

This is a repository copy of *Benefits from Below : Silicon Supplementation Maintains Legume Productivity under Predicted Climate Change Scenarios*.

White Rose Research Online URL for this paper:
<http://eprints.whiterose.ac.uk/129496/>

Version: Accepted Version

Article:

Johnson, Scott N., Ryalls, James M. W., Gherlenda, Andrew N. et al. (2 more authors) (2018) Benefits from Below : Silicon Supplementation Maintains Legume Productivity under Predicted Climate Change Scenarios. *Frontiers in Plant Science*. 202. ISSN 1664-462X

<https://doi.org/10.3389/fpls.2018.00202>

Reuse

This article is distributed under the terms of the Creative Commons Attribution (CC BY) licence. This licence allows you to distribute, remix, tweak, and build upon the work, even commercially, as long as you credit the authors for the original work. More information and the full terms of the licence here:
<https://creativecommons.org/licenses/>

Takedown

If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing eprints@whiterose.ac.uk including the URL of the record and the reason for the withdrawal request.

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

Scott N. Johnson^{1*}, James M. Ryalls¹, Andrew Gherlenda¹, Adam Frew¹, Sue E. Hartley²

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

Submitted to Journal:
Frontiers in Plant Science

Specialty Section:
Functional Plant Ecology

Article type:
Original Research Article

Manuscript ID:
329441

Received on:
10 Nov 2017

Revised on:
25 Jan 2018

Frontiers website link:
www.frontiersin.org

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

The authors are grateful to James Stockdale for conducting the Si analysis and the 2015 class of the Invertebrate Biology (300918) unit for assistance in conducting the experiment. This research was part of a Hawkesbury Institute for the Environment exchange program awarded to SEH and SNJ.

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 eCO₂, 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 (+4°C), separately and in combination, to *M. sativa* growing in Si supplemented (S+) and un-supplemented soil (S-) to determine whether Si moderated the effects of eCO₂ and eT. Plants were either inoculated with the aphid *Acyrtosiphon pisum* or insect-free. In S- 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 S- 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%). *Acyrtosiphon 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. Ryalls¹, Andrew N. Gherlenda¹, Adam Frew^{1,2} and Susan E. Hartley³

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

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

³York 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 (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 eCO₂, 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 (+4°C), 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 *Acyrtosiphon 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%). *Acyrtosiphon 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.

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

45 **Introduction**

46 Projected increases in atmospheric carbon dioxide (CO₂) have been shown experimentally to
47 stimulate biological nitrogen fixation (BNF) in legumes (Soussana and Hartwig, 1996;
48 Zanetti et al., 1996; Hungate et al., 1999; Edwards et al., 2006; Lam et al., 2012). These
49 effects are strongest immediately after exposure to elevated CO₂ (eCO₂) (Hungate et al.,
50 2004) and when other nutrients (especially phosphorus) are not limiting (Rogers et al., 2009).
51 Elevated CO₂ (eCO₂) can promote BNF via several mechanisms, including larger numbers of
52 N₂ fixing symbiotic bacteria in the rhizosphere (Schortemeyer et al., 1996), increased
53 numbers of nodules which house N₂ fixing rhizobia bacteria (Ryle and Powell, 1992) and
54 enhanced nitrogenase activity (Norby, 1987). Broadly speaking, eCO₂ allows legumes to
55 increase rates of photosynthesis and allocate more carbon belowground to support increased
56 root nodulation and therefore BNF (Aranjuelo et al., 2014).

57
58 Researchers are becoming increasingly aware of the importance of testing multiple
59 environmental change factors because they are predicted to occur concurrently and often have
60 either synergistic or antagonistic impacts on one another (Robinson et al., 2012; Johnson and
61 Jones, 2017). Climate models predict, for instance, that air temperatures will increase in
62 tandem with increases in atmospheric CO₂ and warmer temperature may negate any positive
63 impacts of eCO₂ on plant growth (Newman et al., 2011). This may be particularly true in
64 legume systems because higher temperatures can have inhibitory effects on BNF due to the
65 relatively low tolerance of N₂-fixing bacteria to higher temperatures (Zahran, 1999;
66 Whittington et al., 2013; Aranjuelo et al., 2014). The optimal temperature for root nodule
67 symbiosis for temperate legumes is thought to be around 15-25⁰C, above which detrimental
68 effects can become evident (Aranjuelo et al., 2014). Elevated temperature (eT) can directly
69 hinder the development and functionality of root nodulation and accelerate nodule senescence
70 (Piha and Munns, 1987; Aranjuelo et al., 2006). In addition, eT can inhibit nodulation via
71 plant-mediated mechanisms, including reduced root hair formation, fewer nodulation sites
72 and poorer adherence of bacteria to root hairs (Hungria and Franco, 1993; Hungria and
73 Vargas, 2000; Aranjuelo et al., 2014).

