**Author for correspondence:**
James M. W. Ryalls
e-mail: j.ryalls@westernsydney.edu.au

# Impacts of silicon-based grass defences across trophic levels under both current and future atmospheric $CO_2$ scenarios

James M. W. Ryalls[1], Susan E. Hartley[2] and Scott N. Johnson[1]

[1]Hawkesbury Institute for the Environment, Western Sydney University, Bourke Street, Richmond, New South Wales 2753, Australia
[2]York Environmental Sustainability Institute, University of York, Wentworth Way, Heslington, York YO10 5DD, UK

(iD) JMWR, 0000-0003-2015-3605

Silicon (Si) has important functional roles in plants, including resistance against herbivores. Environmental change, such as increasing atmospheric concentrations of $CO_2$, may alter allocation to Si defences in grasses, potentially changing the feeding behaviour and performance of herbivores, which may in turn impact on higher trophic groups. Using Si-treated and untreated grasses (*Phalaris aquatica*) maintained under ambient (400 ppm) and elevated (640 and 800 ppm) $CO_2$ concentrations, we show that Si reduced feeding by crickets (*Acheta domesticus*), resulting in smaller body mass. This, in turn, reduced predatory behaviour by praying mantids (*Tenodera sinensis*), which consequently performed worse. Despite elevated $CO_2$ decreasing Si concentrations in *P. aquatica*, this reduction was not large enough to affect the feeding behaviour of crickets or their predator. Our results suggest that Si-based defences in plants have adverse impacts on both primary and secondary trophic taxa, and these are not likely to decline under future climate change scenarios.

## 1. Introduction

Despite 25% of species being herbivorous insects, we live on a green planet that is dominated by terrestrial plants, so it's clear that plants resist attack. Plant chemical defences restrict damage by insect herbivores both directly (e.g. toxicity) and indirectly by influencing the third trophic level (e.g. recruitment of natural enemies) [1]. While these bottom-up and top-down chemical mechanisms generally protect plants, plant defences can weaken top-down control where they negatively affect the quality of herbivores for their natural enemies [2].

Silicon (Si) is increasingly recognized as playing a range of ecologically significant roles in plants [3], particularly in grasses where it can make up 10% of tissue dry weight. Si can be deposited within or between cells, in the cell wall or as discrete opaline phytoliths. Such Si deposition confers defence against herbivores because of abrasive effects on their mouthparts and diminished nutrient acquisition via reduced palatability and digestibility of foliage, in turn reducing the growth rate and body mass of herbivores feeding on Si-rich plants [4]. Such non-fatal effects could pass up the food chain by, for example, reducing both the palatability and quality of herbivorous insects as food for their predators. This could be detrimental for the foraging behaviour and performance of predators and potentially weaken top-down control of insect herbivores. While Si is widely regarded as an important anti-herbivore defence in grasses, its role higher up the food chain has rarely been tested [5].

Silicon has been hypothesized to act as a metabolically 'cheaper' structural substitute for carbon (C) [6], particularly at times when carbon dioxide ($CO_2$) concentrations were low, such as during the Miocene; it has been suggested that C substitution by Si may have driven the evolution of high Si-accumulating

**Table 1.** Si and $CO_2$ treatment effects on *P. aquatica* foliar characteristics (means $\pm$ s.e.). *p*-values highlighted in bold indicate significance ($p < 0.05$).

| | | biomass (g) | Si concentration (%) | C concentration (%) |
|---|---|---|---|---|
| Si-treatment | control | $2.60 \pm 0.16$ | $0.86 \pm 0.04$ | $41.51 \pm 0.09$ |
| | Si | $4.41 \pm 0.20$ | $1.16 \pm 0.05$ | $41.03 \pm 0.19$ |
| $CO_2$ | 400 ppm | $3.32 \pm 0.25$ | $1.18 \pm 0.07$ | $40.94 \pm 0.12$ |
| | 640 ppm | $3.51 \pm 0.35$ | $0.97 \pm 0.06$ | $41.28 \pm 0.28$ |
| | 800 ppm | $3.30 \pm 0.28$ | $0.89 \pm 0.06$ | $41.57 \pm 0.11$ |
| statistical analysis | $CO_2$ | $F_{2,59} = 2.38$, $p = 0.101$ | $F_{2,58} = 7.14$, $p = \mathbf{0.002}$ | $F_{2,59} = 3.23$, $p = \mathbf{0.046}$ |
| | Si-treatment | $F_{1,59} = 51.68$, $p < \mathbf{0.001}$ | $F_{1,58} = 24.43$, $p < \mathbf{0.001}$ | $F_{1,59} = 5.56$, $p = \mathbf{0.022}$ |
| | $CO_2 \times$ Si-treatment | $F_{2,59} = 0.65$, $p = 0.528$ | $F_{2,58} = 0.15$, $p = 0.860$ | $F_{2,59} = 0.27$, $p = 0.761$ |

