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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://centaur.reading.ac.uk/82353/

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

Publisher: Wiley

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

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Abstract

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

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

Keywords – climate change, global warming, insect herbivore, silicon, silica, leaf toughness

Introduction

There is emerging consensus that silicon (Si) plays an important functional role in plants, particularly in terms of mitigating the impacts of adverse environmental conditions (Cooke & Leishman 2011; Frew, Weston, Reynolds & Gurr 2018). For abiotic stresses, Si can alleviate the effects of drought, salt stress, toxic metals, extreme temperatures and nutrient deficiency (Liang, Sun, Zhu & Christie 2007; Guntzer, Keller & Meunier 2012; Cooke & Leishman 2016). Similarly for biotic stresses, Si can reduce pathogen infection (Van Bockhaven, De Vleesschauwer & Hofte 2013; Wang et al. 2017) and herbivory (Reynolds, Keeping & Meyer 2009; Hartley & DeGabriel 2016). Indeed, the fact that many plants, particularly grasses, are hyper-accumulators of Si (up to 10% of dry mass) and are adapted to increase Si absorption following episodes of stress, points to the functional significance of this non-essential element (Epstein 1999; Massey, Ennos & Hartley 2007; Hartley & DeGabriel 2016).

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

Arguably, Si-based biomechanical defences are more important for resistance to herbivores than pathogens because biomechanical defences can affect herbivores adversely in multiple ways beyond being a physical barrier (Clissold 2008; Massey & Hartley 2009). Indeed, biomechanical properties of plants can influence entire communities of herbivores, with chewing herbivores being especially affected (Peeters, Sanson & Read 2007). Si deposition as discrete phytoliths or other abrasive structures, for example, can wear down insect mouthparts (Massey & Hartley 2009 but see Kvedaras et al. 2009). Ingested Si structures may also hinder efficient food processing and prevent herbivores from extracting adequate nutrition from ingested plant material (Massey & Hartley 2006). Moreover, Si deposition within and between cell walls can make cells more rigid and therefore harder to mechanically crush (Hunt, Dean, Webster, Johnson & Ennos 2008). Cooke, DeGabriel and Hartley (2016) highlight how surprisingly few studies measure biomechanical properties of plant tissues in relation to Si supplementation and herbivore resistance. In the present study we aimed to redress this by measuring two biomechanical properties (i.e. force of fracture and leaf toughness) with relevance to resistance to chewing herbivores (Sanson, Read, Aranwela, Clissold & Peeters 2001).

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

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

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

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

Materials and Methods

Experimental site

Twelve warming frames (Fig.1A) were established on an alluvial floodplain on the Hawkesbury campus of Western Sydney University at 25 m elevation (33°36’ 40″ S, 150°44’26.5″ E). The frames were identical in all respects except that six had the capacity to increase air temperature in the underlying vegetation (c. 1.0 m^2) by up to 3ºC. Frames were constructed by P&C Laws (Richmond, NSW, Australia) and comprised rectangular steel (250 mm × 250 mm) tubing (1.7 m × 1.7 m ×0.90 m; width × length × height). Frames were spaced 2.0 – 2.2 m apart from one another at the site with the warmed plots assigned at random. The plot was warmed using 1000W infra-red ceramic heaters (RS components part 376-2723, Mor Eletric Heating Assoc. Inc. MI, USA) mounted on the frame centrally above the plot (see example in Fig. 1B). This primarily warmed the vegetation and soil. A control system regulated the output of the heating lamps to maintain the canopy surface temperature at the desired set point. The control system comprised a purpose-built temperature sensor and Proportional-Integral-Derivative (PID) controller mounted on each frame and a central data logger (CR1000, Campbell Scientific), which logged the canopy surface temperature of each plot and coordinated the temperature controllers. The temperature controllers were built using a micro controller (Arduino Nano), an infra-red thermometer (Melexis' MLX90614ESF-BAA) and a solid-state relay (FOTEK SSR-40DA). The 12 Arduino controllers were networked with the data logger using RS485 converters (MAX485) and a MODBUS protocol with the logger as the master. The logger polled the six ambient unwarmed frames each minute then calculated the average ambient temperature and added the desired offset (+3°C) before sending this as the set point to the six heated frames. Each controller used a PID control algorithm and pulse width modulation to regulate the output of the heater to maintain the temperature at the set point.

