abundance and reduced their ability to colonise plants, falling by 48% and
218 43% on Si- and Si+ plants, respectively (Table 2). Aphid populations at eT were similar
219 regardless of Si treatments. In short, aphid abundance was always lowest at 30℃ and Si
220 promotion of plant growth and nodulation was decoupled from aphid performance, such that
221 Si+ conditions led to increased nodulation (and potentially BNF) without increasing aphid
222 numbers.
223
224 The key findings of this study are summarized in Fig. 5 which held true when non-significant
225 terms were dropped from models for parsimony (see Table S2). Panels A, B and C in Fig. 5
226 show how aphid abundance mirrors patterns of nodulation and plant growth in non-
227 supplemented soils, but this becomes decoupled in Si+ soils, where Si supplementation
228 restores the fertilising effects of eCO_2 on *M. sativa* at higher temperatures without affecting
229 aphid populations.
230
231 **Discussion**
232 Results from this study suggest that Si supplementation may mitigate the negative impacts of
233 eT on plant growth in *M. sativa* which was potentially due to stimulation of root nodulation,
234 despite the reduction in nodulation at higher temperatures reported in previous studies (e.g.
235 Ryalls et al., 2013b). Even more advantageously, this increased nodulation did not increase
236 susceptibility to an aphid pest at eT, which had previously been observed for Si-induced
237 increases in nodulation at ambient temperatures (Johnson et al., 2017).
238
239 Aphid abundance was strongly suppressed by eT and this most likely explains why aphids did
240 not benefit from increases in plant growth and nodulation that arose under Si+ conditions
241 under eT. While aphid numbers often increase with higher temperatures via faster
242 development and increased fecundity, this increase ceases abruptly over a certain temperature
243 threshold because of the adverse effects on, for example, embryo development and

5

244   maturation (Ryalls and Harrington, 2017). This temperature threshold depends on species,
245   aphid biotype and geographical region (Awmack and Leather, 2007). *Acyrthosiphon pisum*
246   has adapted to the warmer climate of Australia since introduction in the 1970s (Ryalls et al.,
247   2013a). Some populations are able to function at temperatures above 35°C, although their
248   optimum temperature is said to be c. 20-25 °C (Ryalls, 2016) and temperatures above 28°C
249   are likely to reduce aphid growth and development (Bieri et al., 1983; Lamb and MacKay,
250   1988; Mackay et al., 1993). Aphid biotypes with certain secondary bacterial endosymbionts
251   may cope better with higher temperatures, however, since there have been several reports of
252   endosymbionts alleviating the effects of heat stress (Montllor et al., 2002; Russell and Moran,
253   2005; Dunbar et al., 2007). To our knowledge, studies have not yet addressed how bacterial
254   endosymbionts might change in response to eCO_2 *and* eT but endosymbionts could partially
255   facilitate adaptation to climate and atmospheric change (Sun et al., 2016; Ryalls and
256   Harrington, 2017).

258   Several studies using temperature gradient greenhouses have examined the impacts of eCO_2
259   and eT on legume performance, including root nodulation (Aranjuelo et al., 2006; Erice et al.,
260   2006; Erice et al., 2007; Aranjuelo et al., 2008). These studies report a general trend for eCO_2
261   promoting nodulation, but only at the elevated experimental temperatures. This was probably
262   because the elevated temperature range used in experiments (c. 24^0C; Aranjuelo et al., 2008)
263   was still within the optimal range ($19-25^0$C) for nodulation in temperate legumes, so
264   inhibitory effects of temperature on nodulation wouldn't necessarily have occurred
265   (Aranjuelo et al., 2014). When temperature was elevated beyond 25^0C to 30^0C, root
266   nodulation in *M. sativa* has been reported to decrease by 22% under ambient CO_2 (aCO_2) and
267   by 56% under eCO_2 (Ryalls et al., 2013b).

269   Despite increasing evidence that the effects of eCO_2 are often modified by eT, and *vice versa*,
270   comparatively few studies manipulate both factors in tandem (Robinson et al., 2012). In the
271   present study we established that positive impacts of eCO_2 on plant traits were not seen to the
272   same extent when eT conditions were applied. This study therefore lends support to the
273   notion that, wherever feasible, multiple environmental factors should be tested (Newman et
274   al., 2011; Lindroth and Raffa, 2016). Crucially, Si supplementation had consistently stronger
275   impacts on plant traits across a range of environmental conditions and regardless of whether
276   plants were challenged by herbivores.

278   A counterintuitive finding of the study was that Si supplementation actually reduced
279   concentrations of Si in the foliage. Si may have promoted plant growth to such an extent that
280   Si became 'diluted' in foliage, or else had not had time to accumulate in plant tissues over the
281   duration of the study. A similar trend in foliar Si was previously observed in this system
282   however, associated with rapid plant growth, increases in root nodulation and synthesis of
283   amino acids (Johnson et al., 2017) . In addition to any increased nutritional value, the lower
284   concentrations of Si in foliage of Si+ plants may explain why Si supplementation did not
285   increase plant resistance to aphids.

