

Climate warming and plant biomechanical defences: silicon addition contributes to herbivore suppression in a pasture grass

Article

Accepted Version

Johnson, S. N., Ryalls, J. M. W. ORCID: <https://orcid.org/0000-0003-2015-3605>, Barton, C. V. M., Tjoelker, M. G., Wright, I. J. and Moore, B. D. (2019) Climate warming and plant biomechanical defences: silicon addition contributes to herbivore suppression in a pasture grass. *Functional Ecology*, 33 (4). pp. 587-596. ISSN 0269-8463 doi: 10.1111/1365-2435.13295 Available at <https://reading-pure-test.eprints-hosting.org/82353/>

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To link to this article DOI: <http://dx.doi.org/10.1111/1365-2435.13295>

Publisher: Wiley

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2 **Climate warming and plant biomechanical**
3 **defences: silicon addition contributes to**
4 **herbivore suppression in a pasture grass**

5
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15
16
17 **Abstract**

- 18 1. Plants, notably the Poaceae, often accumulate large amounts of silicon (Si) from the
19 soil. Si has multiple functional roles, particularly for alleviating abiotic and biotic
20 stresses (e.g. defence against herbivores). Recent evidence suggests that
21 environmental change, including temperature changes, can diminish Si accumulation
22 which could affect functions such as herbivore defence.
- 23 2. Using a field warming experiment, we grew a pasture grass (*Phalaris aquatica*) that
24 was either supplemented or untreated with Si (+Si and -Si, respectively) under
25 ambient and elevated (+2.8°C above ambient) air temperatures. We quantified soil
26 water, plant growth rates, Si accumulation, leaf biomechanical properties and *in situ*
27 relative growth rates of a herbivorous global insect pest (*Helicoverpa armigera*).

- 28 3. Si supplementation promoted shoot and root biomass by c. 48% and 61%,
29 respectively under ambient temperatures, but these gains were not apparent under
30 warmed conditions.
- 31 4. Warmer temperatures reduced Si uptake by -Si plants by c. 17%, potentially due to
32 the lower levels of soil water content in warmed plots. Si supplementation, however,
33 increased Si accumulation in leaves by c. 24% in warmed plots restoring Si levels to
34 those seen under ambient temperatures.
- 35 5. Si supplementation enhanced biomechanical properties in the leaves, but this was only
36 statistically significant under ambient temperatures; leaves of +Si plants required 42%
37 more force to fracture and were 30% tougher at the midrib than leaves of -Si plants.
38 The relative growth rates of *H. armigera* declined by 56% when feeding on +Si plants
39 under ambient temperatures and while Si supplementation caused a trend towards
40 declining herbivore growth rates under warmer conditions, this was not statistically
41 significant.
- 42 6. We conclude that climate warming may mitigate the beneficial effects of Si on
43 *Phalaris aquatica* in the short term, potentially by reducing Si uptake. While Si
44 uptake can be restored with Si supplementation, Si-enhanced biomechanical defences
45 against a global pest may not be fully restored under warmer temperatures.

46
47 **Keywords** – climate change, global warming, insect herbivore, silicon, silica, leaf toughness
48

49 **Introduction**

50 There is emerging consensus that silicon (Si) plays an important functional role in
51 plants, particularly in terms of mitigating the impacts of adverse environmental conditions
52 (Cooke & Leishman 2011; Frew, Weston, Reynolds & Gurr 2018). For abiotic stresses, Si
53 can alleviate the effects of drought, salt stress, toxic metals, extreme temperatures and

54 nutrient deficiency (Liang, Sun, Zhu & Christie 2007; Guntzer, Keller & Meunier 2012;
55 Cooke & Leishman 2016). Similarly for biotic stresses, Si can reduce pathogen infection
56 (Van Bockhaven, De Vleeschauwer & Hofte 2013; Wang et al. 2017) and herbivory
57 (Reynolds, Keeping & Meyer 2009; Hartley & DeGabriel 2016). Indeed, the fact that many
58 plants, particularly grasses, are hyper-accumulators of Si (up to 10% of dry mass) and are
59 adapted to increase Si absorption following episodes of stress, points to the functional
60 significance of this non-essential element (Epstein 1999; Massey, Ennos & Hartley 2007;
61 Hartley & DeGabriel 2016).

62 Si-based resistance to pathogens and herbivory has been linked to silicification of
63 plant tissues which can affect biomechanical properties such as abrasiveness and penetrability
64 of the plant epidermis (Debona, Rodrigues & Datnoff 2017). In addition to physical
65 resistance, Si is increasingly seen as an agent that can prophylactically moderate defensive
66 responses that are triggered by herbivores and pathogens (Fauteux, Chain, Belzile, Menzies
67 & Bélanger 2006; Van Bockhaven et al. 2013; Ye et al. 2013; Coskun et al. 2018). The
68 mechanisms for this are debatable with Coskun et al. (2018) most recently proposing that,
69 rather than stimulating defensive signalling pathways in plants, Si interfered with pathogen
70 and herbivores' attempts to suppress defensive responses in the plant. In particular, they
71 propose that effector molecules produced by pathogens and herbivores are impeded in the
72 extracellular Si matrix which allows plants to mount defensive responses unhindered.

73 Arguably, Si-based biomechanical defences are more important for resistance to
74 herbivores than pathogens because biomechanical defences can affect herbivores adversely in
75 multiple ways beyond being a physical barrier (Clissold 2008; Massey & Hartley 2009).
76 Indeed, biomechanical properties of plants can influence entire communities of herbivores,
77 with chewing herbivores being especially affected (Peeters, Sanson & Read 2007). Si
78 deposition as discrete phytoliths or other abrasive structures, for example, can wear down

79 insect mouthparts (Massey & Hartley 2009 but see Kvedaras et al. 2009). Ingested Si
80 structures may also hinder efficient food processing and prevent herbivores from extracting
81 adequate nutrition from ingested plant material (Massey & Hartley 2006). Moreover, Si
82 deposition within and between cell walls can make cells more rigid and therefore harder to
83 mechanically crush (Hunt, Dean, Webster, Johnson & Ennos 2008). Cooke, DeGabriel and
84 Hartley (2016) highlight how surprisingly few studies measure biomechanical properties of
85 plant tissues in relation to Si supplementation and herbivore resistance. In the present study
86 we aimed to redress this by measuring two biomechanical properties (i.e. force of fracture
87 and leaf toughness) with relevance to resistance to chewing herbivores (Sanson, Read,
88 Aranwela, Clissold & Peeters 2001).

