Elevated carbon dioxide and warming impact silicon and phenolic-based defences differently in native and exotic grasses

**Running head:** Elevated CO2 and warming impact grass defences

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**Abstract**

Global climate change may increase invasions of exotic plant species by directly promoting the success of invasive/exotic species or by reducing the competitive abilities of native species. Changes in plant chemistry, leading to altered susceptibility to stress, could mediate these effects. Grasses are hyper-accumulators of silicon, which plays a crucial function in the alleviation of diverse biotic and abiotic stresses. It is unknown how predicted increases in atmospheric carbon dioxide (CO2) and air temperature affect silicon accumulation in grasses, especially in relation to primary and secondary metabolites. We tested how elevated CO2 (eCO2) (+240ppm) and temperature (eT) (+4⁰C) affected chemical composition (silicon, phenolics, carbon and nitrogen) and plant growth and in eight grass species, either native or exotic to Australia. eCO2 increased phenolic concentrations by 11%, but caused silicon accumulation to decline by 12%. Moreover, declines in silicon occurred mainly in native species (-19%), but remained largely unchanged in exotic species. Conversely, eT increased silicon accumulation in native species (+19%) but decreased silicon accumulation in exotic species (-10 %). Silicon and phenolic concentrations were negatively correlated with each other, potentially reflecting a defensive trade-off. Moreover, both defences were negatively correlated with plant mass, compatible with a growth-defence trade-off. Grasses responded in a species-specific manner, suggesting that the relative susceptibility of different species may differ under future climates compared to current species rankings of resource quality. For example, the native *Microlaena stipoides* was less well defended under eCO2 in terms of both phenolics and silicon, so could suffer greater vulnerability to herbivores. To our knowledge, this is the first demonstration of the impacts of eCO­2 and eT on silicon accumulation in grasses. We speculate that the greater plasticity in silicon uptake shown by Australian native grasses may be partly a consequence of evolving in a low nutrient and seasonally arid environment.

**Introduction**

Up to a third of global carbon stocks are stored in grasslands, which account for up to 40% of terrestrial land mass ([Gibson, 2009](#_ENREF_23)). This is even higher in the Australian continent, with permanent meadows and pastures accounting for 360 million hectares, 47% of current land use ([FAO, 2016](#_ENREF_18)). Widespread introductions of exotic grass species have occurred throughout the continent and continue to threaten these important ecosystems ([Groves & Willis, 1999](#_ENREF_24)). Globally, introductions of exotic plant species, both deliberate and accidental, are known to be facilitated by climate change through a range of mechanisms ([Dukes & Mooney, 1999](#_ENREF_16); [Walther *et al.*, 2009](#_ENREF_69); [Bradley *et al.*, 2010](#_ENREF_5); [Dukes *et al.*, 2011](#_ENREF_15)). Global warming, for example, can make previously unsuitable regions more conducive to exotic grass species when lower thermal requirements of the invasive plants are exceeded ([Buckland *et al.*, 2001](#_ENREF_7); [Polley *et al.*, 2013](#_ENREF_54)). Another mechanism by which climate change can facilitate the spread of exotic species is when it renders native plant species less competitive than the exotic ones, either in terms of resource acquisition or in the natives’ ability to cope with biotic (e.g. herbivory) and abiotic (e.g. drought) stress ([Manea *et al.*, 2016](#_ENREF_43)).

Grasses are hyper-accumulators of silicon (Si), up to 10% of dry mass, which plays a crucial role in mitigating an extensive array of abiotic stresses, including drought, salt stress, toxic metals, nutrient deficiency ([Ma, 2004](#_ENREF_41); [Liang *et al.*, 2007](#_ENREF_38); [Guntzer *et al.*, 2012](#_ENREF_25)), and biotic stresses including pathogen infection ([Van Bockhaven *et al.*, 2013](#_ENREF_66)) and herbivory ([Massey *et al.*, 2007](#_ENREF_44); [Reynolds *et al.*, 2009](#_ENREF_59); [Cooke & Leishman, 2011](#_ENREF_8); [Hartley & DeGabriel, 2016](#_ENREF_27)). Plants can only acquire silicon via uptake of soluble silicic acid from the soil ([Epstein, 1999](#_ENREF_17)). The process of silicon uptake is still poorly characterised in most plant species, but it is thought to involve both active and passive mechanisms ([Ma *et al.*, 2001](#_ENREF_42); [Hartley, 2015](#_ENREF_26)). Silicon can be deposited within or between cells, in the cell wall or as discrete opaline phytoliths; such deposition varies dramatically between species and in response to damage and Si supply ([Hartley *et al.*, 2015](#_ENREF_28)). Silicon phytoliths are thought to have abrasive effects on herbivore mouthparts and guts, leading to reduced digestive efficiency ([Massey & Hartley, 2009](#_ENREF_45); [Reynolds *et al.*, 2016](#_ENREF_58)). Predicted increases in concentrations of atmospheric carbon dioxide (CO2) and air temperatures can have significant impacts on plant growth as well as primary and secondary metabolite profiles in the plant ([DeLucia *et al.*, 2012](#_ENREF_13); [Ode *et al.*, 2014](#_ENREF_52)), potentially including silicon-based resistance in grasses, though this remains unreported to date ([Hartley & DeGabriel, 2016](#_ENREF_27)). Robinson *et al*. ([2012](#_ENREF_60)) provide the most comprehensive meta-analysis of elevated CO2 (eCO2) impacts on plant-arthropod interactions to date. They specifically highlight silicon as a defence that could be affected by eCO2 and elevated temperature (eT), yet none of the studies available to them had measured silicon. Hence, as yet, evidence-based predictions on the impact of environmental change on silicon have not been possible.