287   Our results demonstrate conclusively the benefits of Si supplementation for root nodulation:
288   root nodule abundance was always increased in plants growing in Si+ soil and other factors,
289   whether CO_2, temperature and aphid presence no longer had significant impacts on nodule
290   abundance. The mechanisms by which Si is so effective at promoting nodulation are not well
291   understood, but could include changes in soil conditions, increased root growth (and potential
292   invasion sites), higher abundance of bacteroids and symbiosomes, together with the synthesis
293   of compounds that upregulate nodulation genes (as discussed by Johnson et al., 2017). The

increased nodule density reported in the present study tentatively suggests that greater nodule abundance was not merely a function of increased root growth. Further work is needed, but Si could provide a useful tool for mitigating some of the negative impacts of climate change on crop production – in this instance maintaining nodulation rates of *M. sativa* in warmer climates. Moreover, other studies suggest Si could redress negative effects of eCO_2 on plant-herbivore interactions. For example, herbivore damage to roots of sugarcane was exacerbated under eCO_2 conditions, but application of Si reversed these effects and stimulated crop growth (Frew et al., 2017). Intervention strategies could include targeted application of Si (e.g. furnace slag), selection of plant lines that naturally take up large amounts of Si (McLarnon et al., 2017) and remediation of soils deficient in bioavailable Si (silicic acid) (Guntzer et al., 2012; Johnson et al., 2016a).

Authors' contributions
SNJ, JMWR, AF and ANG. conceived the experimental design. SNJ, JMWR, AF and ANG acquired and processed data with SEH undertaking Si analysis. JMWR analyzed the data and all authors contributed to the interpretation and drafting of the manuscript.

References
Aranjuelo, I., Arrese-Igor, C., and Molero, G. (2014). Nodule performance within a changing environmental context. *J. Plant Physiol.* 171, 1076-1090. doi: 10.1016/j.jplph.2014.04.002

Aranjuelo, I., Irigoyen, J.J., Perez, P., Martinez-Carrasco, R., and Sanchez-Diaz, M. (2006). Response of nodulated alfalfa to water supply, temperature and elevated CO_2: productivity and water relations. *Environ. Exp. Bot.* 55, 130-141. doi: 10.1016/j.envexpbot.2004.10.007

Aranjuelo, I., Irigoyen, J.J., Sanchez-Diaz, M., and Nogues, S. (2008). Carbon partitioning in N_2 fixing *Medicago sativa* plants exposed to different CO_2 and temperature conditions. *Funct. Plant Biol.* 35, 306-317. doi: 10.1071/fp07296

Awmack, C., and Leather, S.R. (2007). "Growth and development," in *Aphids as Crop Pests,* eds. H.F. Van Emden & R. Harrington. (Wallingford: CABI), 135-151.

Barton, C.V.M., Ellsworth, D.S., Medlyn, B.E., Duursma, R.A., Tissue, D.T., Adams, M.A., Eamus, D., Conroy, J.P., and McMurtrie, R.E. (2010). Whole-tree chambers for elevated atmospheric CO_2 experimentation and tree scale flux measurements in south-eastern Australia: The Hawkesbury Forest Experiment. *Agr. Forest Meteorol.* 150, 941–951. doi: doi.org/10.1016/j.agrformet.2010.03.001

Bieri, M., Baumgartner, J., Bianchi, G., Delucchi, V., and Arx, R.v. (1983). Development and fecundity of pea aphid (*Acyrthosiphon pisum* Harris) as affected by constant temperatures and by pea varieties. *Mitteilungen der Schweizerischen Entomologischen Gesellschaft* 56, 163-171. doi: N/A

Brunner, S.M., Goos, R.J., Swenson, S.J., Foster, S.P., Schatz, B.G., Lawley, Y.E., and Prischmann-Voldseth, D.A. (2015). Impact of nitrogen fixing and plant growth-

promoting bacteria on a phloem-feeding soybean herbivore. *Appl. Soil Ecol.* 86, 71-81. doi: 10.1016/j.apsoil.2014.10.007

Cooke, J., and Leishman, M.R. (2011). Is plant ecology more siliceous than we realise? *Trends Plant Sci.* 16, 61-68. doi: 10.1016/j.tplants.2010.10.003

CSIRO (2007-2016). *Climate change in Australia: technical report* [Online]. Melbourne, Australia: CSIRO. Available: http://www.climatechangeinaustralia.gov.au/ [Accessed 10 November 2017].

Dean, J.M., Mescher, M.C., and De Moraes, C.M. (2009). Plant-rhizobia mutualism influences aphid abundance on soybean. *Plant Soil* 323, 187-196. doi: 10.1007/s11104-009-9924-1

Dean, J.M., Mescher, M.C., and De Moraes, C.M. (2014). Plant dependence on rhizobia for nitrogen influences induced plant defenses and herbivore performance. *Int. J. Mol. Sci.* 15, 1466-1480. doi: 10.3390/ijms15011466

Dunbar, H.E., Wilson, A.C.C., Ferguson, N.R., and Moran, N.A. (2007). Aphid thermal tolerance is governed by a point mutation in bacterial symbionts. *PloS Biol.* 5, 1006-1015. doi: ARTN e96

10.1371/journal.pbio.0050096

Edwards, E.J., McCaffery, S., and Evans, J.R. (2006). Phosphorus availability and elevated CO_2 affect biological nitrogen fixation and nutrient fluxes in a clover-dominated sward. *New Phytol.* 169, 157–167. doi: 10.1111/j.1469-8137.2005.01568.x

Epstein, E. (1999). Silicon. *Annu Rev Plant Physiol. and Plant Molec. Biol.* 50, 641-664. doi: 10.1146/annurev.arplant.50.1.641

Erice, G., Irigoyen, J.J., Perez, P., Martinez-Carrasco, R., and Sanchez-Diaz, M. (2006). Effect of elevated CO_2, temperature and drought on photosynthesis of nodulated alfalfa during a cutting regrowth cycle. *Physiol. Plant.* 126, 458-468. doi: 10.1111/j.1399-3054.2005.00599.x

Erice, G., Irigoyen, J.J., Sanchez-Diaz, M., Avice, J.C., and Ourry, A. (2007). Effect of drought, elevated CO_2 and temperature on accumulation of N and vegetative storage proteins (VSP) in taproot of nodulated alfalfa before and after cutting. *Plant Sci.* 172, 903-912. doi: 10.1016/j.plantsci.2006.12.013

Frew, A., Allsopp, P.G., Gherlenda, A., and Johnson, S.N. (2017). Increased herbivory under elevated atmospheric carbon dioxide concentrations is reversed by silicon-based plant defences *J. Appl. Ecol.* 54, 1310-1319. doi: 10.1111/1365-2664.12822

Friendly, M. (2000). *Visualizing Categorical Data.* Cary, NC: SAS Institute.