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

Experimental Procedure

Two of the four pots in each plot received a one-off silicon supplementation (+Si plants hereafter) in the form of 126g of solid calcium silicate ($CaOSiO_4$) (PlantTuff^{TM} supplied by Australian Steel Mill Services, Port Kembla, NSW, Australia). This equates to an application rate of 10 tonnes ha^{-1} which is at the upper limit of application rates (Personal Communication, NSW Environment Protection Agency). Calcium silicate was lightly tilled into the top 3-5 cm of soil; non-supplemented plants (-Si plants hereafter) had soil lightly tilled without any amendment. In February 2017, c. 200 seeds of a common Australian naturalised pasture grass (Harding grass, *Phalaris aquatica* cultivar Holdfast; Heritage Seeds, NSW) were germinated in potting mix (Native soil mix, Turtle Nursery and Landscape Supplies, NSW) in the glasshouse for three weeks. Plants were then transplanted to the warming frames (48 pots each containing four plants) on 23 February 2017. Each frame therefore contained two +Si pots (2 × 4 plants) and two -Si pots (2 × 4 plants). Plants received natural rainfall (310 mm over six weeks) and supplemental irrigation (c. 200 ml every c. 10 days). Volumetric soil water content meaurements were taken using a hand held moisture probe (HydroSense 2; Campbell Scientific) at weekly intervals. After 50 days, a plant was selected at random and removed from each pot and washed free of soil. The roots and shoots were freeze dried, weighed and then ground for analysis of Si concentrations (see below). The remaining plants had been selected at random to determine leaf biomechanical properties (measured on day 50) and herbivore performance (*in situ* between days 44–50).

Silicon uptake in the plant

Si concentrations in the shoots and roots were determined with an X-ray fluorescence spectrometer (Epsilon-3x, PANalytical-Almelo, The Netherlands) using a method similar to that of Reidinger, Ramsey & Hartley (2012). In summary, root and leaf material was ground to a fine powder and analyzed according to the procedure described in Hiltpold et al. (2017). Si levels were expressed as shoot and root Si concentration (as % of dry mass) and calibrated against plant-certified reference material of known Si content (Garbuzov, Reidinger & Hartley 2011; Hiltpold et al. 2017).

Force of fracture and leaf toughness

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

Insect herbivore performance

Of the two remaining plants in each pot, one was selected at random and used to determine the effects of Si supplementation on herbivore performance. This was conducted six days prior to harvesting all of the plants. Approximately 100 *Helicoverpa amigera* eggs (supplied by CSIRO Agriculture & Food, Narrabri, Australia) were individually hatched on growing media (modified from Teakle & Jensen 1985) at 20ºC and 15:9 hour photoperiod (light:dark). Upon reaching second instar (c. 7 days later), one herbivore was weighed and then applied to each of the remaining plants in all 48 pots in the field. The insect was confined to one leaf blade using a circular (25.4 mm diameter) mesh clip cage (BioQuip, CA, USA) supported by a tethered post. Cages were moved on alternate days to undamaged leaf tissue to ensure food availability was not limiting. Six days later, all larvae were removed and re-weighed. Relative growth rates (RGR) were calculated: RGR = ((final mass – initial mass) / initial mass) / 6 days.