grass species during this period [6]. Conversely, within the next century, $CO_2$ concentrations are expected to double, potentially resulting in higher C, and hence lower Si concentrations within plants. There is already some evidence for negative correlations between Si- and C-based defences [7] but the impacts of these changes on herbivores and their predators have not yet been addressed.

This research determined how elevated $CO_2$ concentrations and Si supplementation affected Si-accumulation in a grass, and how this impacted feeding and performance of an insect herbivore and its predator. Grasses subjected to increasing $CO_2$ concentrations, from current (400 ppm) through to impending concentrations (640 and 800 ppm predicted for 2050 and 2100, respectively), were expected to progressively accumulate less Si as C availability and fixation increased (i.e. elevated $CO_2$ was expected to decrease plant Si-uptake). This would make them more susceptible to herbivores, whose performance and quality to predators would increase. Regardless of $CO_2$ effects, we hypothesized that higher Si-uptake reduces feeding and performance of herbivores which, in turn, reduces predation and performance of higher trophic levels.

## 2. Material and methods

The house cricket (*Acheta domesticus*) and the Chinese praying mantid (*Tenodera sinensis*) were used as a model herbivore and predator, respectively, maintained in culture at 26°C, 75% relative humidity. Three plant growth chambers (TPG-1260TH, Thermoline Scientific), maintained at $CO_2$ concentrations of 400, 640 and 800 ppm were used. Chambers were lit with five 400 W Sunmaster Dual Spectrum High Pressure Sodium globes at 350 $\mu$mol m$^{-2}$ s$^{-1}$ at plant level. $CO_2$ was measured using a single Vaisala GMP222 probe positioned behind the return air fans, and temperature and humidity were measured using an Elektronik EE08 sensor. Air temperatures within each chamber were maintained at 26/18°C day/night on a 15 L : 9 D cycle. Humidity was controlled at 60%.

Eight seeds of a common Australian pasture grass (Harding grass, *Phalaris aquatica* cultivar Holdfast; Heritage Seeds, NSW), were planted in 48 pots (70 mm diameter) filled with local field soil [8] in each of the three growth chambers. Half of the pots had Si-supplementation, involving watering the plants with 500 mg l$^{-1}$ solution of dissolved sodium metasilicate ($Na_2SiO_3 \cdot 9H_2O$), and half received deionized water alone (approx.

80 ml three times a week in both cases). Chambers were swapped every two weeks and the plants were rotated within the chambers twice-weekly to minimize chamber effects and pseudo-replication, as previously demonstrated [9].

Eight weeks after sowing, shoot material from each of 11 pots in each Si-treatment (i.e. Si-treated or control plants) per chamber was cut to soil level and herbivore performance was assessed by following the procedure of Massey *et al.* [10]. Sixty crickets were placed in each of six terrariums (two per chamber). In each chamber, crickets in one terrarium were fed on Si-treated grasses and crickets in the other terrarium were fed on control plants with no Si. Sixteen grass tillers were placed in the terrariums daily to maintain a constant food supply. After one week, six crickets from each of the two terrariums per chamber were weighed and placed in the culture cage with each of 12 praying mantids for 24 h. Six of the mantids were fed crickets reared on Si-supplemented plants and the other six received crickets reared on control plants. Mantids were starved for 72 h beforehand and weighed before the assay. After 24 h, the remaining crickets were counted and weighed. Mantids and cricket remnants were also weighed. Mantid frass was weighed after a further 24 h. The mass of crickets eaten was calculated by subtracting the mass of cricket frass and their remains from the total cricket mass.

Dried shoot material was ball-milled to a fine powder. Carbon concentrations were determined with a Carlo Erba CE1110 elemental analyser and Si concentrations were determined with an X-ray fluorescence spectrometer (Epsilon-3x, PANalytical-Almelo, The Netherlands). See [11] for full protocol.

Si-treatment and $CO_2$ effects on grass characteristics and cricket performance parameters were analysed using generalized linear models within R v. 3.1.1. Mantid performance parameters were analysed using mixed models in the *nlme* statistical package within R. The fixed terms included Si-treatment (control and Si) and $CO_2$ (400, 640 and 800 ppm) as well as the interaction between these terms. The random term within the mixed models included mantid ID to account for any mantid preference bias.