Garbuzov, M., Reidinger, S., and Hartley, S.E. (2011). Interactive effects of plant-available soil silicon and herbivory on competition between two grass species. *Ann. Bot.* 108, 1355-1363. doi: 10.1093/aob/mcr230

Gerard, P.J. (2001). Dependence of *Sitona lepidus* (Coleoptera: Curculionidae) larvae on abundance of white clover *Rhizobium* nodules. *B. Entomol. Res.* 91, 149–152. doi: 10.1079/BER200076

Guntzer, F., Keller, C., and Meunier, J.D. (2012). Benefits of plant silicon for crops: a review. *Agron. Sust. Dev.* 32, 201-213. doi: 10.1007/s13593-011-0039-8

Guo, Z.G., Liu, H.X., Tian, F.P., Zhang, Z.H., and Wang, S.M. (2006). Effect of silicon on the morphology of shoots and roots of alfalfa (*Medicago sativa*). *Aust. J. Exp. Agric.* 46, 1161-1166. doi: 10.1071/ea05117

Horst, W.J., and Marschner, H. (1978). Effect of silicon on manganese tolerance of bean-plants (*Phaseolus vulgaris* L.). *Plant Soil* 50, 287-303. doi: 10.1007/bf02107179

Hungate, B.A., Dijkstra, P., Johnson, D.W., Hinkle, C.R., and Drake, B.G. (1999). Elevated CO_2 increases nitrogen fixation and decreases soil nitrogen mineralization in Florida scrub oak. *Glob. Change Biol.* 5, 781–789. doi: 10.1046/j.1365-2486.1999.00275.x

Hungate, B.A., Stiling, P.D., Dijkstra, P., Johnson, D.W., Ketterer, M.E., Hymus, G.J., Hinkle, C.R., and Drake, B.G. (2004). CO_2 elicits long-term decline in nitrogen fixation. *Science* 304, 1291-1291. doi: 10.1126/science.1095549

Hungria, M., and Franco, A.A. (1993). Effects of high-temperature on nodulation and nitrogen-fixation by *Phaseolus vulgaris* L. *Plant Soil* 149, 95-102. doi: 10.1007/bf00010766

Hungria, M., and Vargas, M.A.T. (2000). Environmental factors affecting N-$_2$ fixation in grain legumes in the tropics, with an emphasis on Brazil. *Field Crop. Res.* 65, 151-164. doi: 10.1016/s0378-4290(99)00084-2

Johnson, S.N., Benefer, C.M., Frew, A., Griffiths, B.S., Hartley, S.E., Karley, A.J., Rasmann, S., Schumann, M., Sonnemann, I., and Robert, C.A.M. (2016a). New frontiers in belowground ecology for plant protection from root-feeding insects. *Appl. Soil Ecol.* 108, 96-107. doi: dx.doi.org/10.1016/j.apsoil.2016.07.017

Johnson, S.N., Gherlenda, A.N., Frew, A., and Ryalls, J.M.W. (2016b). The importance of testing multiple environmental factors in legume-insect research: replication, reviewers and rebuttal. *Front. Plant Sci.* 7, 489. doi: 10.3389/fpls.2016.00489

Johnson, S.N., Hartley, S.E., Ryalls, J.M.W., Frew, A., DeGabriel, J.L., Duncan, M., and Gherlenda, A. (2017). Silicon-induced root nodulation and synthesis of essential amino acids in a legume is associated with higher herbivore abundance. *Funct. Ecol.* 31, 1903-1909. doi: 10.1111/1365-2435.12893

Johnson, S.N., and Jones, T.H. (2017). "Emerging Issues and Future Perspectives for Global Climate Change Studies," in *Global Climate Change and Terrestrial Invertebrates,* eds. S.N. Johnson & T.H. Jones. (Chichester, UK: John Wiley & Sons, Ltd), 368-377.

Johnson, S.N., and McNicol, J.W. (2010). Elevated CO_2 and aboveground-belowground herbivory by the clover root weevil. *Oecologia* 162, 209–216. doi: 10.1007/s00442-009-1428-4

Katayama, N., Nishida, T., Zhang, Z.Q., and Ohgushi, T. (2010). Belowground microbial symbiont enhances plant susceptibility to a spider mite through change in soybean leaf quality. *Popul. Ecol.* 52, 499-506. doi:

Kempel, A., Brandl, R., and Schadler, M. (2009). Symbiotic soil microorganisms as players in aboveground plant-herbivore interactions - the role of rhizobia. *Oikos* 118, 634-640. doi: 10.1111/j.1600-0706.2009.17418.x

Lam, S.K., Chen, D.L., Norton, R., Armstrong, R., and Mosier, A.R. (2012). Nitrogen dynamics in grain crop and legume pasture systems under elevated atmospheric carbon dioxide concentration: A meta-analysis. *Glob. Change Biol.* 18, 2853-2859. doi: 10.1111/j.1365-2486.2012.02758.x

Lamb, R.J., and MacKay, P.A. (1988). Effects of temperature on developmental rate and adult weight of Australian populations of *Acyrthosiphon pisum* (Harris) (Homoptera: Aphididae). *Mem. Entomol. Soc. Can.* 120, 49-55. doi:

Lindroth, R.L., and Raffa, K.F. (2016). "Experimental approaches for assessing invertebrate responses to global change factors," in *Global Climate Chane and Terrestrial Invertebrates,* eds. S.N. Johnson & T.H. Jones. (Chichester, UK: John Wiley & Sons), 30-45.