74
75 Soil conditions play an important role in determining the extent to which eCO₂ and eT affect
76 root nodulation in legumes (Aranjuelo et al., 2014). Several studies report that
77 supplementation of soil silicon (Si) levels promotes growth in legumes (Horst and Marschner,
78 1978; Miyake and Takahashi, 1985; Guo et al., 2006; Johnson et al., 2017), though we know
79 less about the functional role of Si in legumes compared to other plant families such as the
80 Poaceae (Epstein, 1999; Cooke and Leishman, 2011). Moreover, Si supplementation can
81 increase rates of root nodulation and symbiosis with nitrogen fixing bacteria (Nelwamondo
82 and Dakora, 1999; Mali and Aery, 2008). However, how these positive effects of Si on
83 nodulation are affected by eCO₂ or eT, alone or in combination, have not yet been addressed.
84 If Si could maintain nodulation rates under future climate change scenarios, such as eT,
85 which usually decrease it, then such supplementation could be important in the mitigation of
86 climate change impacts on agriculture.

87
88 While rhizobial colonization promotes legume growth and vigour, this improved host quality
89 can also increase susceptibility to belowground (Quinn and Hower, 1986; Gerard, 2001;
90 Johnson and McNicol, 2010) and aboveground insect herbivores (Dean et al., 2009; Kempel
91 et al., 2009; Katayama et al., 2010; Dean et al., 2014; Whitaker et al., 2014). Beneficial
92 effects of rhizobia on herbivores most likely arise through increased provision of nitrogen,
93 which is frequently limiting in insect herbivore diets (Mattson, 1980). Increased provision of
94 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 Poaceae; 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 eCO₂ 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 *Acyrtosiphon*
104 *pisum*). We hypothesised that eCO₂ 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 CO₂ 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 CO₂ and temperature conditions.

109

110 **Methods and Materials**

111 *Insect cultures and plant material*

112 Four *Acyrtosiphon 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 CO₂ 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 CO₂ and temperature-controlled glasshouse
127 chambers (320 plants in total) using a fully factorial design of ambient CO₂ (aCO₂; 400 μmol
128 mol⁻¹) and eCO₂ (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 NaSiO₃.9H₂O 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 (CO₂, 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 CO₂, 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 eCO₂ 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 CO₂ and temperature in
195 the latter, with eCO₂ promoting plant growth more at eT than under aT conditions (Fig. 1B).

196
197 Root nodulation increased when plants grew under eCO₂ (+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 (CO₂, 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 CO₂ (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 reversed under Si+ conditions (Table S1,
209 Fig. S1). There was a very weak interaction between Si, aphids and CO₂.

210
211 Si concentration in the foliage was unaffected by CO₂, 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 eCO₂ (Fig. 4), although colonization
216 success increased by 14% under eCO₂ (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°C 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 eCO₂ 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

244 maturation (Ryalls and Harrington, 2017). This temperature threshold depends on species,
245 aphid biotype and geographical region (Awmack and Leather, 2007). *Acyrtosiphon 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 eCO₂ and eT but endosymbionts could partially
255 facilitate adaptation to climate and atmospheric change (Sun et al., 2016; Ryalls and
256 Harrington, 2017).

257
258 Several studies using temperature gradient greenhouses have examined the impacts of eCO₂
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 eCO₂
261 promoting nodulation, but only at the elevated experimental temperatures. This was probably
262 because the elevated temperature range used in experiments (c. 24°C; Aranjuelo et al., 2008)
263 was still within the optimal range (19-25°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°C to 30°C, root
266 nodulation in *M. sativa* has been reported to decrease by 22% under ambient CO₂ (aCO₂) and
267 by 56% under eCO₂ (Ryalls et al., 2013b).

268
269 Despite increasing evidence that the effects of eCO₂ 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 eCO₂ 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.

277
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.

286
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 CO₂, 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

294 increased nodule density reported in the present study tentatively suggests that greater nodule
295 abundance was not merely a function of increased root growth. Further work is needed, but Si
296 could provide a useful tool for mitigating some of the negative impacts of climate change on
297 crop production – in this instance maintaining nodulation rates of *M. sativa* in warmer
298 climates. Moreover, other studies suggest Si could redress negative effects of eCO₂ on plant-
299 herbivore interactions. For example, herbivore damage to roots of sugarcane was exacerbated
300 under eCO₂ conditions, but application of Si reversed these effects and stimulated crop
301 growth (Frew et al., 2017). Intervention strategies could include targeted application of Si
302 (e.g. furnace slag), selection of plant lines that naturally take up large amounts of Si
303 (McLarnon et al., 2017) and remediation of soils deficient in bioavailable Si (silicic acid)
304 (Guntzer et al., 2012; Johnson et al., 2016a).

305

306 **Acknowledgements**

307 The authors are grateful to James Stockdale for conducting the Si analysis and the 2015 class
308 of the Invertebrate Biology (300918) unit for assistance in conducting the experiment. This
309 research was part of a Hawkesbury Institute for the Environment exchange program awarded
310 to SEH and SNJ.