Statistical analysis

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

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

Results

Air temperature and soil moisture

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

Plant growth

Shoot and root biomass were unaffected by warming, but increased significantly in +Si plants overall (Fig. 3), although a significant warming by Si treatment interaction was observed for shoot biomass (P = 0.04). Contrast analysis showed that Si increased shoot and root biomass in ambient plots ($F_{1,33} = 11.61$, $P = 0.002$ and $F_{1,33} = 7.34$, $P = 0.011$, respectively) whereas shoot and root biomass were unaffected by Si supplementation in warmed plots ($F_{1,33} = 0.22$, $P = 0.645$ and $F_{1,33} = 2.81$, $P = 0.103$, respectively). Total plant biomass reflected the same responses as shoot biomass; Si promoted total biomass ($F_{1,34} = 7.40$, $P = 0.010$), temperature had no direct effect ($F_{1,10} = 2.90$, $P = 0.119$). Again, contrast analysis showed that Si impacts on plant growth were only apparent in ambient plots ($F_{1,33} = 11.11$, $P = 0.002$) and not warmed ($F_{1,33} = 0.21$, $P = 0.650$) plots.

Silicon uptake in the plant

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

Leaf biomechanical properties

Overall, the force required to fracture leaves at the midrib was c. 32% higher in +Si plants than -Si plants (Fig. 5A). Midrib and lamina thickness were not significantly affected by Si supplementation or warming (data not shown) and midribs of leaves from +Si plants were consequently c. 21% tougher (i.e. they had a higher specific work of fracture) than leaves from -Si plants (Fig. 5B). Si supplementation significantly increased midrib force to fracture and midrib toughness in ambient plots (t = -2.61, $P = 0.013$ and t = -2.24, $P = 0.032$, respectively) but these increases in +Si plants were less pronounced in warmed plots (t = -1.31, $P = 0.200$ and t = -0.60, $P = 0.551$, respectively). Overall leaf toughness was not significantly affected by Si supplementation (Table 1). Warming had a negligible impact on leaf biomechanical measures (Fig. 5, Table 1).

Insect herbivore performance

Relative growth rate (RGR) of *H. armigera* was halved when feeding on +Si plants compared to herbivores feeding on -Si plants, though this was only statistically significant under ambient conditions ($F_{1,33} = 9,53$, $P = 0.004$) and not under warmed conditions ($F_{1,33} = 2.11$, $P = 0.156$) (Fig. 6). Herbivore RGR was also negatively affected by warming compared to those feeding on plants grown in ambient plots (c. -55%). Analysing herbivore mass with ANCOVA (as recommended by Raubenheimer & Simpson 1992) gave similar results for temperature ($F_{1,9} = 5.81$, $P = 0.039$) and Si supplementation ($F_{1,33} = 9.86$, $P = 0.004$), with no interactive effect between the two ($F_{1,33} = 1.16$, $P = 0.289$).

Discussion

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

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

Global warming and Si uptake

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

In a glasshouse study using eight grass species, elevated temperatures were reported not to affect Si concentrations overall, but some species showed significant responses (Johnson & Hartley 2018). In particular, grass species native to Australia contained higher concentrations of Si, which rose in response to warmer conditions (c. +19%) whereas exotic species showed declines (c. -10%) in Si concentrations. It remains speculative, but having the capacity to absorb large amounts of Si might be a selective advantage to plants evolving in low-nutrient soils and seasonally arid climates such as Australia (Johnson & Hartley 2018). *Phalaris aquatica* originated in Southern Europe and the Caucasus and while now naturalised, it is exotic to Australia (Popay 2015). The observed declines in Si at warmer temperatures observed in the present study are therefore consistent with patterns for exotic species tested by Johnson and Hartley (2018). We should caution, however, that comparatively few grass species have been investigated in terms of global warming and Si uptake, and most of these (like the current study) were conducted over relatively short time periods. Moreover, the effects of warming on Si uptake can be further reduced by factors such as elevated atmospheric carbon dioxide concentrations (Ryalls, Hartley & Johnson 2017; Johnson & Hartley 2018) so warming effects could be context dependent.