Mackay, P.A., Lamb, R.J., and Smith, M.A.H. (1993). Variability in life-history traits of the aphid, *Acyrthosiphon pisum* (Harris), from sexual and asexual populations. *Oecologia* 94, 330-338. doi: Doi 10.1007/Bf00317106

440 Mali, M., and Aery, N.C. (2008). Silicon effects on nodule growth, dry-matter production,
441   and mineral nutrition of cowpea (*Vigna unguiculata*). *J. Plant Nutr. Soil Sci.* 171,
442   835-840. doi: 10.1002/jpln.200700362

443 Mattson, W.J. (1980). Herbivory in relation to plant nitrogen content. *Annu. Rev. Ecol. Syst.*
444   11, 119–161. doi: 10.1146/annurev.es.11.110180.001003

445 McLarnon, E., McQueen-Mason, S., Lenk, I., and Hartley, S.E. (2017). Evidence for active
446   uptake and deposition of Si-based defenses in tall fescue. *Front. Plant Sci.* 8, 1199.
447   doi: 10.3389/fpls.2017.01199

448 Miyake, Y., and Takahashi, E. (1985). Effect of silicon on the growth of soybean plants in a
449   solution culture. *Soil Sci. Plant Nutr.* 31, 625-636. doi:
450   10.1080/00380768.1985.10557470

451 Montllor, C.B., Maxmen, A., and Purcell, A.H. (2002). Facultative bacterial endosymbionts
452   benefit pea aphids *Acyrthosiphon pisum* under heat stress. *Ecol. Entomol.* 27, 189–
453   195. doi:

454 Nelwamondo, A., and Dakora, F.D. (1999). Silicon promotes nodule formation and nodule
455   function in symbiotic cowpea (*Vigna unguiculata*). *New Phytol.* 142, 463-467. doi:
456   10.1046/j.1469-8137.1999.00409.x

457 Newman, J.A., Anand, M., Henry, H.A.L., Hunt, S., and Gedalof, Z. (2011). *Climate Change*
458   *Biology.* Wallingford, UK: CABI.

459 Norby, R.J. (1987). Nodulation and nitrogenase activity in nitrogen-fixing woody-plants
460   stimulated by CO_2 enrichment of the atmosphere. *Physiol. Plantarum* 71, 77–82. doi:
461   10.1111/j.1399-3054.1987.tb04620.x

462 Piha, M.I., and Munns, D.N. (1987). Sensitivity of the common bean (*Phaseolus vulgaris* L)
463   symbiosis to high soil-temperature. *Plant Soil* 98, 183-194. doi: 10.1007/bf02374822

464 Pineda, A., Zheng, S.-J., van Loon, J.J.A., Pieterse, C.M.J., and Dicke, M. (2010). Helping
465   plants to deal with insects: the role of beneficial soil-borne microbes. *Trends Plant Sci*
466   15, 507-514. doi: 10.1016/j.tplants.2010.05.007

467 Quinn, M.A., and Hower, A.A. (1986). Effects of root nodules and taproots on survival and
468   abundance of *Sitona hispidulus* (Coleoptera, Curculionidae) on *Medicago sativa*.
469   *Ecol. Entomol.* 11, 391–400. doi: 10.1111/j.1365-2311.1986.tb00318.x

470 Reidinger, S., Ramsey, M.H., and Hartley, S.E. (2012). Rapid and accurate analyses of
471   silicon and phosphorus in plants using a portable X-ray fluorescence spectrometer.
472   *New Phytol.* 195, 699-706. doi: 10.1111/j.1469-8137.2012.04179.x

473 Reynolds, O.L., Keeping, M.G., and Meyer, J.H. (2009). Silicon-augmented resistance of
474   plants to herbivorous insects: a review. *Ann. Appl. Biol.* 155, 171–186. doi:
475   10.1111/j.1744-7348.2009.00348.x

476 Robinson, E.A., Ryan, G.D., and Newman, J.A. (2012). A meta-analytical review of the
477   effects of elevated CO_2 on plant-arthropod interactions highlights the importance of
478   interacting environmental and biological variables. *New Phytol.* 194, 321–336. doi:
479   10.1111/j.1469-8137.2012.04074.x

480 Rogers, A., Ainsworth, E.A., and Leakey, A.D.B. (2009). Will elevated carbon dioxide
481   concentration amplify the benefits of nitrogen fixation in legumes? *Plant Physiol.*
482   151, 1009–1016. doi: 10.1104/pp.109.144113

483 Russell, J.A., and Moran, N.A. (2005). Horizontal transfer of bacterial symbionts: heritability
484   and fitness effects in a novel aphid host. *Appl. Environ. Microbiol.* 71, 7987–7994.
485   doi:

486 Ryalls, J.M.W. (2016). The impacts of climate change and belowground herbivory on aphids
487   via primary metabolites. [PhD Thesis], Western Sydney University.

488 Ryalls, J.M.W., and Harrington, R. (2017). "Climate and atmospheric change impacts on
489   aphids as vectors of plant diseases," in *Global Climate Change and Terrestrial*

490   *Invertebrates,* eds. S.N. Johnson & T.H. Jones. (Chichester, UK: John Wiley & Son),
491   148-175.