## 3. Results

### (a) Plant responses

Si-treatment increased the biomass of *P. aquatica*, but $CO_2$ had no significant effect (table 1). Si and C concentrations were negatively correlated ($r = -0.471$, $p < 0.001$, d.f. $= 62$). Si-treated plants contained higher and lower concentrations of Si and C, respectively, compared with control plants.
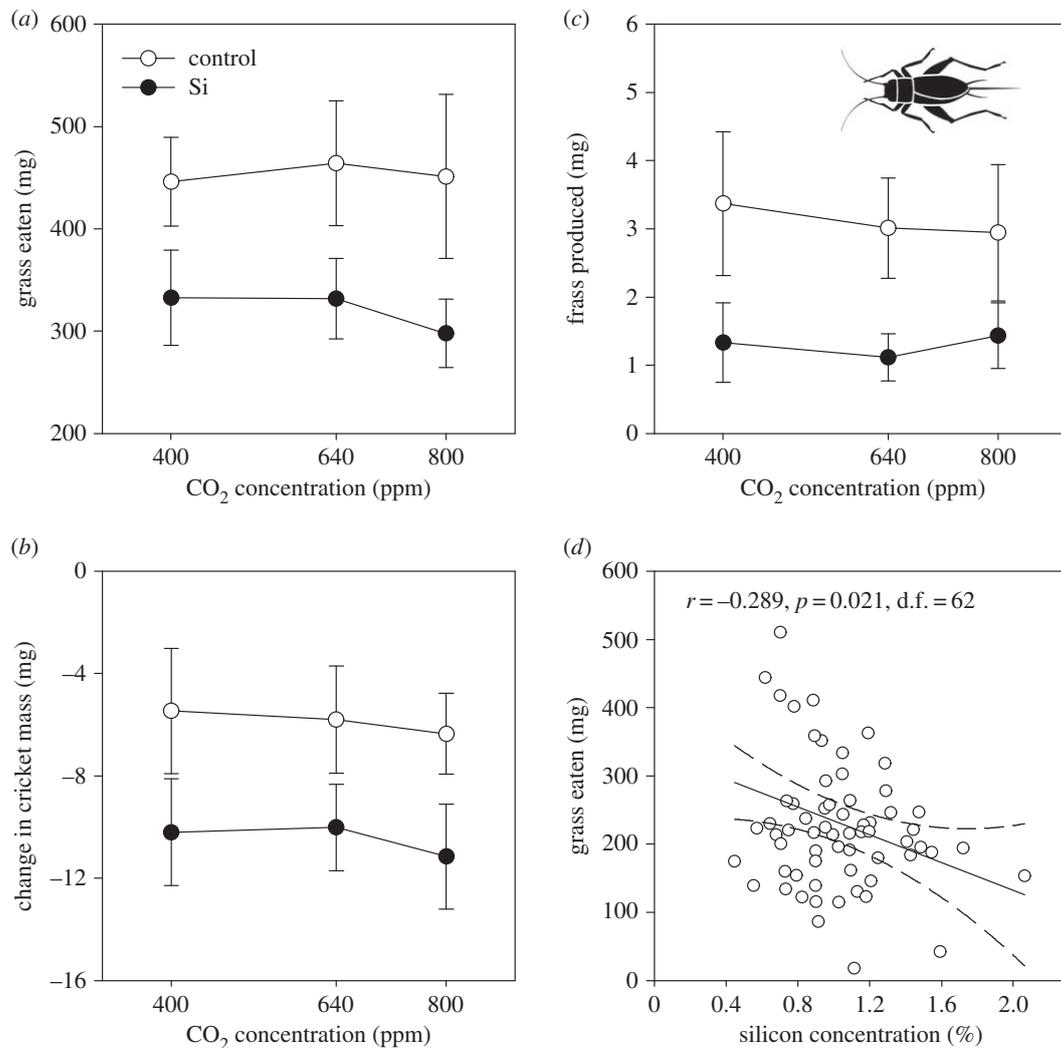
**Figure 1.** The impacts of Si-treatment on (*a*) the mass of grass eaten, (*b*) the change in cricket mass and (*c*) cricket frass production under three $CO_2$ concentrations. Mean values ($\pm$ s.e.) are shown. (*d*) The correlation (with 95% CI) between silicon concentration and the mass of grass consumed by crickets.