89 Plants accumulate Si from the soil in the form of soluble silicic acid which is then
90 distributed throughout the plant (Currie & Perry 2007; Ma & Yamaji 2015). Initially thought
91 to be a passive uptake process largely driven by transpiration rates, a number of specialized
92 influx and efflux Si transporters have now been identified (reviewed by Ma & Yamaji 2015;
93 Deshmukh & Bélanger 2016). Some of these transporters are water channel aquaporins,
94 which mediate passive transport, but several are active anion transporters, which plants can
95 control to some extent (Ma, Yamaji, Tamai & Mitani 2007; Yamaji, Mitatni & Ma 2008).
96 The extent to which plants can actively control Si accumulation relative to passive uptake that
97 is hydraulically and osmotically driven is still debated (Quigley & Anderson 2014; Kumar,
98 Milstein, Bрами, Elbaum & Elbaum 2017; McLarnon, McQueen-Mason, Lenk & Hartley
99 2017). There is, however, wider recognition that climatic factors such as water availability
100 and temperature influence Si accumulation (Schoelynck et al. 2014; Hartley 2015; Maguire,
101 Templer, Battles & Fulweiler 2017).

102 Depending on emissions of greenhouse gases, global mean air temperatures are
103 predicted to increase by 0.3-1.7 °C (low emissions), 1.1-3.1 °C (moderate emissions) and 2.6-

104 4.8 °C (high emissions) by 2081-2100 (IPCC 2013). Given the important role played by Si in
105 plant stress alleviation, it is surprising that so little is known about how global warming will
106 affect Si uptake in plants (Cooke et al. 2016; Johnson & Hartley 2018). Warming often
107 increases transpiration rates in plants which may increase Si uptake, as could higher
108 metabolically-driven uptake of nutrients in general. Alternatively, warming may decrease soil
109 water availability, either through direct drying effects or increased water uptake due to
110 increased vegetation growth, both of which may reduce the pool of soluble Si in soil (Hartley
111 2015; Cooke et al. 2016).

112 We adopted the hypothesis that (1) warming promotes plant growth and Si uptake.
113 Consequently, (2) increased Si uptake, whether due to temperature or Si supplementation,
114 increases biomechanical resistance of leaves and reduces herbivore performance (relative
115 growth rates). The aim of this study was therefore to determine the effects of warming on
116 growth of an important pasture grass (*Phalaris aquatica*), Si accumulation under low and
117 high Si availability, biomechanical defences in the leaves and how these changes affected the
118 performance of a global herbivore pest, the cotton bollworm (*Helicoverpa armigera*). In
119 order to include stochastic variation in ambient and elevated (c. +3°C) temperatures (as would
120 occur under field conditions), we conducted the experiment in outdoor plots with controlled
121 infra-red heating.

122

123 **Materials and Methods**

124 *Experimental site*

125 Twelve warming frames (Fig.1A) were established on an alluvial floodplain on the
126 Hawkesbury campus of Western Sydney University at 25 m elevation (33°36' 40" S,
127 150°44'26.5" E). The frames were identical in all respects except that six had the capacity to
128 increase air temperature in the underlying vegetation (c. 1.0 m²) by up to 3°C. Frames were

129 constructed by P&C Laws (Richmond, NSW, Australia) and comprised rectangular steel (250
130 mm × 250 mm) tubing (1.7 m × 1.7 m × 0.90 m; width × length × height). Frames were
131 spaced 2.0 – 2.2 m apart from one another at the site with the warmed plots assigned at
132 random. The plot was warmed using 1000W infra-red ceramic heaters (RS components part
133 376-2723, Mor Electric Heating Assoc. Inc. MI, USA) mounted on the frame centrally above
134 the plot (see example in Fig. 1B). This primarily warmed the vegetation and soil. A control
135 system regulated the output of the heating lamps to maintain the canopy surface temperature
136 at the desired set point. The control system comprised a purpose-built temperature sensor and
137 Proportional-Integral-Derivative (PID) controller mounted on each frame and a central data
138 logger (CR1000, Campbell Scientific), which logged the canopy surface temperature of each
139 plot and coordinated the temperature controllers. The temperature controllers were built using
140 a micro controller (Arduino Nano), an infra-red thermometer (Melexis' MLX90614ESF-
141 BAA) and a solid-state relay (FOTEK SSR-40DA). The 12 Arduino controllers were
142 networked with the data logger using RS485 converters (MAX485) and a MODBUS protocol
143 with the logger as the master. The logger polled the six ambient unwarmed frames each
144 minute then calculated the average ambient temperature and added the desired offset (+3°C)
145 before sending this as the set point to the six heated frames. Each controller used a PID
146 control algorithm and pulse width modulation to regulate the output of the heater to maintain
147 the temperature at the set point.

148 Four pots (410mm x 410 mm and 310 mm deep) were submerged at ground-level in a
149 2 × 2 formation identical to the rain exclusion shelters depicted in Johnson et al. (2016). Pots
150 were filled with the soil from the excavated trenches, which was of the Clarendon Formation
151 type (Isbell 2002) (see Table S1 for full details). It was a low-moderate fertility sandy loam
152 soil with low organic matter and relatively low bioavailable silicon (with reference to Haynes
153 2017).

154 *Experimental Procedure*

155 Two of the four pots in each plot received a one-off silicon supplementation (+Si
156 plants hereafter) in the form of 126g of solid calcium silicate (CaOSiO₄) (PlantTuff™
157 supplied by Australian Steel Mill Services, Port Kembla, NSW, Australia). This equates to an
158 application rate of 10 tonnes ha⁻¹ which is at the upper limit of application rates (Personal
159 Communication, NSW Environment Protection Agency). Calcium silicate was lightly tilled
160 into the top 3-5 cm of soil; non-supplemented plants (-Si plants hereafter) had soil lightly
161 tilled without any amendment. In February 2017, c. 200 seeds of a common Australian
162 naturalised pasture grass (Harding grass, *Phalaris aquatica* cultivar Holdfast; Heritage Seeds,
163 NSW) were germinated in potting mix (Native soil mix, Turtle Nursery and Landscape
164 Supplies, NSW) in the glasshouse for three weeks. Plants were then transplanted to the
165 warming frames (48 pots each containing four plants) on 23 February 2017. Each frame
166 therefore contained two +Si pots (2 × 4 plants) and two -Si pots (2 × 4 plants). Plants
167 received natural rainfall (310 mm over six weeks) and supplemental irrigation (c. 200 ml
168 every c. 10 days). Volumetric soil water content measurements were taken using a hand held
169 moisture probe (HydroSense 2; Campbell Scientific) at weekly intervals. After 50 days, a
170 plant was selected at random and removed from each pot and washed free of soil. The roots
171 and shoots were freeze dried, weighed and then ground for analysis of Si concentrations (see
172 below). The remaining plants had been selected at random to determine leaf biomechanical
173 properties (measured on day 50) and herbivore performance (*in situ* between days 44–50).

174

175 *Silicon uptake in the plant*

176 Si concentrations in the shoots and roots were determined with an X-ray fluorescence
177 spectrometer (Epsilon-3x, PANalytical-Almelo, The Netherlands) using a method similar to
178 that of Reidinger, Ramsey & Hartley (2012). In summary, root and leaf material was ground

179 to a fine powder and analyzed according to the procedure described in Hiltbold et al. (2017).
180 Si levels were expressed as shoot and root Si concentration (as % of dry mass) and calibrated
181 against plant-certified reference material of known Si content (Garbuzov, Reidinger &
182 Hartley 2011; Hiltbold et al. 2017).