492 Ryalls, J.M.W., Riegler, M., Moore, B.D., and Johnson, S.N. (2013a). Biology and trophic
493   interactions of lucerne aphids. *Agr. Forest Entomol.* 15, 335-350. doi:
494   10.1111/afe.12024

495 Ryalls, J.M.W., Riegler, M., Moore, B.D., Lopaticki, G., and Johnson, S.N. (2013b). Effects
496   of elevated temperature and CO_2 on aboveground-belowground systems: a case study
497   with plants, their mutualistic bacteria and root/shoot herbivores. *Front. Plant Sci.* 4:
498   445. doi: 10.3389/fpls.2013.00445

499 Ryle, G.J.A., and Powell, C.E. (1992). The influence of elevated CO_2 and temperature on
500   biomass production of continuously defoliated white clover. *Plant Cell Environ.* 15,
501   593–599. doi: 10.1111/j.1365-3040.1992.tb01493.x

502 Schortemeyer, M., Hartwig, U.A., Hendrey, G.R., and Sadowsky, M.J. (1996). Microbial
503   community changes in the rhizospheres of white clover and perennial ryegrass
504   exposed to Free Air Carbon dioxide Enrichment (FACE). *Soil Biol. Biochem.* 28,
505   1717–1724. doi: 10.1016/S0038-0717(96)00243-X

506 Soussana, J.F., and Hartwig, U.A. (1996). The effects of elevated CO_2 on symbiotic N_2
507   fixation: a link between the carbon and nitrogen cycles in grassland ecosystems. *Plant*
508   *Soil* 187, 321–332. doi: 10.1007/BF00017097

509 Sun, Y., Guo, H., and Ge, F. (2016). Plant–aphid interactions under elevated CO_2: some cues
510   from aphid feeding behavior. *Front. Plant Sci.* 7, 502. doi: 10.3389/fpls.2016.00502

511 Whitaker, M.R.L., Katayama, N., and Ohgushi, T. (2014). Plant-rhizobia interactions alter
512   aphid honeydew composition. *Arthropod-Plant Interact.* 8, 213-220. doi:
513   10.1007/s11829-014-9304-5

514 Whittington, H.R., Tilman, D., and Powers, J.S. (2013). Consequences of elevated
515   temperatures on legume biomass and nitrogen cycling in a field warming and
516   biodiversity experiment in a North American prairie. *Func. Plant Biol.* 40, 1147-1158.
517   doi: 10.1071/FP12345

518 Zahran, H.H. (1999). Rhizobium-legume symbiosis and nitrogen fixation under severe
519   conditions and in an arid climate. *Microbiol. Mol. Biol R.* 63, 968-989. doi: 1092-
520   2172/99/

521 Zanetti, S., Hartwig, U.A., Luscher, A., Hebeisen, T., Frehner, M., Fischer, B.U., Hendrey,
522   G.R., Blum, H., and Nosberger, J. (1996). Stimulation of symbiotic N_2 fixation in
523   *Trifolium repens* L. under elevated atmospheric pCO_2 in a grassland ecosystem.
524   *Plant Physiol.* 112, 575–583. doi: doi.org/10.1104/pp.112.2.575

525

Figure Legends

Fig. 1. Impacts of CO_2, temperature and aphid presence on dry mass of *M. sativa* when growing in (A) non-supplemented and (B) Si supplemented soil. Mean values ± standard error shown (N = 20) with statistically significant effects indicated *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$. Significant factors for the whole experiment given in the upper panel (see Table 1 for full results) and for Si- and Si+ plants separately in the respective graphical panels.

Fig. 2. Impacts of CO_2, temperature and aphid presence on root nodulation (number per plant) of *M. sativa* when growing in (A) non-supplemented and (B) Si supplemented soil. Mean values ± standard error shown (N = 20) with statistically significant effects indicated *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$. Significant factors shown as per Fig. 1 legend.

Fig. 3. Impacts of CO_2, temperature and aphid presence on Si concentrations of *M. sativa* foliage (% dry mass) when growing in (A) non-supplemented and (B) Si supplemented soil. Mean values ± standard error shown (N = 9) with statistically significant effects indicated ***$P < 0.001$. Significant factors shown as per Fig. 1 legend.

Fig. 4. Impacts of CO_2 and temperature on aphid (*A. pisum*) abundance (number per plant) when feeding on *M. sativa* plants growing in (A) non-supplemented and (B) Si supplemented soil. Mean values ± standard error shown (N = 20) with statistically significant effects indicated ***$P < 0.001$. Significant factors shown as per Fig. 1 legend.

Fig. 5. Graphical summary showing how Si supplementation affects *M. sativa* growth, root nodulation and susceptibility to *A. pisum* in current and predicted changes to the climate and atmosphere. Compared with ambient CO_2 and temperature conditions (panel A), eCO_2 had beneficial effects on plant growth and nodulation (panel B), but these were negated when acting in combination with predicted increases in temperature (panel C). Si supplementation (panel D) restored root nodulation to comparable levels as those seen under eCO_2 conditions (without warming) and stimulated plant growth beyond this. Aphid responses were decoupled from changes in nodulation and growth with Si supplementation.