**Table 2.** Cricket and mantid responses to $CO_2$ and Si-treatment from generalized linear models (herbivore performance tests) and mixed models (predator performance tests).

| response variable | $CO_2$ | | | Si-treatment | | | $CO_2 \times$ Si-treatment | | |
|---|---|---|---|---|---|---|---|---|---|
| | *F* | *p*-value | d.f. | *F* | *p*-value | d.f. | *F* | *p*-value | d.f. |
| herbivore performance | | | | | | | | | |
| grass eaten (figure 1*a*) | 0.08 | 0.923 | 2,59 | 9.54 | **0.003** | 1,59 | 0.07 | 0.929 | 2,59 |
| $\Delta$ cricket mass (figure 1*b*) | 0.05 | 0.948 | 2,59 | 4.12 | **0.047** | 1,59 | 0.03 | 0.974 | 2,59 |
| frass produced (figure 1*c*) | 1.82 | 0.171 | 2,59 | 5.54 | **0.022** | 1,59 | 0.01 | 0.986 | 2,59 |
| predator performance | | | | | | | | | |
| no. crickets eaten | 1.42 | 0.265 | 2,19 | 27.47 | **<0.001** | 1,19 | 1.21 | 0.320 | 2,19 |
| mass crickets eaten[a] (figure 2*a*) | 0.05 | 0.949 | 2,19 | 16.35 | **<0.001** | 1,19 | 0.11 | 0.898 | 2,19 |
| $\Delta$ mantid mass (figure 2*b*) | 0.73 | 0.496 | 2,19 | 9.68 | **0.006** | 1,19 | 0.99 | 0.389 | 2,19 |
| frass produced[b] (figure 2*c*) | 0.61 | 0.555 | 2,19 | 5.73 | **0.027** | 1,19 | 1.15 | 0.338 | 2,19 |

*p*-values highlighted in bold indicate significance ($p < 0.05$). Where appropriate, response variables were transformed ([a]exponential, [b]square root) before analysis.

Moreover, increasing $CO_2$ concentrations decreased and increased Si and C concentrations, respectively (table 1). No interactive (Si $\times$ $CO_2$) effects were observed.

## (b) Herbivore performance

Crickets ate less grass, lost more weight and produced less frass when they were fed on Si-treated grasses compared
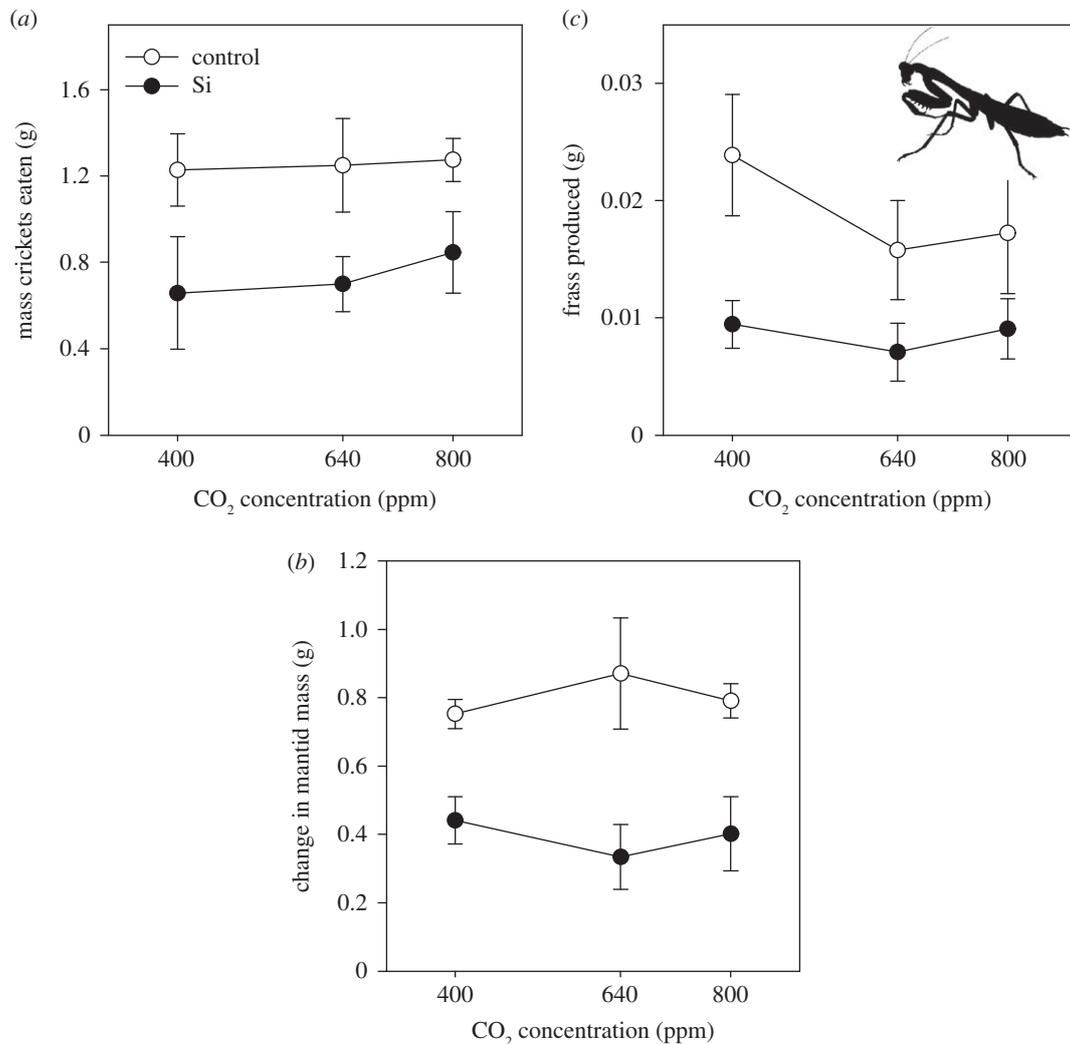
**Figure 2.** The impacts of Si-fed crickets (i.e. crickets fed on Si-supplemented grasses) on (a) the mass of crickets eaten by mantids, (b) the change in mantid mass and (c) mantid frass production under three $CO_2$ concentrations. Mean values ($\pm$ s.e.) are shown.