311

312 **Authors' contributions**

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

316

317

318

319 **References**

- 320 Aranjuelo, I., Arrese-Igor, C., and Molero, G. (2014). Nodule performance within a changing
321 environmental context. *J. Plant Physiol.* 171, 1076-1090. doi:
322 10.1016/j.jplph.2014.04.002
- 323 Aranjuelo, I., Irigoyen, J.J., Perez, P., Martinez-Carrasco, R., and Sanchez-Diaz, M. (2006).
324 Response of nodulated alfalfa to water supply, temperature and elevated CO₂:
325 productivity and water relations. *Environ. Exp. Bot.* 55, 130-141. doi:
326 10.1016/j.envexpbot.2004.10.007
- 327 Aranjuelo, I., Irigoyen, J.J., Sanchez-Diaz, M., and Nogues, S. (2008). Carbon partitioning in
328 N₂ fixing *Medicago sativa* plants exposed to different CO₂ and temperature
329 conditions. *Funct. Plant Biol.* 35, 306-317. doi: 10.1071/fp07296
- 330 Awmack, C., and Leather, S.R. (2007). "Growth and development," in *Aphids as Crop Pests*,
331 eds. H.F. Van Emden & R. Harrington. (Wallingford: CABI), 135-151.
- 332 Barton, C.V.M., Ellsworth, D.S., Medlyn, B.E., Duursma, R.A., Tissue, D.T., Adams, M.A.,
333 Eamus, D., Conroy, J.P., and McMurtrie, R.E. (2010). Whole-tree chambers for
334 elevated atmospheric CO₂ experimentation and tree scale flux measurements in south-
335 eastern Australia: The Hawkesbury Forest Experiment. *Agr. Forest Meteorol.* 150,
336 941–951. doi: doi.org/10.1016/j.agrformet.2010.03.001
- 337 Bieri, M., Baumgartner, J., Bianchi, G., Delucchi, V., and Arx, R.v. (1983). Development and
338 fecundity of pea aphid (*Acyrtosiphon pisum* Harris) as affected by constant
339 temperatures and by pea varieties. *Mitteilungen der Schweizerischen*
340 *Entomologischen Gesellschaft* 56, 163-171. doi: N/A
- 341 Brunner, S.M., Goos, R.J., Swenson, S.J., Foster, S.P., Schatz, B.G., Lawley, Y.E., and
342 Prischmann-Voldseth, D.A. (2015). Impact of nitrogen fixing and plant growth-

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

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

347 CSIRO (2007-2016). *Climate change in Australia: technical report* [Online]. Melbourne,
348 Australia: CSIRO. Available: <http://www.climatechangeinaustralia.gov.au/> [Accessed
349 10 November 2017].

350 Dean, J.M., Mescher, M.C., and De Moraes, C.M. (2009). Plant-rhizobia mutualism
351 influences aphid abundance on soybean. *Plant Soil* 323, 187-196. doi:
352 10.1007/s11104-009-9924-1

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

356 Dunbar, H.E., Wilson, A.C.C., Ferguson, N.R., and Moran, N.A. (2007). Aphid thermal
357 tolerance is governed by a point mutation in bacterial symbionts. *PloS Biol.* 5, 1006-
358 1015. doi: ARTN e96
359 10.1371/journal.pbio.0050096

360 Edwards, E.J., McCaffery, S., and Evans, J.R. (2006). Phosphorus availability and elevated
361 CO₂ affect biological nitrogen fixation and nutrient fluxes in a clover-dominated
362 sward. *New Phytol.* 169, 157–167. doi: 10.1111/j.1469-8137.2005.01568.x

363 Epstein, E. (1999). Silicon. *Annu Rev Plant Physiol. and Plant Molec. Biol.* 50, 641-664. doi:
364 10.1146/annurev.arplant.50.1.641

365 Erice, G., Irigoyen, J.J., Perez, P., Martinez-Carrasco, R., and Sanchez-Diaz, M. (2006).
366 Effect of elevated CO₂, temperature and drought on photosynthesis of nodulated
367 alfalfa during a cutting regrowth cycle. *Physiol. Plant.* 126, 458-468. doi:
368 10.1111/j.1399-3054.2005.00599.x

369 Erice, G., Irigoyen, J.J., Sanchez-Diaz, M., Avice, J.C., and Ourry, A. (2007). Effect of
370 drought, elevated CO₂ and temperature on accumulation of N and vegetative storage
371 proteins (VSP) in taproot of nodulated alfalfa before and after cutting. *Plant Sci.* 172,
372 903-912. doi: 10.1016/j.plantsci.2006.12.013

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

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

377 Garbuzov, M., Reidinger, S., and Hartley, S.E. (2011). Interactive effects of plant-available
378 soil silicon and herbivory on competition between two grass species. *Ann. Bot.* 108,
379 1355-1363. doi: 10.1093/aob/mcr230

380 Gerard, P.J. (2001). Dependence of *Sitona lepidus* (Coleoptera: Curculionidae) larvae on
381 abundance of white clover *Rhizobium* nodules. *B. Entomol. Res.* 91, 149–152. doi:
382 10.1079/BER200076