183

184 *Force of fracture and leaf toughness*

185 The force to fracture leaves was measured with a shear test (Aranwela, Sanson &
186 Read 1999) using the purpose-built machine described by Wright and Cannon (2001). The
187 machine measured the force required to cut a leaf at a constant cutting angle (20°) and speed.
188 For each leaf, one cut was made perpendicular to the midrib. For most leaves, force to
189 fracture increased as the blade progressed through the lamina, to a clear peak at the midrib
190 (Fig. S1). Once the midrib had been severed, the remaining leaf blade most often folded back
191 under the blade, increasing friction on the blade and making measurements of force of
192 fracture from the point onwards unreliable. Consequently, we calculated maximum force of
193 fracture up to and including the midrib – this was always occurred when the midrib was
194 severed (Fig. S1). Midrib and lamina thickness were measured at two points each with a
195 digital micrometer. Midrib toughness was determined by dividing maximum force of fracture
196 by midrib thickness. Overall leaf toughness was estimated by dividing the average force to
197 fracture from initial blade contact with the leaf to the point of midrib fracture (Fig. S1) by the
198 average thickness of the midrib and lamina combined. In total, force to fracture
199 measurements were taken from 2-4 leaves per plant.

200

201 *Insect herbivore performance*

202 Of the two remaining plants in each pot, one was selected at random and used to
203 determine the effects of Si supplementation on herbivore performance. This was conducted

204 six days prior to harvesting all of the plants. Approximately 100 *Helicoverpa amigera* eggs
205 (supplied by CSIRO Agriculture & Food, Narrabri, Australia) were individually hatched on
206 growing media (modified from Teakle & Jensen 1985) at 20°C and 15:9 hour photoperiod
207 (light:dark). Upon reaching second instar (c. 7 days later), one herbivore was weighed and
208 then applied to each of the remaining plants in all 48 pots in the field. The insect was
209 confined to one leaf blade using a circular (25.4 mm diameter) mesh clip cage (BioQuip, CA,
210 USA) supported by a tethered post. Cages were moved on alternate days to undamaged leaf
211 tissue to ensure food availability was not limiting. Six days later, all larvae were removed and
212 re-weighed. Relative growth rates (RGR) were calculated: $RGR = ((\text{final mass} - \text{initial mass})$
213 $/ \text{initial mass}) / 6 \text{ days}$.

214

215 *Statistical analysis*

216 Mean daily canopy surface temperature in ambient and warmed plots was analysed
217 with a paired *t*-test. Analysis of variance (ANOVA) was conducted with temperature
218 (ambient or warmed) and Si status (+Si or -Si) included as fixed main and interactive effects
219 for all other tests. Frame (number) was included as a random term and observations from
220 individual frames nested within this. Contrast analysis was included within each model to
221 determine differences between temperature and Si treatment combinations. For plant
222 biophysical responses, where multiple measurements (2–4) were taken from the same plant
223 and the design was unbalanced we implemented split-plot ANOVAs in which pot (number)
224 was nested within frame (number). Least square means tests were implemented for contrast
225 analysis for these tests. For herbivore performance, we conducted an additional ANCOVA
226 test in which initial mass was included as a quantitative covariate and final mass was the
227 response variable. This was to validate inferences from the ANOVA test conducted on
228 herbivore RGR which can be problematic (see discussion by Raubenheimer & Simpson

229 1992). Unless otherwise stated (see figure or table legends), analysis was conducted on
230 untransformed data. Where necessary, transformations were applied according to goodness-
231 of-fit tests using the ‘goodfit’ function in the vcd package in R (Friendly 2000).
232 Transformations were selected on the basis of residual diagnostic plots which fitted a normal
233 distribution and with least heteroscedasticity. All analysis was conducted in the R statistical
234 package or Genstat (version 18, VSN International, Hemel Hempstead, UK).

235

236 **Results**

237 *Air temperature and soil moisture*

238 The warming frames caused a significant increase in canopy temperature by $2.8\text{ }^{\circ}\text{C} \pm$
239 0.10 (mean \pm standard error) during the experiment (Fig 2A). An example of diurnal patterns
240 in temperature between the two types of frame is shown in Fig. S2. The increased
241 temperature resulted in a significant decline (c. -3% absolute change, c. -22% relative
242 change) in soil water (Fig. 2B). There was a marginally statistically significant impact of Si
243 in terms of reduced mean soil water content during the experiment ($P = 0.051$; Fig 2B).
244 Contrast analysis suggested this occurred in warmed plots ($F_{1,33} = 3.24$, $P = 0.081$) rather
245 than ambient plots ($F_{1,33} = 1.03$, $P = 0.318$), but neither were significant at a 95% confidence
246 interval.

247

248 *Plant growth*

249 Shoot and root biomass were unaffected by warming, but increased significantly in
250 +Si plants overall (Fig. 3), although a significant warming by Si treatment interaction was
251 observed for shoot biomass ($P = 0.04$). Contrast analysis showed that Si increased shoot and
252 root biomass in ambient plots ($F_{1,33} = 11.61$, $P = 0.002$ and $F_{1,33} = 7.34$, $P = 0.011$,
253 respectively) whereas shoot and root biomass were unaffected by Si supplementation in

254 warmed plots ($F_{1,33} = 0.22$, $P = 0.645$ and $F_{1,33} = 2.81$, $P = 0.103$, respectively). Total plant
255 biomass reflected the same responses as shoot biomass; Si promoted total biomass ($F_{1,34} =$
256 7.40 , $P = 0.010$), temperature had no direct effect ($F_{1,10} = 2.90$, $P = 0.119$). Again, contrast
257 analysis showed that Si impacts on plant growth were only apparent in ambient plots ($F_{1,33} =$
258 11.11 , $P = 0.002$) and not warmed ($F_{1,33} = 0.21$, $P = 0.650$) plots.

259

260 *Silicon uptake in the plant*

261 The overall model indicated that Si concentrations in leaves were lower under warmer
262 conditions (Fig. 4), whereas Si supplementation caused a significant increase in Si
263 concentration in the leaves (Fig. 4). Contrast analysis, however, showed that warming caused
264 foliar Si concentrations to be lower in -Si plants only ($F_{1,44} = 6.46$, $P = 0.015$) and had no
265 impact on +Si plants ($F_{1,44} = 0.28$, $P = 0.600$). Denominator degrees of freedom are higher
266 because frame could not be included as block term in these contrasts due to confounding
267 effects. While -Si plants in warmed plots had the lowest concentrations of foliar Si,
268 supplementation with Si increased concentrations in warmed plots ($F_{1,33} = 7.09$, $P = 0.012$)
269 and restored levels to those seen in ambient plots. Si supplementation had no impact in
270 ambient plots ($F_{1,33} = 0.49$, $P = 0.489$). Si concentrations in the roots were unaffected by
271 warming or Si supplementation (Table 1).