Si and leaf biomechanical properties

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

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

Warming and herbivore performance

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

Conclusions

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

Acknowledgements

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

Authors' contributions

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

Data accessibility

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

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

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	$F_{1,34} = 1.85$, $P = 0.182$	$F_{1,30} = 0.73$, $P = 0.398$

Figure Legends

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.

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 ± 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 ± standard error shown (N = 12; six frames containing two pots). Result of statistical analysis for temperature (T) and Si supplementation (Si) provided.

Figure 3. Impacts of warming and Si supplementation on shoot and root growth[1]. Mean values ± 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 $^*P < 0.05$. [1]Data log transformed prior to analysis.

Figure 4. Impacts of warming and Si supplementation on Si concentrations in the leaves. Mean values ± 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 $^*P < 0.05$. [1]Data logit transformed prior to analysis.

Figure 5. Impacts of warming and Si supplementation on **(A)** force to fracture the midrib and **(B)** midrib toughness. Mean values ± 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 $^*P < 0.05$.

Figure 6. Relative growth rates (RGR) of *H. armigera* feeding on -Si and +Si plants under ambient and warmed air temperatures. Mean values ± 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 $^{**}P < 0.01$.

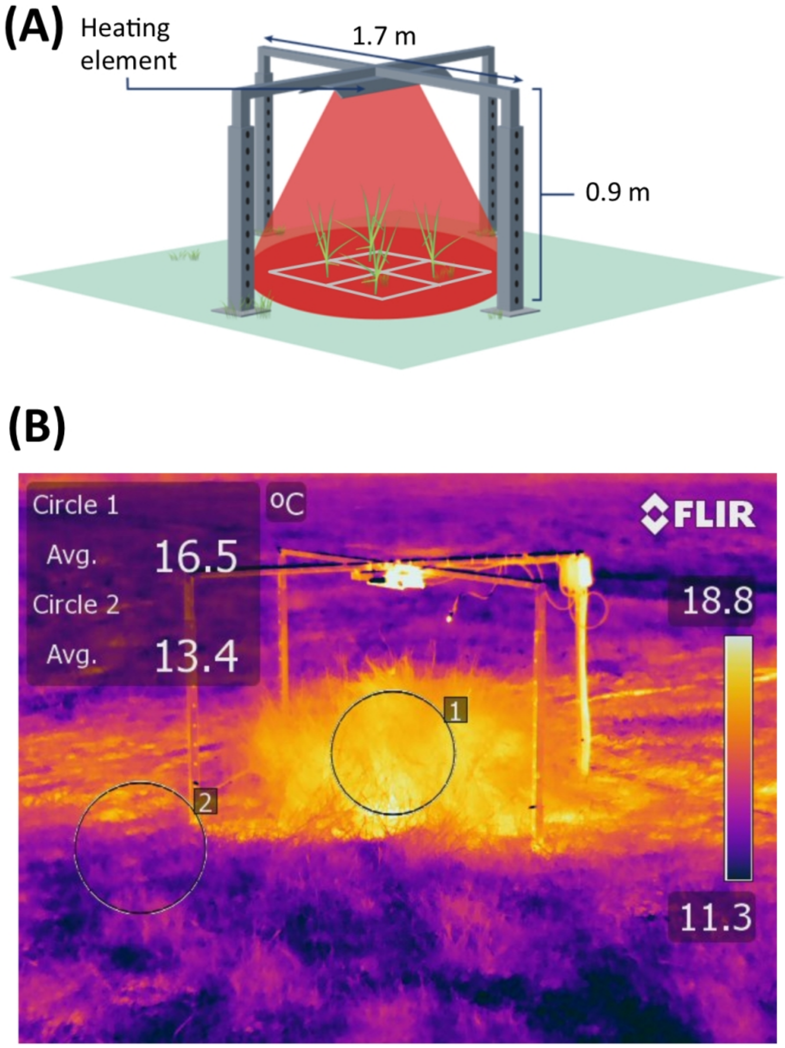

Figure 1. (A) Schematic of warming frames (one plant per pot shown for clarity) used in the study and (B) typical thermal image of a warmed area showing temperature within (circle 1) and outside (circle 2) the frames.