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

385 Guo, Z.G., Liu, H.X., Tian, F.P., Zhang, Z.H., and Wang, S.M. (2006). Effect of silicon on
386 the morphology of shoots and roots of alfalfa (*Medicago sativa*). *Aust. J. Exp. Agric.*
387 46, 1161-1166. doi: 10.1071/ea05117

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

390 Hungate, B.A., Dijkstra, P., Johnson, D.W., Hinkle, C.R., and Drake, B.G. (1999). Elevated
391 CO₂ increases nitrogen fixation and decreases soil nitrogen mineralization in Florida
392 scrub oak. *Glob. Change Biol.* 5, 781–789. doi: 10.1046/j.1365-2486.1999.00275.x

393 Hungate, B.A., Stiling, P.D., Dijkstra, P., Johnson, D.W., Ketterer, M.E., Hymus, G.J.,
394 Hinkle, C.R., and Drake, B.G. (2004). CO₂ elicits long-term decline in nitrogen
395 fixation. *Science* 304, 1291-1291. doi: 10.1126/science.1095549

396 Hungria, M., and Franco, A.A. (1993). Effects of high-temperature on nodulation and
397 nitrogen-fixation by *Phaseolus vulgaris* L. *Plant Soil* 149, 95-102. doi:
398 10.1007/bf00010766

399 Hungria, M., and Vargas, M.A.T. (2000). Environmental factors affecting N₂ fixation in
400 grain legumes in the tropics, with an emphasis on Brazil. *Field Crop. Res.* 65, 151-
401 164. doi: 10.1016/s0378-4290(99)00084-2

402 Johnson, S.N., Benefer, C.M., Frew, A., Griffiths, B.S., Hartley, S.E., Karley, A.J., Rasmann,
403 S., Schumann, M., Sonnemann, I., and Robert, C.A.M. (2016a). New frontiers in
404 belowground ecology for plant protection from root-feeding insects. *Appl. Soil Ecol.*
405 108, 96-107. doi: dx.doi.org/10.1016/j.apsoil.2016.07.017

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

409 Johnson, S.N., Hartley, S.E., Ryalls, J.M.W., Frew, A., DeGabriel, J.L., Duncan, M., and
410 Gherlenda, A. (2017). Silicon-induced root nodulation and synthesis of essential
411 amino acids in a legume is associated with higher herbivore abundance. *Funct. Ecol.*
412 31, 1903-1909. doi: 10.1111/1365-2435.12893

413 Johnson, S.N., and Jones, T.H. (2017). "Emerging Issues and Future Perspectives for Global
414 Climate Change Studies," in *Global Climate Change and Terrestrial Invertebrates*,
415 eds. S.N. Johnson & T.H. Jones. (Chichester, UK: John Wiley & Sons, Ltd), 368-
416 377.

417 Johnson, S.N., and McNicol, J.W. (2010). Elevated CO₂ and aboveground-belowground
418 herbivory by the clover root weevil. *Oecologia* 162, 209–216. doi: 10.1007/s00442-
419 009-1428-4

420 Katayama, N., Nishida, T., Zhang, Z.Q., and Ohgushi, T. (2010). Belowground microbial
421 symbiont enhances plant susceptibility to a spider mite through change in soybean
422 leaf quality. *Popul. Ecol.* 52, 499-506. doi:
423 Kempel, A., Brandl, R., and Schadler, M. (2009). Symbiotic soil microorganisms as players
424 in aboveground plant-herbivore interactions - the role of rhizobia. *Oikos* 118, 634-
425 640. doi: 10.1111/j.1600-0706.2009.17418.x

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

430 Lamb, R.J., and MacKay, P.A. (1988). Effects of temperature on developmental rate and
431 adult weight of Australian populations of *Acyrtosiphon pisum* (Harris) (Homoptera:
432 Aphididae). *Mem. Entomol. Soc. Can.* 120, 49-55. doi:
433 Lindroth, R.L., and Raffa, K.F. (2016). "Experimental approaches for assessing invertebrate
434 responses to global change factors," in *Global Climate Change and Terrestrial*
435 *Invertebrates*, eds. S.N. Johnson & T.H. Jones. (Chichester, UK: John Wiley &
436 Sons), 30-45.

437 Mackay, P.A., Lamb, R.J., and Smith, M.A.H. (1993). Variability in life-history traits of the
438 aphid, *Acyrtosiphon pisum* (Harris), from sexual and asexual populations. *Oecologia*
439 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 *Acyrtosiphon 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 CO₂ 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 CO₂ 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 CO₂ 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 CO₂ 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 CO₂ on symbiotic N₂
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 CO₂: 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 N₂ fixation in
523 *Trifolium repens* L. under elevated atmospheric pCO₂ in a grassland ecosystem.
524 *Plant Physiol.* 112, 575–583. doi: doi.org/10.1104/pp.112.2.575

525

526 **Figure Legends**

527 **Fig. 1.** Impacts of CO₂, temperature and aphid presence on dry mass of *M. sativa* when growing in (A) non-
528 supplemented and (B) Si supplemented soil. Mean values ± standard error shown (N = 20) with statistically
529 significant effects indicated **P* < 0.05, ***P* < 0.01 and ****P* < 0.001. Significant factors for the whole
530 experiment given in the upper panel (see Table 1 for full results) and for Si- and Si+ plants separately in the
531 respective graphical panels.