with control grasses (figure 1a–c). Similarly, grass consumption was negatively correlated with Si concentration (figure 1d). $CO_2$ had no significant effect (table 2).

### (c) Predator performance

Mantids ate significantly more control crickets ($4.94 \pm 0.23$) than Si-fed crickets ($3.22 \pm 0.26$; table 2). This was reflected in the mass of crickets consumed by mantids, the change in mantid mass and the amount of frass produced by mantids, which all increased when they were fed on control crickets relative to Si-fed crickets (figure 2a–c). $CO_2$ had no significant effect (table 2).

## 4. Discussion

This study highlights how the impacts of Si-defence in grasses can extend up the food chain to affect a chewing folivore and its natural enemy. Results demonstrated a clear reduction in cricket (*A. domestica*) performance on Si-fertilized plants, which subsequently reduced the consumption rate of its predator, *T. sinensis*. The anticipated (and observed) decrease in grass Si concentrations under elevated $CO_2$,

however, was not sufficient to alter the feeding performance of crickets or their predator.

### (a) Grass responses to Si and $CO_2$

Silicon promotes growth in many plant species, especially among the Poaceae, which can increase in biomass by up to 85% in Si-fertilized soils [5]. In our case, grass biomass increased by 70% in Si-supplemented plants compared with non-Si controls. Si supplementation significantly increased Si concentrations, whereas increasing $CO_2$ concentrations decreased Si concentrations. Moreover, Si concentrations were negatively correlated with C concentrations, suggesting a possible trade-off between Si and C, as was observed in a study of Si-defences in roots [7].

### (b) Herbivore performance

Calculating approximate digestibility of Si-supplemented plants by crickets (see [12]) suggested digestibility of leaves was not different from control plants ($98.05 \pm 0.44\%$ and $98.40 \pm 0.31\%$, respectively). Instead, it seems more likely that Si supplementation caused crickets to avoid these plants, potentially because of increased toughness and/or abrasiveness of leaf tissues, as observed in other studies

using chewing herbivores [12]. Increases in other chemical defences may also accompany increased Si-accumulation [5]. Regardless, foliar Si concentrations were associated with clear declines in herbivore feeding and body mass. Elevated $CO_2$ concentrations did not strongly affect herbivore performance despite causing a significant decrease in foliar Si concentrations, suggesting that these shifts in Si concentrations were not enough to alter the feeding behaviour of crickets or their predator in the current study. Plant species with higher baselines of Si-accumulation, however, may show greater declines and become more susceptible to herbivory under elevated $CO_2$ [4].