Plant response	Dry mass[1]		Root Nodules		Si Concentration[1]	
Model fixed effect	$F_{1,304}$	P	$RD_{1,304}$	P	$F_{1,94}$	P
CO_2	**51.86**	**<0.001**	**409.15**	**<0.001**	1.32	0.25
Temp	**10.28**	**<0.001**	**384.58**	**<0.001**	0.37	0.54
Aphids	**7.36**	**0.01**	384.57	0.90	1.17	0.28
Si	**150.55**	**<0.001**	**352.27**	**<0.001**	**15.22**	**<0.001**
$CO_2 \times$ Temp	3.19	0.07	351.81	0.50	2.09	0.15
$CO_2 \times$ Aphids	0.11	0.74	349.06	0.10	0.01	0.99
Temp $\times$ Aphids	0.63	0.43	345.60	0.06	1.03	0.31
$CO_2 \times$ Si	**4.06**	**0.04**	344.47	0.29	0.07	0.80
Temp $\times$ Si	2.00	0.16	**336.69**	**0.01**	0.01	0.92
Aphids $\times$ Si	1.83	0.18	336.66	0.86	0.15	0.70
$CO_2 \times$ Temp $\times$ Aphids	1.07	0.30	335.82	0.36	0.42	0.52
$CO_2 \times$ Temp $\times$ Si	**8.70**	**<0.001**	334.95	0.35	0.43	0.51
$CO_2 \times$ Aphids $\times$ Si	0.26	0.61	333.17	0.18	0.51	0.48
Temp $\times$ Aphids $\times$ Si	0.25	0.61	332.97	0.65	0.06	0.81
$CO_2 \times$ Temp $\times$ Aphids $\times$ Si	0.06	0.81	332.95	0.90	0.84	0.36

563 [1]Log transformed. Degrees of freedom in each column apply to all effects.

564 **Table 2.** Results of statistical tests examining the effects of CO_2, temperature and Si supplementation on
565 aphid abundance and colonisation success. Statistically significant (P <0.05) indicated in **bold** with residual
566 deviation (RD) given.