532 **Fig. 2.** Impacts of CO₂, temperature and aphid presence on root nodulation (number per plant) of *M. sativa*
533 when growing in (A) non-supplemented and (B) Si supplemented soil. Mean values ± standard error shown
534 (N = 20) with statistically significant effects indicated **P* < 0.05, ***P* < 0.01 and ****P* < 0.001. Significant
535 factors shown as per Fig. 1 legend.

536 **Fig. 3.** Impacts of CO₂, temperature and aphid presence on Si concentrations of *M. sativa* foliage (% dry
537 mass) when growing in (A) non-supplemented and (B) Si supplemented soil. Mean values ± standard error
538 shown (N = 9) with statistically significant effects indicated ****P* < 0.001. Significant factors shown as per
539 Fig. 1 legend.

540 **Fig. 4.** Impacts of CO₂ and temperature on aphid (*A. pisum*) abundance (number per plant) when feeding on
541 *M. sativa* plants growing in (A) non-supplemented and (B) Si supplemented soil. Mean values ± standard
542 error shown (N = 20) with statistically significant effects indicated ****P* < 0.001. Significant factors shown
543 as per Fig. 1 legend.

544 **Fig. 5.** Graphical summary showing how Si supplementation affects *M. sativa* growth, root nodulation and
545 susceptibility to *A. pisum* in current and predicted changes to the climate and atmosphere. Compared with
546 ambient CO₂ and temperature conditions (panel A), eCO₂ had beneficial effects on plant growth and
547 nodulation (panel B), but these were negated when acting in combination with predicted increases in
548 temperature (panel C). Si supplementation (panel D) restored root nodulation to comparable levels as those
549 seen under eCO₂ conditions (without warming) and stimulated plant growth beyond this. Aphid responses
550 were decoupled from changes in nodulation and growth with Si supplementation.

551 **Table 1.** Results of statistical tests examining the effects of CO₂, temperature (Temp), aphid presence and Si supplementation (Si) on plant growth, root nodulation,
 552 and foliar Si concentrations. Statistically significant ($P < 0.05$) factors indicated in **bold** with Fisher's (F) or residual deviation (RD) given depending on the models
 553 used. Analysis conducted on transformed data as indicated.

Plant response Model fixed effect	Dry mass ¹		Root Nodules		Si Concentration ¹ ⁵⁵⁴	
	F _{1,304}	P	RD _{1,304}	P	F _{1,94}	P
CO ₂	51.86	<0.001	409.15	<0.001	1.32	0.25 ⁵⁵⁵
Temp	10.28	<0.001	384.58	<0.001	0.37	0.54
Aphids	7.36	0.01	384.57	0.90	1.17	0.28 ⁵⁵⁶
Si	150.55	<0.001	352.27	<0.001	15.22	<0.001
CO ₂ × Temp	3.19	0.07	351.81	0.50	2.09	0.15 ⁵⁵⁷
CO ₂ × Aphids	0.11	0.74	349.06	0.10	0.01	0.99
Temp × Aphids	0.63	0.43	345.60	0.06	1.03	0.31 ⁵⁵⁸
CO ₂ × Si	4.06	0.04	344.47	0.29	0.07	0.80
Temp × Si	2.00	0.16	336.69	0.01	0.01	0.92 ⁵⁵⁹
Aphids × Si	1.83	0.18	336.66	0.86	0.15	0.70
CO ₂ × Temp × Aphids	1.07	0.30	335.82	0.36	0.42	0.52 ⁵⁶⁰
CO ₂ × Temp × Si	8.70	<0.001	334.95	0.35	0.43	0.51
CO ₂ × Aphids × Si	0.26	0.61	333.17	0.18	0.51	0.48 ⁵⁶¹
Temp × Aphids × Si	0.25	0.61	332.97	0.65	0.06	0.81
CO ₂ × Temp × Aphids × Si	0.06	0.81	332.95	0.90	0.84	0.36 ⁵⁶²

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

564 **Table 2.** Results of statistical tests examining the effects of CO₂, 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}	<i>P</i>	RD _{1,159}	<i>P</i>
CO ₂	177.62	0.16	215.23	0.026
Temp	162.39	<0.001	197.21	<0.001
Si	160.42	0.16	196.16	0.30
CO ₂ × Temp	160.24	0.68	195.90	0.61
CO ₂ × Si	160.24	0.93	195.90	0.99
Temp × Si	160.03	0.65	105.89	0.95
CO ₂ × Temp × Si	159.91	0.73	194.50	0.24