272

273 *Leaf biomechanical properties*

274 Overall, the force required to fracture leaves at the midrib was c. 32% higher in +Si
275 plants than -Si plants (Fig. 5A). Midrib and lamina thickness were not significantly affected
276 by Si supplementation or warming (data not shown) and midribs of leaves from +Si plants
277 were consequently c. 21% tougher (i.e. they had a higher specific work of fracture) than
278 leaves from -Si plants (Fig. 5B). Si supplementation significantly increased midrib force to

279 fracture and midrib toughness in ambient plots ($t = -2.61$, $P = 0.013$ and $t = -2.24$, $P = 0.032$,
280 respectively) but these increases in +Si plants were less pronounced in warmed plots ($t = -$
281 1.31 , $P = 0.200$ and $t = -0.60$, $P = 0.551$, respectively). Overall leaf toughness was not
282 significantly affected by Si supplementation (Table 1). Warming had a negligible impact on
283 leaf biomechanical measures (Fig. 5, Table 1).

284

285 *Insect herbivore performance*

286 Relative growth rate (RGR) of *H. armigera* was halved when feeding on +Si plants
287 compared to herbivores feeding on -Si plants, though this was only statistically significant
288 under ambient conditions ($F_{1,33} = 9.53$, $P = 0.004$) and not under warmed conditions ($F_{1,33} =$
289 2.11 , $P = 0.156$) (Fig. 6). Herbivore RGR was also negatively affected by warming compared
290 to those feeding on plants grown in ambient plots (c. -55%). Analysing herbivore mass with
291 ANCOVA (as recommended by Raubenheimer & Simpson 1992) gave similar results for
292 temperature ($F_{1,9} = 5.81$, $P = 0.039$) and Si supplementation ($F_{1,33} = 9.86$, $P = 0.004$), with no
293 interactive effect between the two ($F_{1,33} = 1.16$, $P = 0.289$).

294

295 **Discussion**

296 This study has shown that under ambient temperatures Si supplementation increased
297 plant growth and leaf biomechanical resistance of a pasture grass, which was associated with
298 a sharp decline in herbivore performance, but without a significant increase in foliar Si
299 concentrations. Warming reduced Si uptake in plants, potentially due to lower levels of soil
300 water, but this was remedied with Si supplementation which produced foliar Si
301 concentrations similar to those in plants growing under ambient conditions. While
302 biomechanical properties were enhanced and herbivore performance diminished in warmed
303 +Si plants, this was not quite to the same extent as plants growing under ambient conditions.

304 It is therefore possible that Si was being used by plants to alleviate other adverse effects of
305 temperature and/or water stress under warmed conditions.

306

307 *Global warming and Si uptake*

308 We hypothesised that warming increases Si uptake under the expectation that
309 warming would enhance plant growth. Our results showed this not to be the case and
310 warming generally had negative impacts on plant growth and Si uptake. Plants take up Si in a
311 soluble form (silicic acid) which is influenced, at least in part, by hydraulic and osmotic
312 processes in the plant (Quigley & Anderson 2014; McLarnon et al. 2017). Maintaining Si
313 uptake via such mechanisms when Si was plentiful but with reduced soil water under
314 warming, however, probably comes at a physiological cost and may explain why leaf
315 biomechanical properties (and herbivore resistance) were enhanced to a lesser extent.
316 Increasing Si uptake may also reflect a strategy to alleviate the effects of mild water stress
317 when Si was plentiful since Si is well documented to relieve the effects of drought in plants
318 (Debona et al. 2017). Indeed, a rain exclusion experiment reported that two grasses (*Cynodon*
319 *dactylon* and *Eragrostis curvula*) took up significantly more Si when experiencing drought
320 conditions in conjunction with root herbivory (Power et al. 2016).

321 In a glasshouse study using eight grass species, elevated temperatures were reported
322 not to affect Si concentrations overall, but some species showed significant responses
323 (Johnson & Hartley 2018). In particular, grass species native to Australia contained higher
324 concentrations of Si, which rose in response to warmer conditions (c. +19%) whereas exotic
325 species showed declines (c. -10%) in Si concentrations. It remains speculative, but having the
326 capacity to absorb large amounts of Si might be a selective advantage to plants evolving in
327 low-nutrient soils and seasonally arid climates such as Australia (Johnson & Hartley 2018).
328 *Phalaris aquatica* originated in Southern Europe and the Caucasus and while now

329 naturalised, it is exotic to Australia (Popay 2015). The observed declines in Si at warmer
330 temperatures observed in the present study are therefore consistent with patterns for exotic
331 species tested by Johnson and Hartley (2018). We should caution, however, that
332 comparatively few grass species have been investigated in terms of global warming and Si
333 uptake, and most of these (like the current study) were conducted over relatively short time
334 periods. Moreover, the effects of warming on Si uptake can be further reduced by factors
335 such as elevated atmospheric carbon dioxide concentrations (Ryalls, Hartley & Johnson
336 2017; Johnson & Hartley 2018) so warming effects could be context dependent.

337

338 *Si and leaf biomechanical properties*

339 Si supplementation is known to change biomechanical properties of plants with
340 consequent negative impacts on herbivores, either through physical effects or reduced
341 digestion (Hochuli 1996; Hanley, Lamont, Fairbanks & Rafferty 2007). Silicification of
342 leaves can wear down insect mouthparts which ultimately inhibits feeding (e.g. Massey &
343 Hartley 2009), although this not the case for all mandibulate herbivores (e.g. Kvedaras,
344 Byrne, Coombes & Keeping 2009). The relatively short period that herbivores fed on plants
345 in the present study may have been sufficient to cause mandibular wear due to silicification
346 resulting in lower RGR (e.g. Massey & Hartley 2009). Alternatively, silicification of leaves
347 can also immediately inhibit the ability of chewing herbivores to fracture plant tissue, for
348 instance by absorbing energy which reduces fracture propagation (Clissold 2008). This seems
349 a more likely reason for the observed reductions in herbivore performance although this does
350 not exclude the possibility that Si enhanced the efficacy of other chemical defences (Coskun
351 et al. 2018; Frew et al. 2018) or otherwise reduced the digestibility of plant tissue (Massey
352 and Hartley 2009).

353 It was noteworthy that toughness increased in the midrib of +Si plants, but not for the
354 leaf overall, indicating less, if any, toughening of the lamina. This may explain why we saw
355 modest increases in concentrations of leaf Si overall with supplementation. While Si
356 supplementation was clearly associated with greater biomechanical strength, this may have
357 been due to localised Si deposition which was less detectable when we analysed all of the
358 tissue collectively (i.e. dilution effects masked differences). Using 17 species of rice (*Oryza*
359 spp.), Whang, Kim and Hess (1998) showed that silica bodies of the midrib differed
360 markedly from those of other leaf veins. The significance of these differences is unknown,
361 but our findings show that Si supplementation does not result in uniformly tougher leaves and
362 biomechanical resistance to herbivory may be based largely within the midrib.