567

568

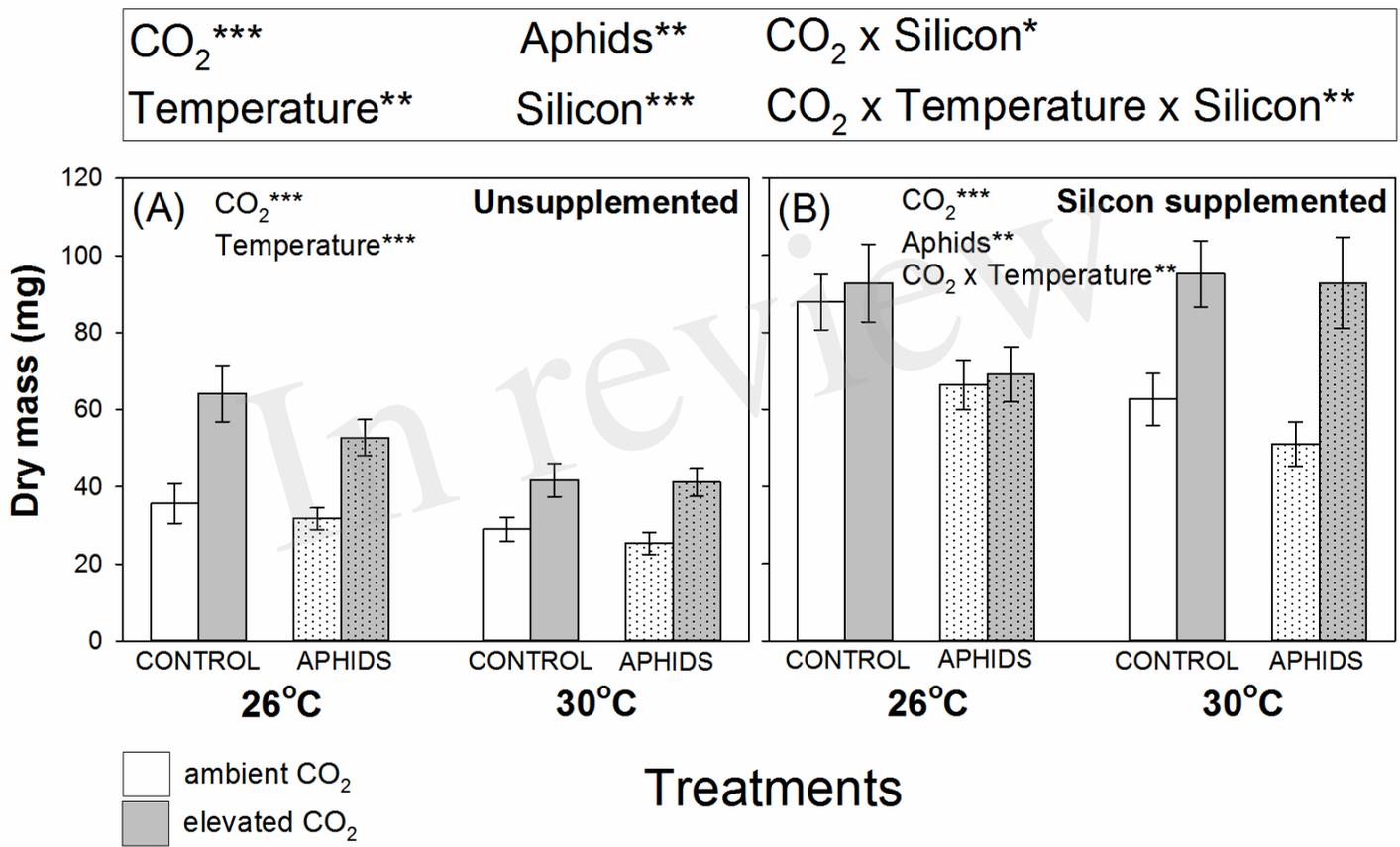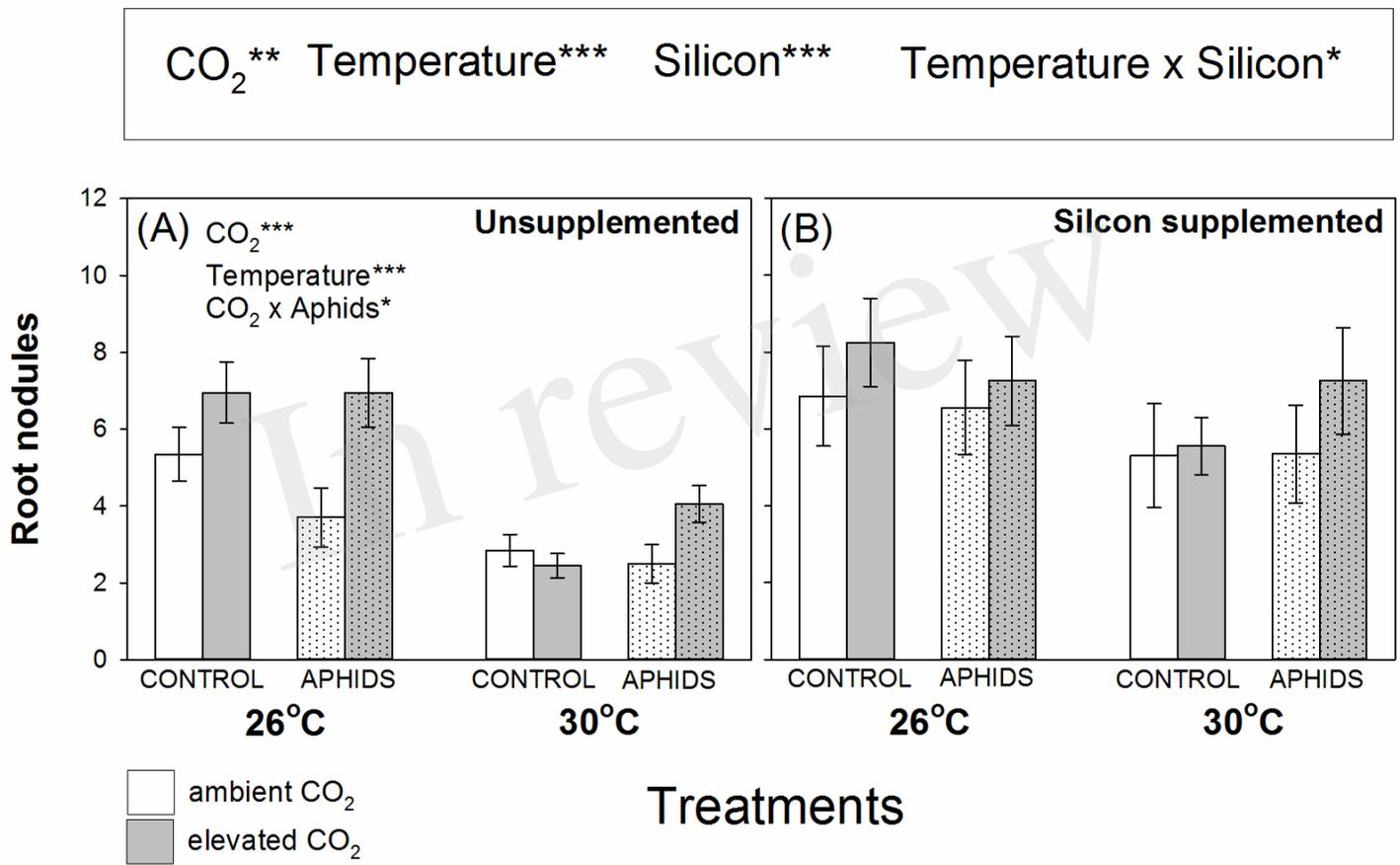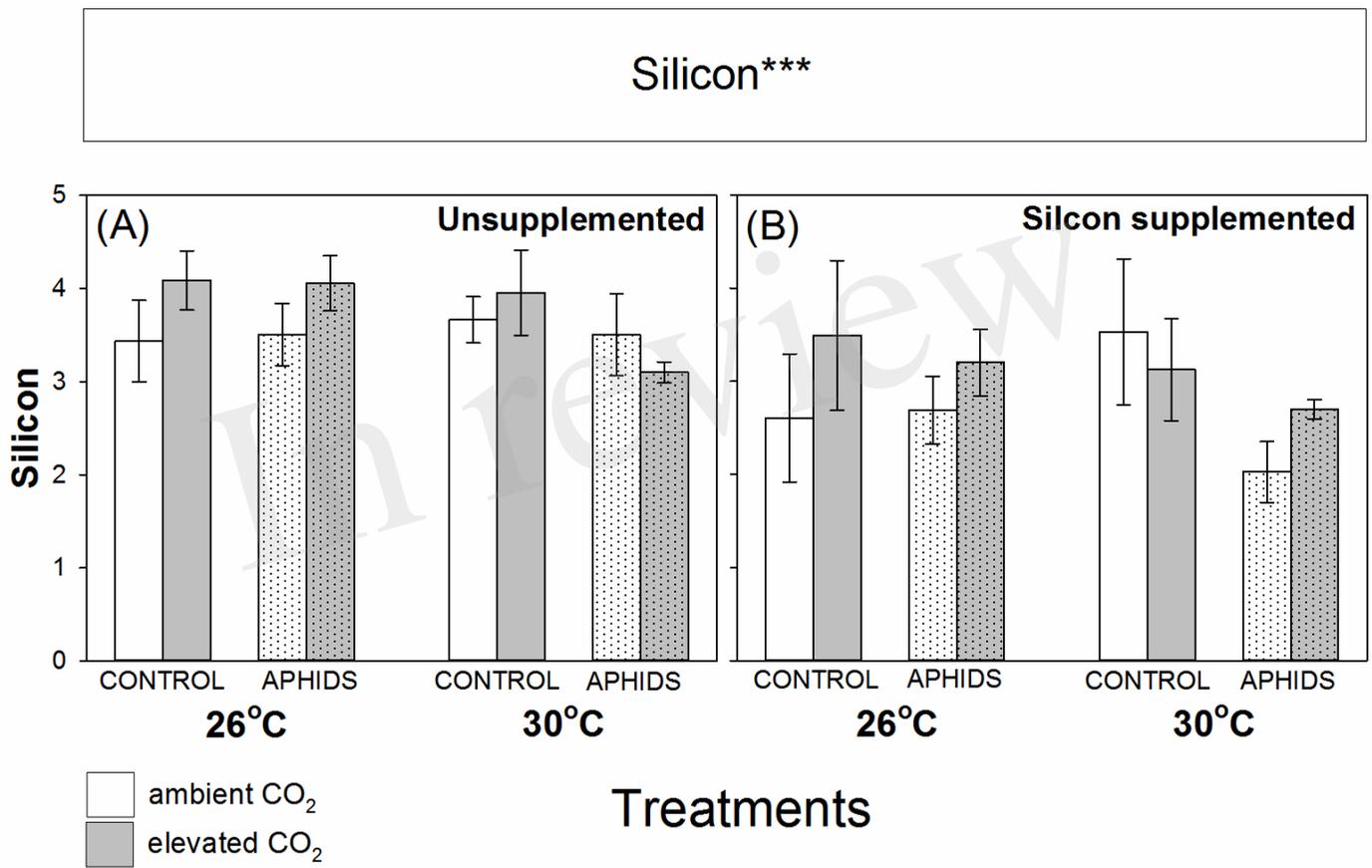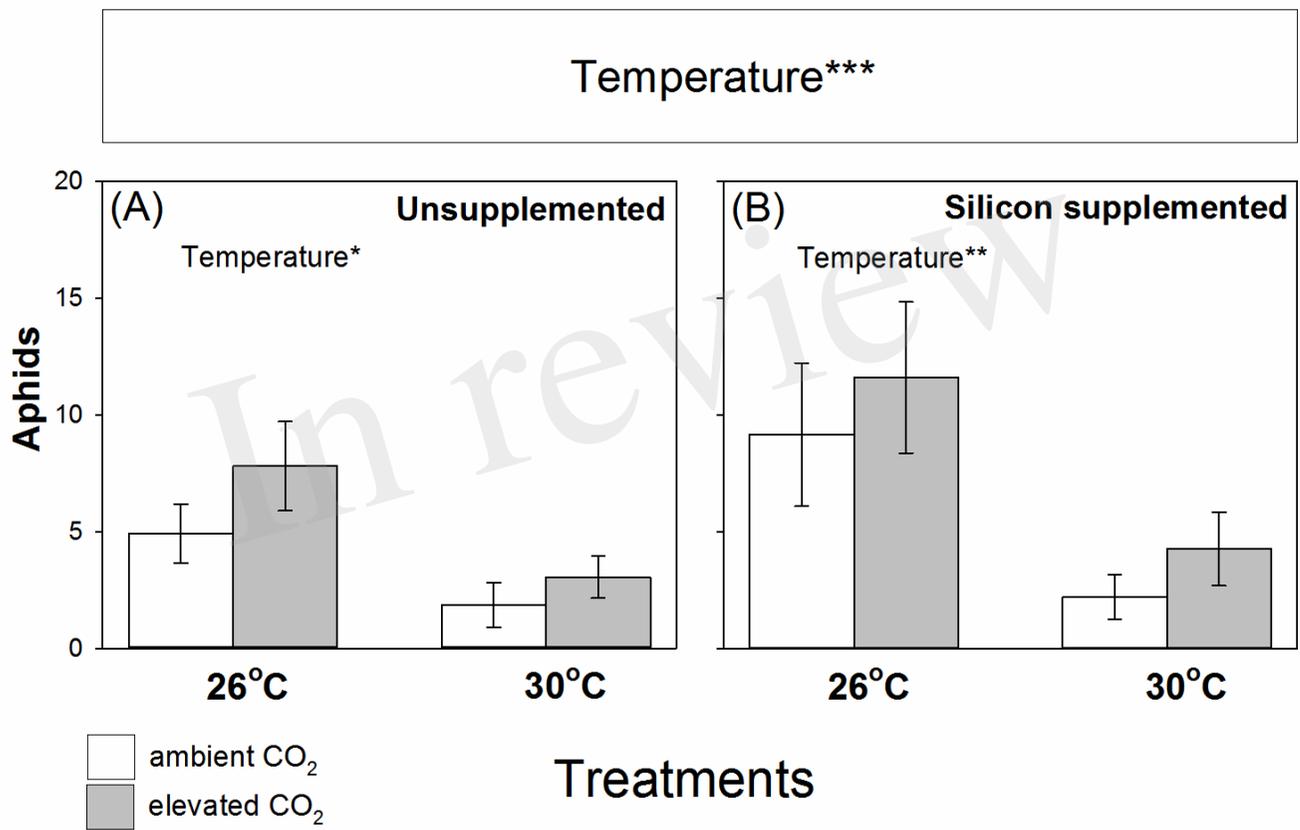| Plant response Model fixed effect	Aphid abundance		Aphid colonisation	
	$RD_{1,159}$	P	$RD_{1,159}$	P 569
CO_2	177.62	0.16	**215.23**	**0.026**
				570
Temp	**162.39**	**<0.001**	**197.21**	**<0.001**
Si	160.42	0.16	196.16	0.30 571
$CO_2 \times$ Temp	160.24	0.68	195.90	0.61 572
$CO_2 \times$ Si	160.24	0.93	195.90	0.99
Temp $\times$ Si	160.03	0.65	105.89	0.95 573
$CO_2 \times$ Temp $\times$ Si	159.91	0.73	194.50	0.24 574

575

Figure 1.TIF

Figure 2.TIF

Figure 3.TIF

Figure 4.TIF

Figure 5.JPEG