575

In review

Figure 1.TIF

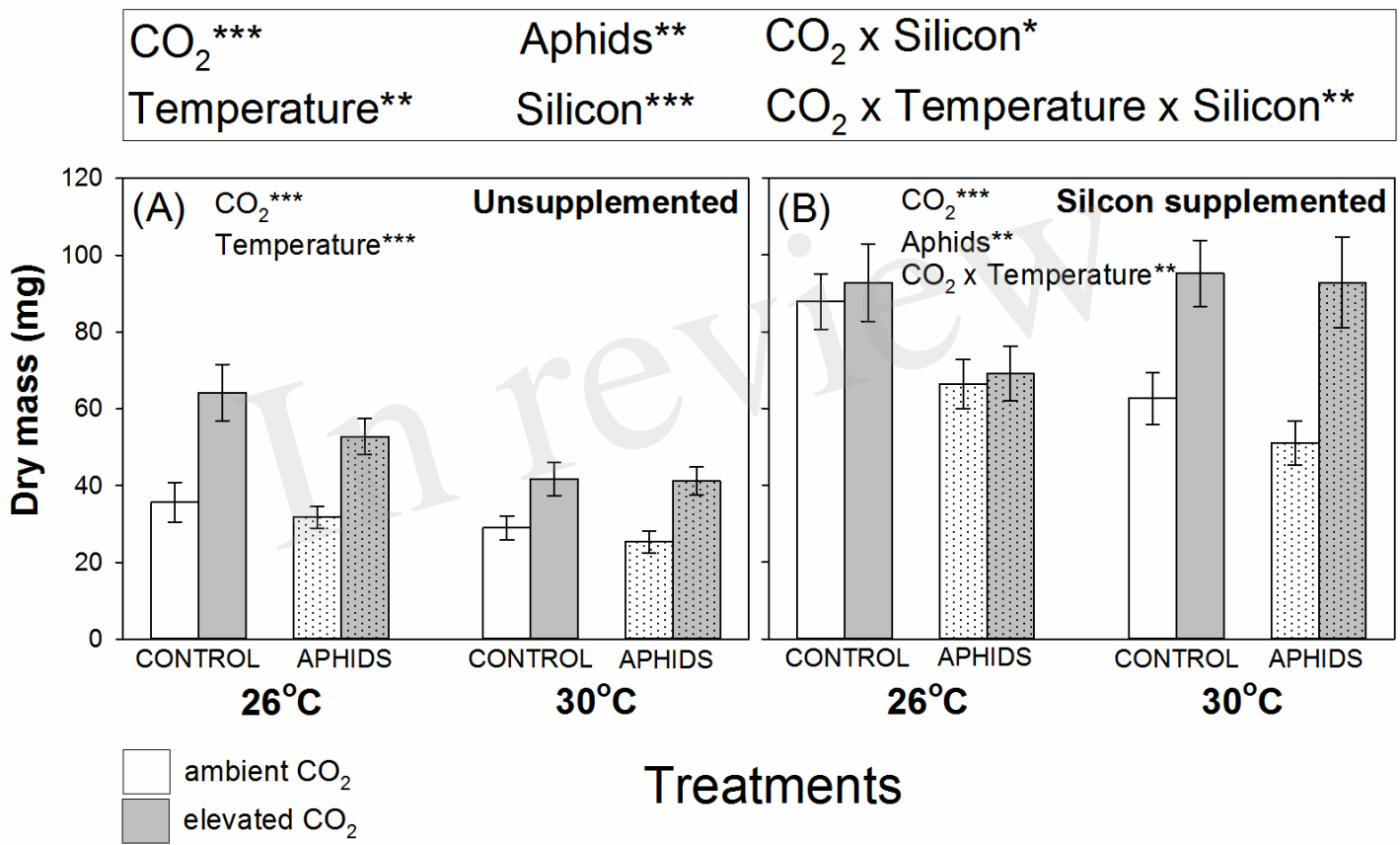


Figure 2.TIF