363

364 *Warming and herbivore performance*

365 Warming also reduced the performance of herbivores, perhaps reflecting a decline in
366 plant vigour as the effects of warming and soil drying took effect. The nitrogen content of
367 foliage, for example, often decreases with increased temperature (Zvereva & Kozlov 2006),
368 although this is a highly variable response (Sardans, Rivas-Ubach & Penuelas 2012; Johnson
369 & Hartley 2018). While warming may also increase tissue toughness, Zvereva and Kozlov
370 (2006) found no statistically significant effects across plant taxa in their meta-analysis.
371 Warming may have also directly affected insect physiology, with warmed plots often
372 exceeding 35°C for short periods. Temperatures of 35°C are considered to be highly adverse
373 for this species (Mironidis 2014) and may have increased risk of desiccation. Regardless,
374 warming clearly had negative impacts on herbivores, which may explain why performance
375 tended to decline on warmed –Si plants despite them having lower concentrations of Si.

376

377 *Conclusions*

378 Every model released by the Intergovernmental Panel on Climate Change predicts
379 increases in mean air temperature by 2100 (IPCC 2013). It is therefore important to
380 understand the effects of warming on grasslands, particularly using realistic warming
381 conditions that incorporate natural stochastic variation in air temperature (Lindroth & Raffa
382 2016). In Australia alone, *P. aquatica* based pastures account for 1.6 million hectares of land
383 (Popay 2015) yet little is known about how this species responds to climate warming. As a
384 temperate grass usually sown in Autumn, the optimum temperature for *P. aquatica* seedling
385 growth is 15–20°C (Watson 1995). Mean daily temperature exceeded this in 42 of the 50 days
386 in warmed plots, which suggests that even modest increases in Autumn temperatures may
387 adversely affect this species. Given the known benefits of Si for stress alleviation in grasses,
388 maximising Si uptake may be important for climate change mitigation (Frew, Allsopp,
389 Gherlenda & Johnson 2017). This could include irrigation measures, use of cultivars that are
390 naturally high accumulators of Si, supplementation with sources of Si (e.g. silicon fertilisers)
391 and targeted remediation of Si deficient soils (Guntzer et al. 2012; Liang, Nikolic, Belanger,
392 Gong & Song 2015; Haynes 2017).

393

394 *Acknowledgements*

395 The warming frames were built with a E.A. Southee Award to JMWR from the
396 Hawkesbury Foundation. The steel frames were constructed by P&C Laws (Richmond,
397 NSW). The research was further funded by grants from the Australian Research Council to
398 SNJ and BDM (DP14100363) and SNJ (DP170102278 and FT170100342). Jenni Kremer
399 and Jessica Ezweld are thanked for undertaking technical assistance. Gavin Tory and Marc
400 Smith of Australian Steel Mill Services are thanked for advice and provision of the calcium
401 silicate.

402

403 *Authors' contributions*

404 SNJ, BDM and JMWR conceived the ideas for the study with methodological design
405 also including CVB, MGT and IJW. SNJ and JMWR oversaw data collection with BDM
406 overseeing biomechanical testing with direction from IJW. SNJ led manuscript preparation
407 with all authors contributing critically to drafts before giving approval for submission.

408

409 *Data accessibility*

410 Datasets are available through Dryad [DOI: to follow].

411

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- 588

589 **Table 1.** Impacts of warming and Si supplementation on root silicon concentrations and overall leaf toughness (mean ± standard error) with
 590 results of statistical analysis below. N = 12 (six frames containing two pots).

591

Temperature	Si treatment	Root silicon (% dry mass)	Overall leaf toughness (N m ⁻¹)
Ambient	-Si	6.26 ± 0.47	918.50 ± 87.72
	+Si	8.56 ± 0.98	1067.41 ± 65.24
Warmed	-Si	8.35 ± 1.13	881.50 ± 107.56
	+Si	7.96 ± 1.17	949.00 ± 54.32
Statistical analysis	T	F _{1,10} = 0.61, P = 0.453	F _{1,8} = 0.13, P = 0.731
	Si	F _{1,34} = 0.93, P = 0.342	F _{1,30} = 1.12, P = 0.299
	T x Si	F _{1,34} = 1.85, P = 0.182	F _{1,30} = 0.73, P = 0.398

592

593

594 **Figure Legends**

595 **Figure 1. (A)** Schematic of warming frames (one plant per pot shown for clarity) used in the study
596 and **(B)** typical thermal image of a warmed area showing temperature within (circle 1) and outside
597 (circle 2) the frames.

598 **Figure 2. (A)** Mean daily plant canopy surface temperature within ambient (open circles) and
599 warmed (closed circles) frames during the experiment (Austral Autumn). Mean values \pm standard
600 error (latter mostly obscured) for frames ($N = 6$) shown. **(B)** Soil water content of pots in ambient
601 and warmed frames supplemented with Si (grey bars) and untreated (white bars). Mean values \pm
602 standard error shown ($N = 12$; six frames containing two pots). Result of statistical analysis for
603 temperature (T) and Si supplementation (Si) provided.

604 **Figure 3.** Impacts of warming and Si supplementation on shoot and root growth¹. Mean values \pm
605 standard error shown ($N = 12$; six frames containing two pots). Results of statistical analyses for
606 temperature (T) and Si supplementation (Si) provided with statistically significant differences
607 between $-Si$ and $+Si$ plants determined by contrast analysis indicated $*P < 0.05$. ¹Data log
608 transformed prior to analysis.

609 **Figure 4.** Impacts of warming and Si supplementation on Si concentrations in the leaves. Mean
610 values \pm standard error shown ($N = 12$; six frames containing two pots). Results of statistical
611 analyses for temperature (T) and Si supplementation (Si) provided with statistically significant
612 differences between $-Si$ and $+Si$ plants determined by contrast analysis indicated $*P < 0.05$. ¹Data
613 logit transformed prior to analysis.

614 **Figure 5.** Impacts of warming and Si supplementation on **(A)** force to fracture the midrib and **(B)**
615 midrib toughness. Mean values \pm standard error shown. Results of statistical analyses for
616 temperature (T) and Si supplementation (Si) provided with statistically significant differences
617 between $-Si$ and $+Si$ plants determined by contrast analysis indicated $*P < 0.05$.

618

619 **Figure 6.** Relative growth rates (RGR) of *H. armigera* feeding on -Si and +Si plants under ambient
620 and warmed air temperatures. Mean values \pm standard error shown (N = 12; six frames containing
621 two pots). Results of statistical analyses for temperature (T) and Si supplementation (Si) provided
622 with statistically significant differences between -Si and +Si plants determined by contrast analysis
623 indicated $**P < 0.01$.