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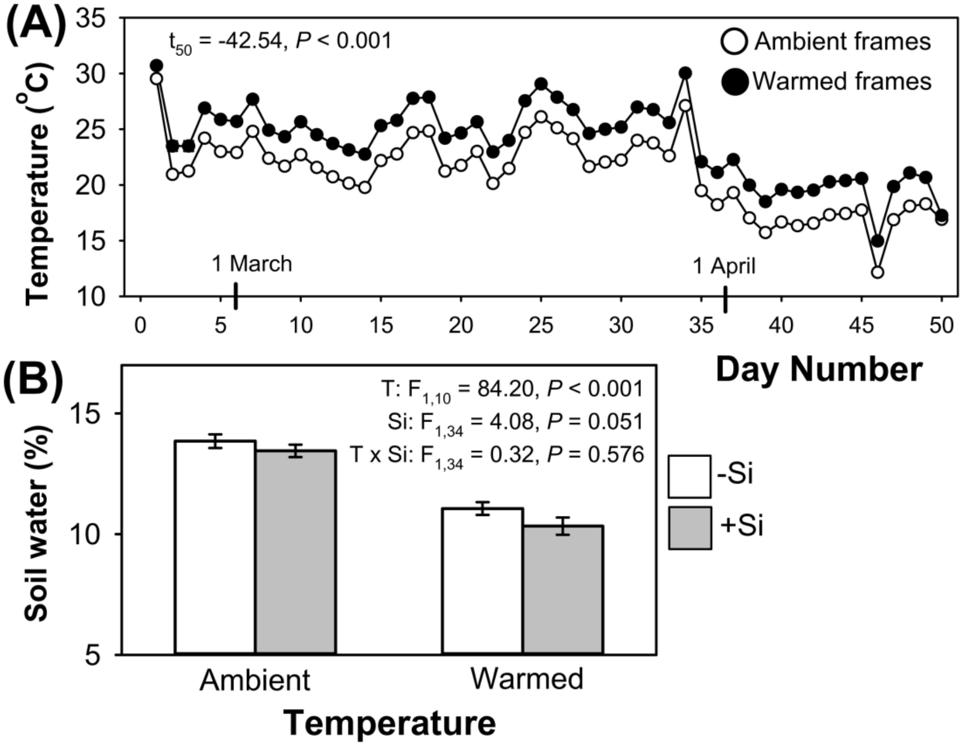

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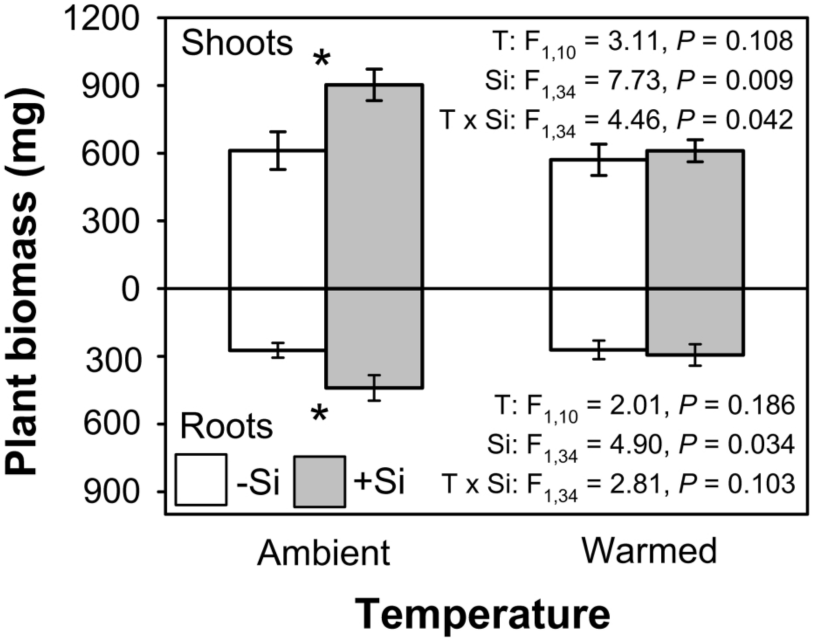