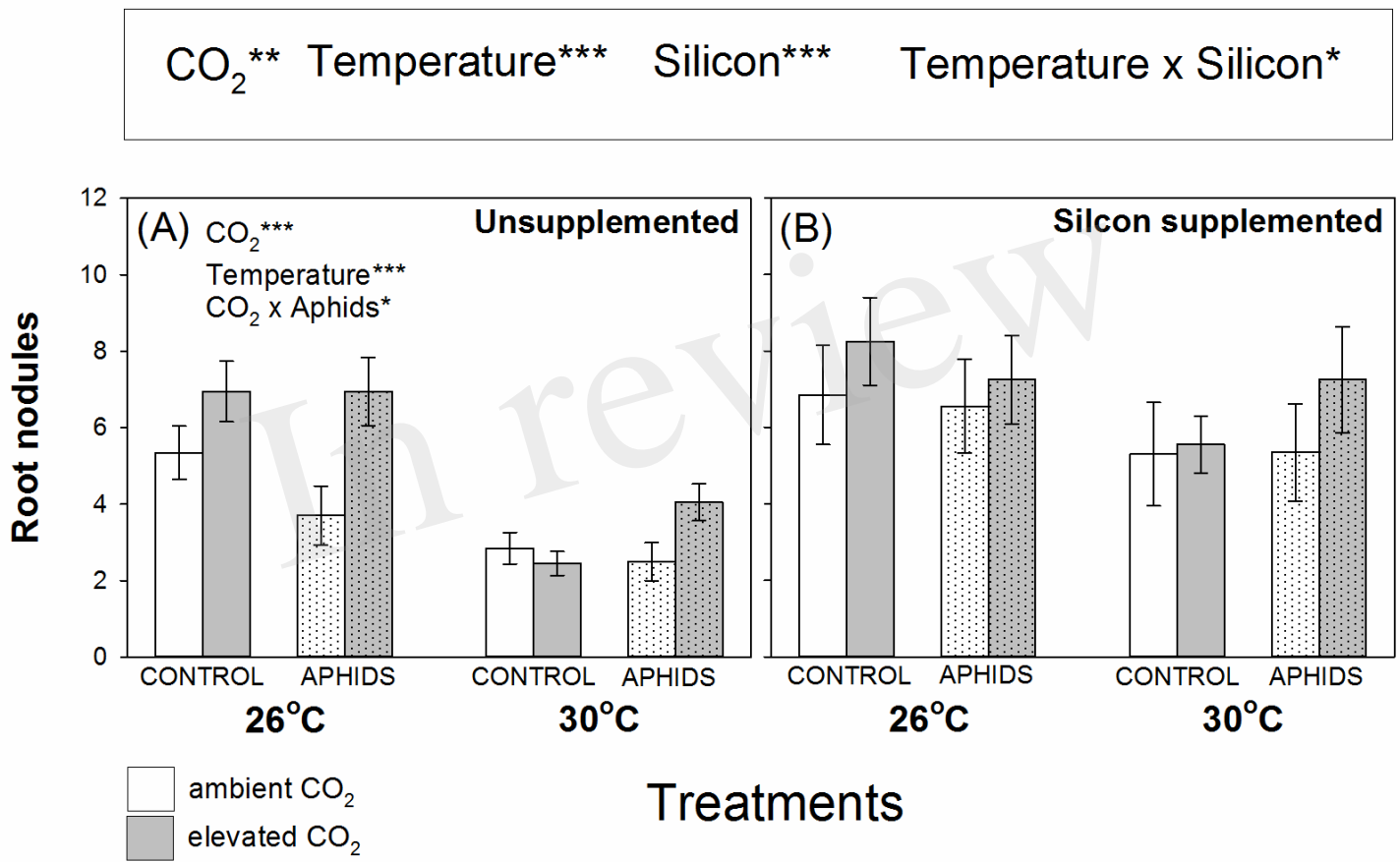


Figure 3.TIF

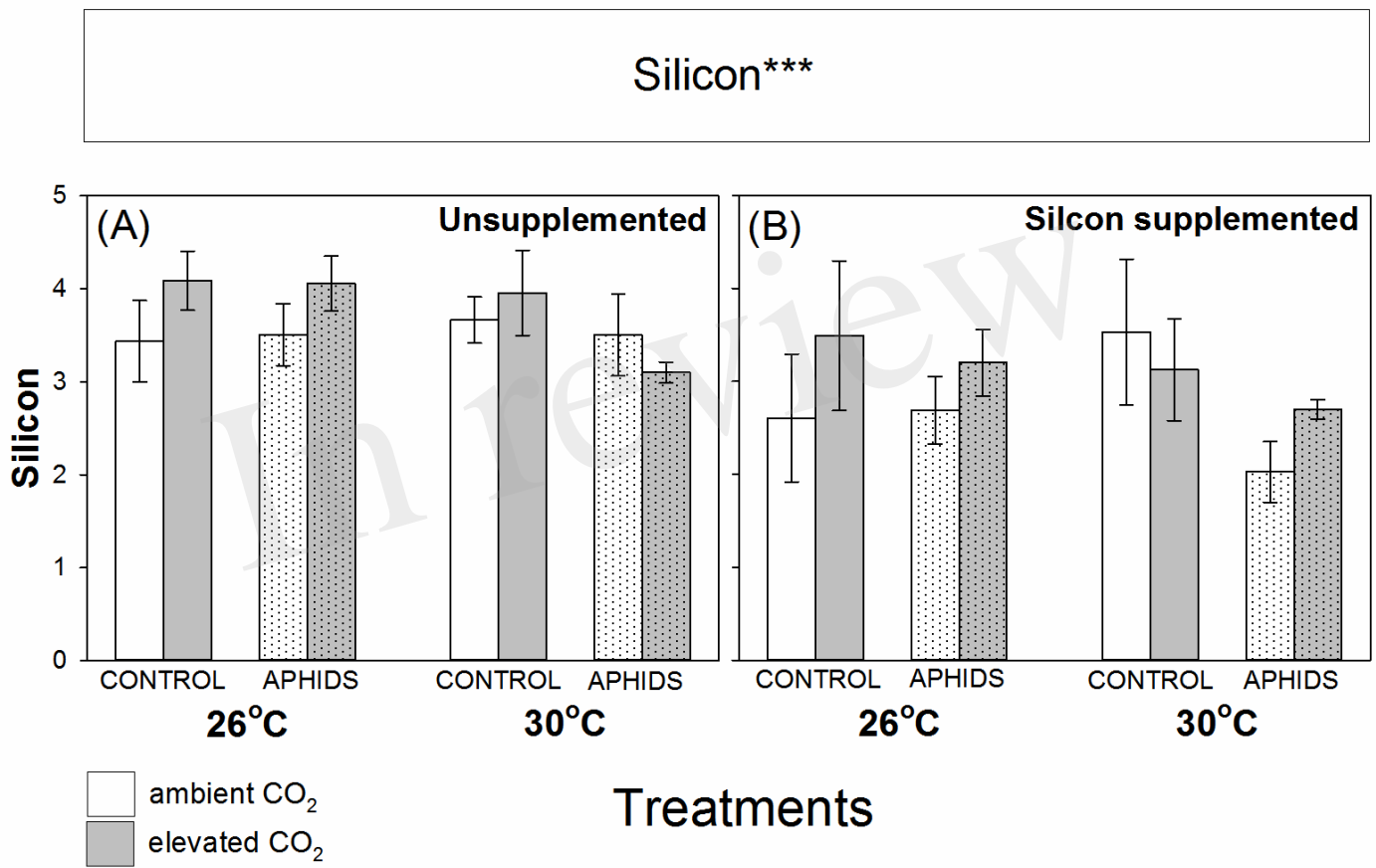