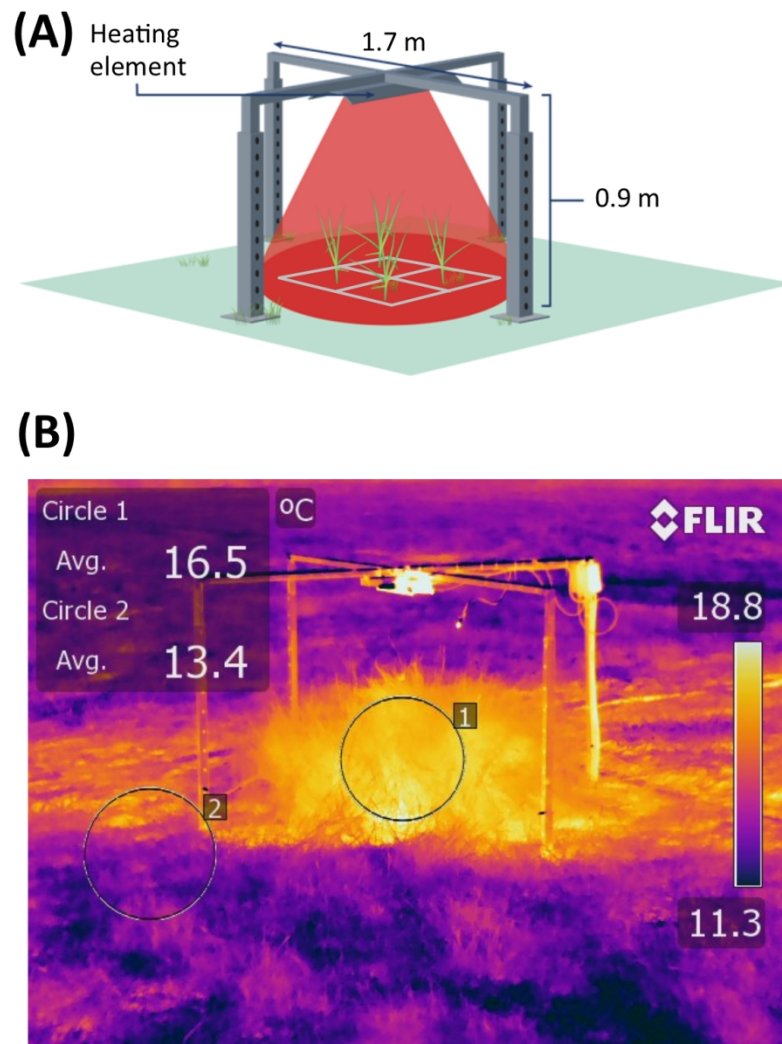


Figure 1. (A) Schematic of warming frames (one plant per pot shown for clarity) used in the study and (B) typical thermal image of a warmed area showing temperature within (circle 1) and outside (circle 2) the frames.

209x297mm (150 x 150 DPI)

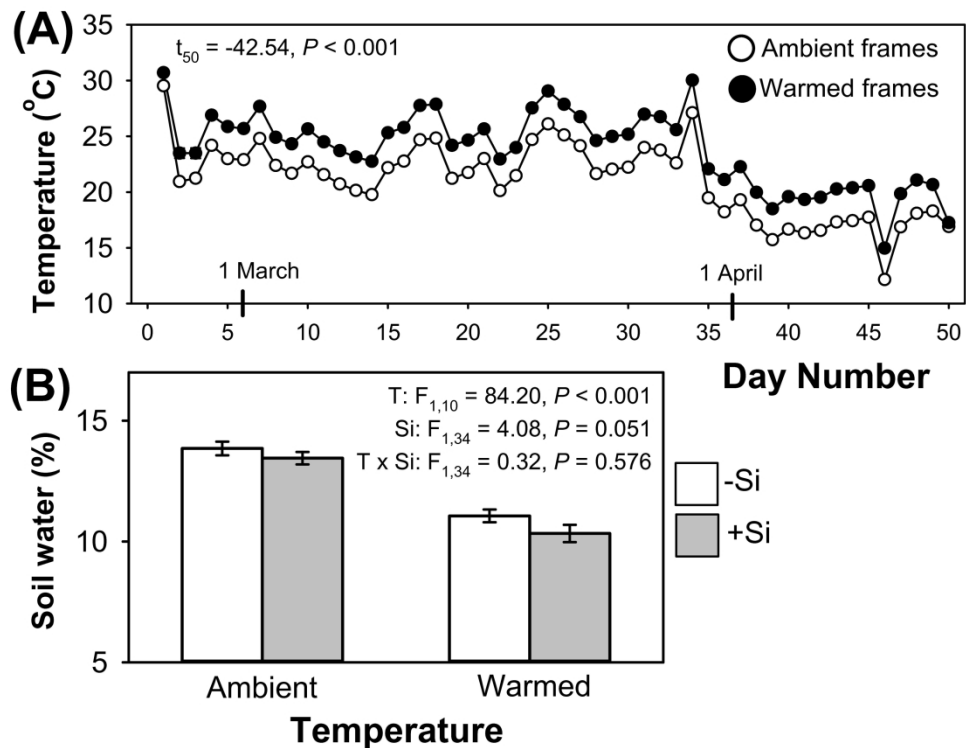


Figure 2. (A) Mean daily plant canopy surface temperature within ambient (open circles) and warmed (closed circles) frames during the experiment (Austral Autumn). Mean values \pm standard error (latter mostly obscured) for frames ($N = 6$) shown. (B) Soil water content of pots in ambient and warmed frames supplemented with Si (grey bars) and untreated (white bars). Mean values \pm standard error shown ($N = 12$; six frames containing two pots). Result of statistical analysis for temperature (T) and Si supplementation (Si) provided.

279x215mm (300 x 300 DPI)

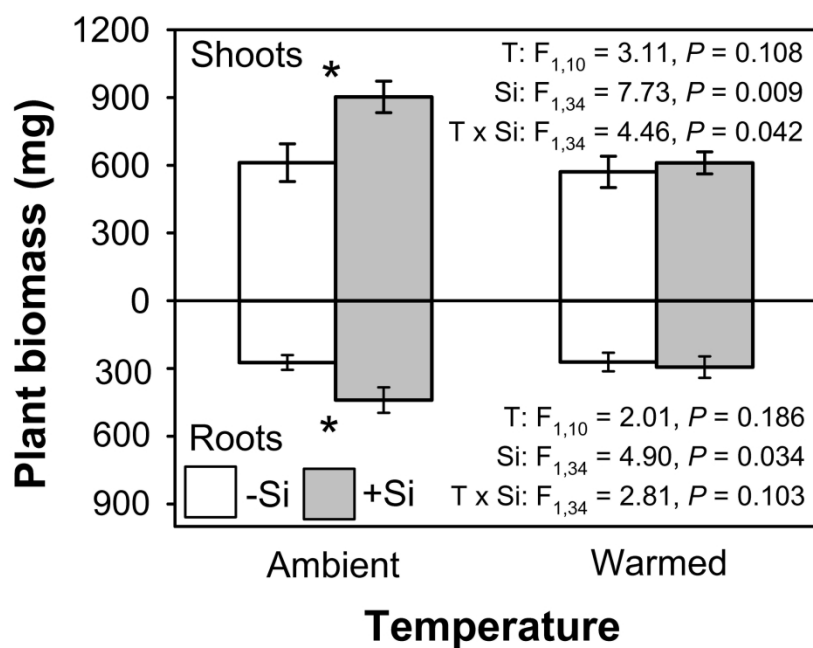


Figure 3. Impacts of warming and Si supplementation on shoot and root growth¹. Mean values \pm standard error shown (N = 12; six frames containing two pots). Results of statistical analyses for temperature (T) and Si supplementation (Si) provided with statistically significant differences between -Si and +Si plants determined by contrast analysis indicated * $P < 0.05$. ¹Data log transformed prior to analysis.