Figure 3. Impacts of warming and Si supplementation on shoot and root growth1. Mean values ± standard error shown (N = 12; six frames containing two pots). Results of statistical analyses for temperature (T) and Si supplementation (Si) provided with statistically significant differences between –Si and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

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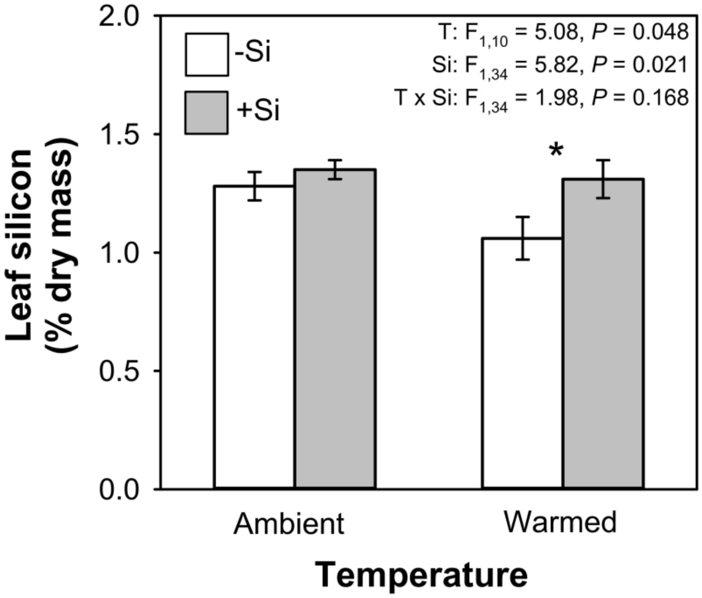

Figure 4. Impacts of warming and Si supplementation on Si concentrations in the leaves. Mean values ± standard error shown (N = 12; six frames containing two pots). Results of statistical analyses for temperature (T) and Si supplementation (Si) provided with statistically significant differences between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

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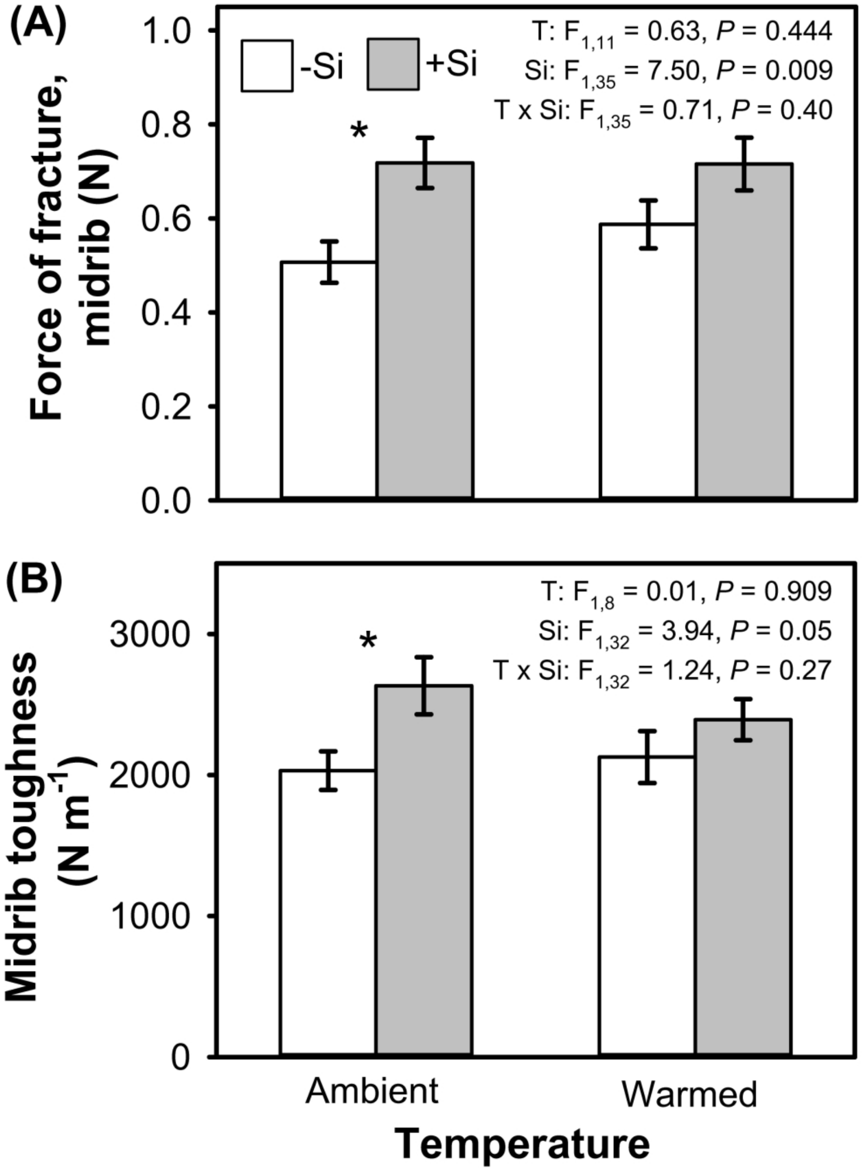