Figure 4.TIF

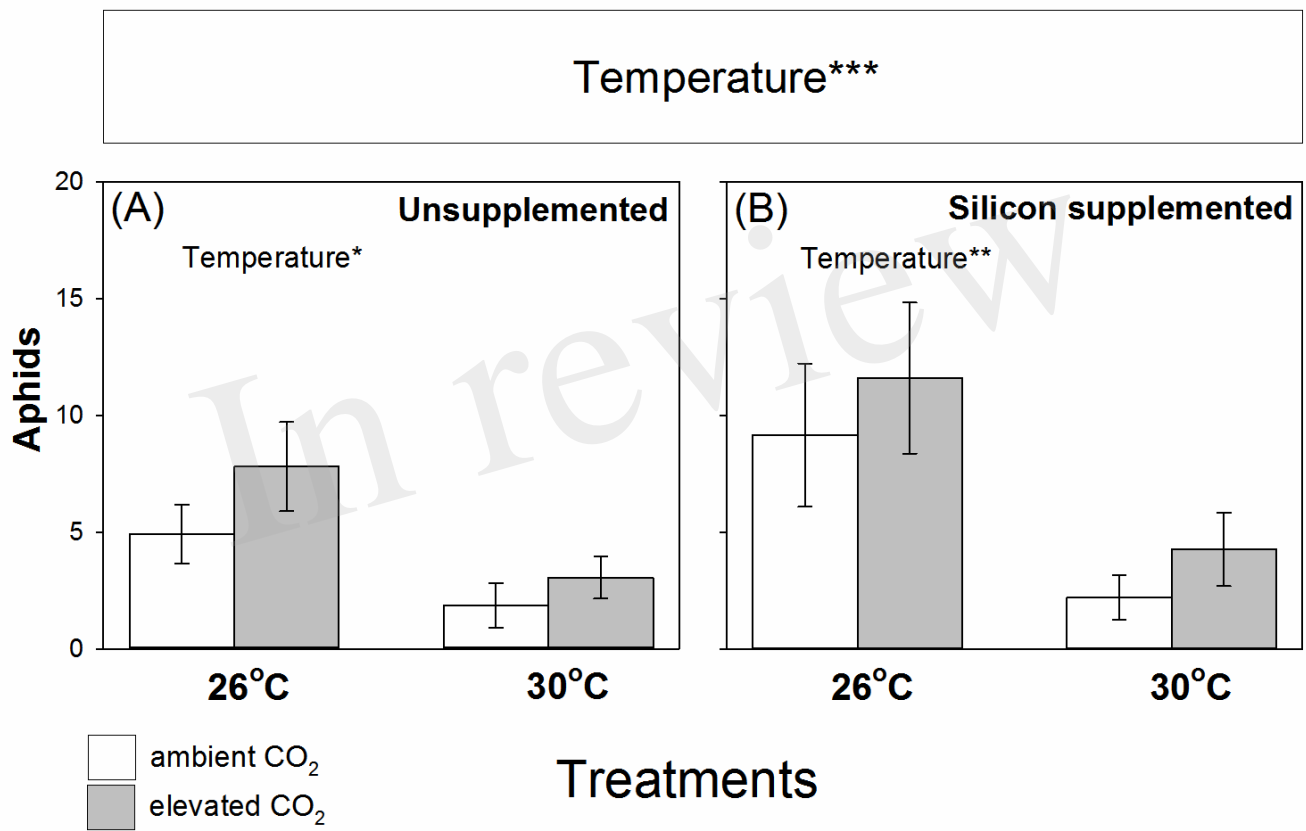


Figure 5.JPEG