215x279mm (300 x 300 DPI)

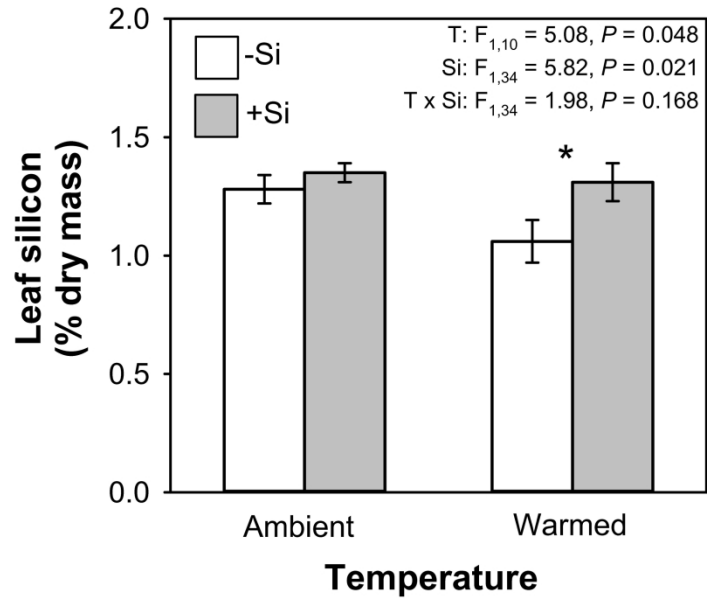


Figure 4. Impacts of warming and Si supplementation on Si concentrations in the leaves. Mean values \pm standard error shown ($N = 12$; six frames containing two pots). Results of statistical analyses for temperature (T) and Si supplementation (Si) provided with statistically significant differences between $-Si$ and $+Si$ plants determined by contrast analysis indicated $*P < 0.05$. 1Data logit transformed prior to analysis.

279x215mm (300 x 300 DPI)

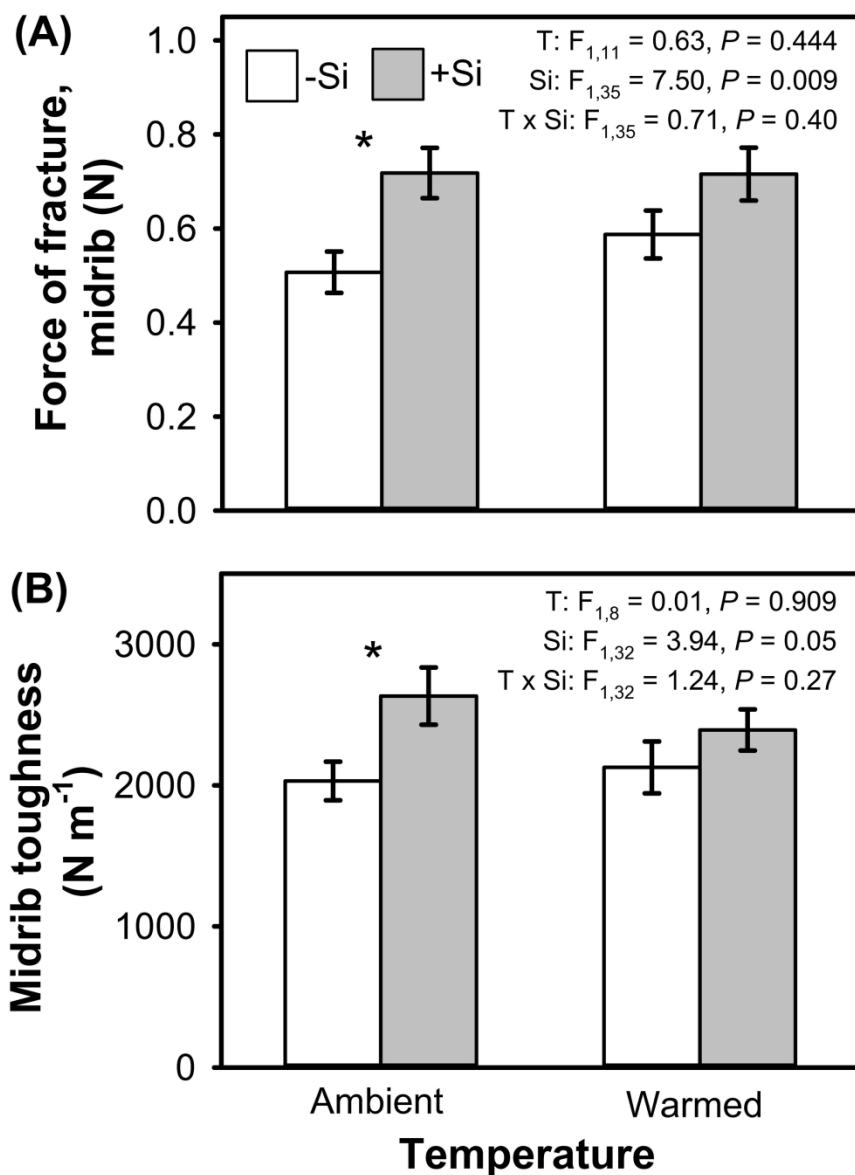


Figure 5. Impacts of warming and Si supplementation on (A) force to fracture the midrib and (B) midrib toughness. Mean values \pm standard error shown. Results of statistical analyses for temperature (T) and Si supplementation (Si) provided with statistically significant differences between -Si and +Si plants determined by contrast analysis indicated * $P < 0.05$.