## (c) Higher trophic interactions

Surprisingly few studies have considered the effect of Si on the natural enemies of insect herbivores [5]. Si-uptake in plants may impact up the food chain to affect natural enemies directly, by modifying their behaviour, or indirectly, by changing the nutritional quality of their prey and/or host [13]. We identified an indirect effect of Si on the predator of a chewing herbivore, with a reduction in the consumption rate of crickets by predatory mantids, probably because of the diminished body size of herbivores and reduced nutritional quality (e.g. protein per unit of herbivore body mass). Regardless of mechanism, we have shown that Si-based defences, despite negative impacts on the herbivores themselves, can potentially weaken top-down control of herbivore populations by their predators. Furthermore, we have shown that these effects are not likely to be diminished under future climate change scenarios. The impacts of Si on higher trophic-level interactions in plants that employ Si-defences is, therefore, an important, but hitherto neglected, aspect of our understanding of the selective pressures determining how plants invest in silicon- and non-silicon-based defences [2].

## References

1. Price PW, Bouton CE, Gross P, McPheron BA, Thompson JN, Weis AE. 1980 Interactions among three trophic levels: influence of plants on interactions between insect herbivores and natural enemies. *Annu. Rev. Ecol. Syst.* **11**, 41–65. (doi:10.1146/annurev.es.11.110180.000353)

2. Ode PJ. 2006 Plant chemistry and natural enemy fitness: effects on herbivore and natural enemy interactions. *Annu. Rev. Entomol.* **51**, 163–185. (doi:10.1146/annurev.ento.51.110104.151110)

3. Cooke J, DeGabriel JL, Hartley SE. 2016 The functional ecology of plant silicon: geoscience to genes. *Funct. Ecol.* **30**, 1270–1276. (doi:10.1111/1365-2435.12711)

4. Hartley SE, DeGabriel JL. 2016 The ecology of herbivore-induced silicon defences in grasses. *Funct. Ecol.* **30**, 1311–1322. (doi:10.1111/1365-2435.12706)

5. Reynolds OL, Padula MP, Zeng R, Gurr GM. 2016 Silicon: potential to promote direct and indirect effects on plant defense against arthropod pests in agriculture. *Front. Plant Sci.* **7**, 484. (doi:10.3389/fpls.2016.00744)

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

7. Frew A, Powell JR, Sallam N, Allsopp PG, Johnson SN. 2016 Trade-offs between silicon and phenolic defenses may explain enhanced performance of root herbivores on phenolic-rich plants. *J. Chem. Ecol.* **42**, 768–771. (doi:10.1007/s10886-016-0734-7)

8. Barton CVM *et al.* 2010 Whole-tree chambers for elevated atmospheric $CO_2$ experimentation and tree scale flux measurements in south-eastern Australia: the Hawkesbury Forest Experiment. *Agric. For. Meteorol.* **150**, 941–951. (doi:10.1016/j.agrformet.2010.03.001)

9. Johnson SN, Gherlenda AN, Frew A, Ryalls JMW. 2016 The importance of testing multiple environmental factors in legume–insect research: replication, reviewers and rebuttal. *Front. Plant Sci.* **7**, 489. (doi:10.3389/fpls.2016.00489)

10. Massey FP, Ennos AR, Hartley SE. 2006 Silica in grasses as a defence against insect herbivores: contrasting effects on folivores and a phloem feeder. *J. Anim. Ecol.* **75**, 595–603. (doi:10.1111/j.1365-2656.2006.01082.x)

11. Hiltpold I, Demarta L, Johnson SN, Moore BD, Power SA, Mitchell C. 2016 Silicon and other essential element composition in roots using X-ray fluorescence spectroscopy: a high throughput approach. In *Invertebrate ecology of Australasian grasslands* (ed. SN Johnson). Hawkesbury, Australia: Western Sydney University.

12. Massey FP, Hartley SE. 2009 Physical defences wear you down: progressive and irreversible impacts of silica on insect herbivores. *J. Anim. Ecol.* **78**, 281–291. (doi:10.1111/j.1365-2656.2008.01472.x)

13. Kvedaras OL, An M, Choi Y, Gurr G. 2010 Silicon enhances natural enemy attraction and biological control through induced plant defences. *Bull. Entomol. Res.* **100**, 367–371. (doi:10.1017/S0007485309990265)

14. Ryalls JMW, Hartley SE, Johnson SN. 2017 Data from: Impacts of silicon-based grass defences across trophic levels under both current and future atmospheric $CO_2$ scenarios. Dryad Digital Repository. (doi:10.5061/dryad.j1d56)