Figure 5. Impacts of warming and Si supplementation on (A) force to fracture the midrib and (B) midrib toughness. Mean values ± standard error shown. Results of statistical analyses for temperature (T) and Si supplementation (Si) provided with statistically significant differences between –Si and +Si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

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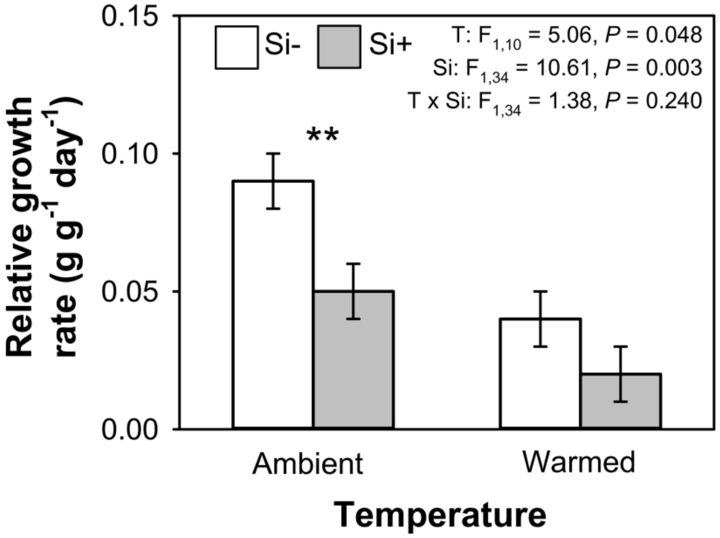

Figure 6. Relative growth rates (RGR) of H. armigera feeding on -Si and +Si plants under ambient and warmed air temperatures. Mean values ± standard error shown (N = 12; six frames containing two pots). Results of statistical analyses for temperature (T) and Si supplementation (Si) provided with statistically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