215x279mm (300 x 300 DPI)

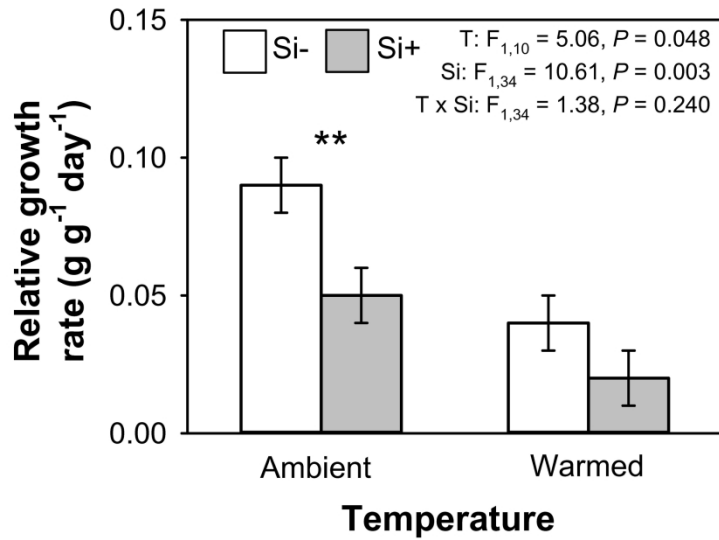


Figure 6. Relative growth rates (RGR) of *H. armigera* feeding on -Si and +Si plants under ambient and warmed air temperatures. Mean values \pm standard error shown (N = 12; six frames containing two pots). Results of statistical analyses for temperature (T) and Si supplementation (Si) provided with statistically significant differences between -Si and +Si plants determined by contrast analysis indicated **P < 0.01.

279x215mm (300 x 300 DPI)

Supplemental Material

Table S1. Chemical composition and characteristics of the homogenised soil (analysed by Environmental Analysis Laboratory at Southern Cross University, Australia).

Method	Nutrient / Property	Units	Mean	Standard Error
Colwell	Phosphorus	mg/kg	17.38	1.43
	Nitrate Nitrogen	mg/kg	16.74	13.29
KCl	Ammonium Nitrogen	mg/kg	2.75	0.37
	Sulfur	mg/kg	4.82	1.30
1:5 Water	pH	units	6.12	0.24
	Conductivity	dS/m	0.05	0.02
Calculation	Estimated Organic Matter	% OM	2.66	0.71
		cmol ⁺ /kg	3.46	0.81
	Calcium	kg/ha	1552.04	364.26
		mg/kg	692.87	162.62
		cmol ⁺ /kg	0.78	0.10
Ammonium	Magnesium	kg/ha	213.13	26.62
Acetate +		mg/kg	95.15	11.88
Calculations		cmol ⁺ /kg	0.42	0.05
	Potassium	kg/ha	369.84	41.43
		mg/kg	165.11	18.50
		cmol ⁺ /kg	0.09	0.02
	Sodium	kg/ha	44.91	8.53
		mg/kg	20.05	3.81
		cmol ⁺ /kg	0.02	0.00
KCl	Aluminium	kg/ha	3.66	0.62
		mg/kg	1.63	0.28
		cmol ⁺ /kg	0.05	0.01
Acidity Titration	Hydrogen	kg/ha	1.18	0.27
		mg/kg	0.53	0.12
Calculation	Effective Cation Exchange Capacity (ECEC)	cmol ⁺ /kg	4.82	0.89
	Calcium	%	70.46	3.55
	Magnesium	%	16.70	1.63
Base Saturation	Potassium	%	9.30	1.65
Calculations	Sodium - ESP	%	1.96	0.61
	Aluminium	%	0.41	0.12
	Hydrogen	%	1.17	0.34
Calculation	Calcium / Magnesium Ratio	ratio	4.35	0.69
CaCl ₂	Soil pH (CaCl ₂)	units	5.50	0.22
	Zinc	mg/kg	2.36	0.74
DTPA	Manganese	mg/kg	87.45	22.77
	Iron	mg/kg	70.46	5.00
	Copper	mg/kg	1.00	0.22
CaCl ₂	Boron	mg/kg	0.29	0.03
	Silicon (bioavailable)	mg/kg	23.42	0.83
LECO IR	Total Carbon	%	1.52	0.41
Analyser	Total Nitrogen	%	0.12	0.03

Fig. S1. A representative force-displacement curve for a *Phalaris aquatica* leaf. The red line indicates the portion over which the average force was calculated and maximum force applied to sever the leaf's midrib is indicated 'max'. The baseline is indicated by the black dashed line; note the failure of the force measurement to return to the baseline after the leaf was severed due to the leaf blade having folded between the blade and anvil.

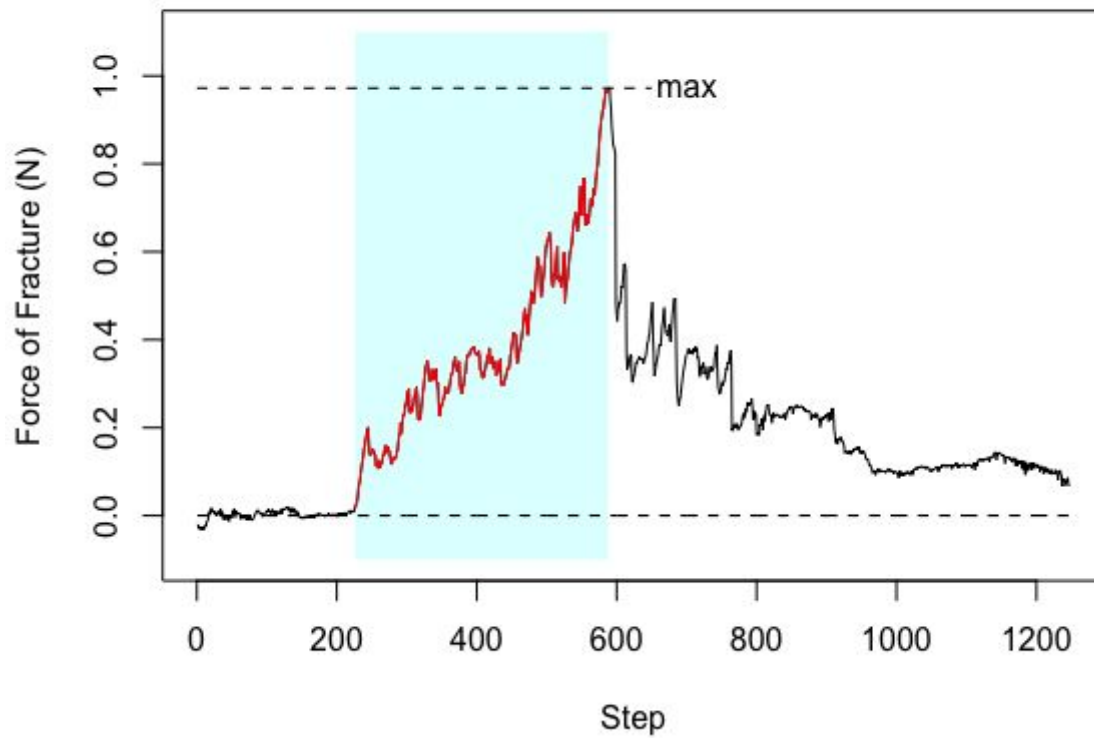


Fig. S2. Temperatures for all 12 frames running under ambient (grey lines) and warmed (black lines) conditions over a three-day period during the experiment. Note the higher variation in warmed frames during the middle of March 21, which was a windy day and therefore harder to consistently warm.