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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
KCl	Nitrate Nitrogen	mg/kg	16.74	13.29
	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
Ammonium Acetate + Calculations	Calcium	cmol$^+$/kg	3.46	0.81
		kg/ha	1552.04	364.26
		mg/kg	692.87	162.62
	Magnesium	cmol$^+$/kg	0.78	0.10
		kg/ha	213.13	26.62
		mg/kg	95.15	11.88
	Potassium	cmol$^+$/kg	0.42	0.05
		kg/ha	369.84	41.43
		mg/kg	165.11	18.50
	Sodium	cmol$^+$/kg	0.09	0.02
		kg/ha	44.91	8.53
		mg/kg	20.05	3.81
KCl	Aluminium	cmol$^+$/kg	0.02	0.00
		kg/ha	3.66	0.62
		mg/kg	1.63	0.28
Acidity Titration	Hydrogen	cmol$^+$/kg	0.05	0.01
		kg/ha	1.18	0.27
		mg/kg	0.53	0.12
Calculation	Effective Cation Exchange Capacity (ECEC)	cmol$^+$/kg	4.82	0.89
Base Saturation Calculations	Calcium	%	70.46	3.55
	Magnesium	%	16.70	1.63
	Potassium	%	9.30	1.65
	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
DTPA	Zinc	mg/kg	2.36	0.74
	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 Analyser	Total Carbon	%	1.52	0.41
	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.

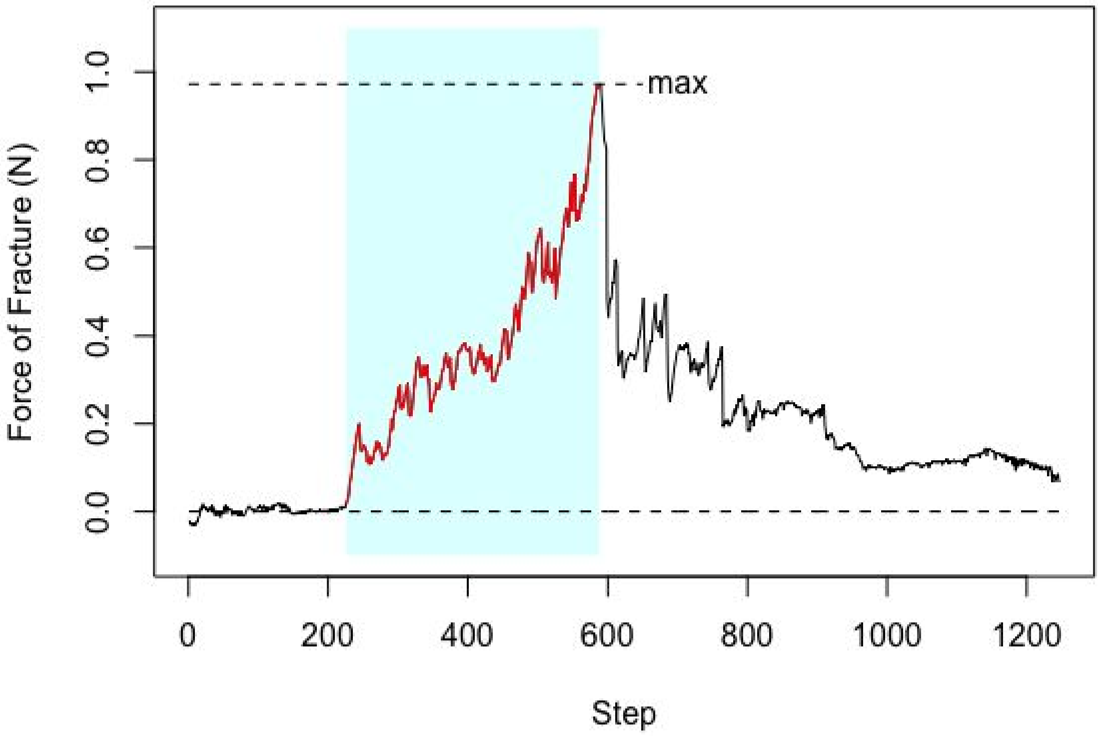

Fig. S2. Temperatures for all 12 frames running under ambient (grey lines) and warmed (black lines) conditions over a three-day period during the experiment. Note the higher variation in warmed frames during the middle of March 21, which was a windy day and therefore harder to consistently warm.