What has been demonstrated is potential trade-offs between silicon and other defences. Silicon accumulation in grasses has been shown to be negatively correlated with phenolic compounds in both the shoots ([Cooke & Leishman, 2012](#_ENREF_9); [Schaller *et al.*, 2012](#_ENREF_63)) and roots ([Frew *et al.*, 2016](#_ENREF_20)). Cooke & Leishman ([2012](#_ENREF_9)) showed this convincingly using 47 plant species, and suggested that there could be a trade-off between silicon and carbon-based (e.g. phenolics and terpenoids) anti-herbivore defences in plants. Since phenolic compounds are widely reported to respond to environmental change ([Zvereva & Kozlov, 2006](#_ENREF_73); [Veteli *et al.*, 2007](#_ENREF_67); [Robinson *et al.*, 2012](#_ENREF_60)), such changes could affect silicon accumulation.

Broadly speaking, plant growth and photosynthesis increase in response to eCO2, with stronger responses seen in C3 than C4 plants ([Wand *et al.*, 1999](#_ENREF_70); [Long *et al.*, 2004](#_ENREF_39); [Ainsworth & Rogers, 2007](#_ENREF_1)). While both carbon and nitrogen often increase in plants grown under eCO2, carbon concentrations often increase at an accelerated rate relative to nitrogen concentrations, leading to an average increase in C:N ratios ranging from 11% ([Luo *et al.*, 2006](#_ENREF_40)) to 19% ([Robinson *et al.*, 2012](#_ENREF_60)), effectively reducing nitrogen concentrations, either by reallocation or dilution ([DeLucia *et al.*, 2012](#_ENREF_13)), although the latter mechanism has been contested ([Feng *et al.*, 2015](#_ENREF_19)). The most recent meta-analysis to examine the effects of eCO2 on plant secondary metabolites reported that nitrogen-based secondary metabolites decreased (-16%), whereas total phenolics increased by 19% ([Robinson *et al.*, 2012](#_ENREF_60)). Phenolic compounds, in particular, are a type of plant defence shown to increase fairly consistently across plant taxa in response to eCO2 ([Robinson *et al.*, 2012](#_ENREF_60)). Elevated temperature (eT) increases growth and photosynthesis to an optimum, beyond which it has adverse effects ([Newman *et al.*, 2011](#_ENREF_51)). The impacts of eT on primary and secondary metabolites are less clear cut. The meta-analysis by Zvereva and Kozlov ([2006](#_ENREF_73)) reported that eT led to decreased leaf soluble sugars, starch and total non-structural carbohydrates whereas nitrogen concentrations were unaffected. Generally, concentrations of foliar terpenes increased whereas phenolic compounds decreased ([Zvereva & Kozlov, 2006](#_ENREF_73)).

Studies involving both eCO2 and eT are rare compared to single factor studies ([Robinson *et al.*, 2012](#_ENREF_60)), despite the fact that they are predicted increase in tandem ([IPCC, 2014](#_ENREF_31)) and often have interactive impacts on plant chemistry when they have been investigated (e.g. [Murray *et al.*, 2013](#_ENREF_50); [Gherlenda *et al.*, 2015](#_ENREF_22); [Ryalls *et al.*, 2015](#_ENREF_61)). When eCO2 and eT were considered together, for example, temperature negated the increase in leaf carbohydrates caused by eCO2, but amplified decreases in leaf nitrogen concentrations, causing substantial increases in leaf C:N ratio ([Zvereva & Kozlov, 2006](#_ENREF_73)). Similarly, increased concentration of phenolics in trees observed under eCO2 were reduced when eT conditions were simultaneously applied ([Veteli *et al.*, 2007](#_ENREF_67)). The reasons for counteracting effects are unclear, but Kuokkanen *et al*. ([2001](#_ENREF_36)) speculated that higher temperatures may increase losses of carbon via maintenance respiration ([Amthor, 1984](#_ENREF_2)), potentially attenuating increased rates of carbon fixation under eCO2.

How might eCO2 and eT affect silicon uptake in grasses? Silicon is thought to provide plants with structural support at a lower metabolic cost than carbon-based compounds such as lignin ([Raven, 1983](#_ENREF_56); [McNaughton *et al.*, 1985](#_ENREF_47)), so higher carbon availability under eCO2 may reduce this advantage. Indeed, it has been suggested that silification of the Poaceae during the Miocene, when atmospheric CO2 levels were low, was a competitive strategy ([Craine, 2009](#_ENREF_11)) that allowed grasses to maintain growth and defence ([Cooke & Leishman, 2011](#_ENREF_8)). While this remains speculative ([see recent review by Stromberg *et al.*, 2016](#_ENREF_65)), it at least raises the prospect that the reverse may occur in a high CO2 world and plants could reduce silicon accumulation. In support of the hypothesis that eCO2 decreases silicon uptake is the fact that eCO2 usually depresses the jasmonate signalling pathway in plants ([Zavala *et al.*, 2013](#_ENREF_72); [Ode *et al.*, 2014](#_ENREF_52)). In rice at least, silicon uptake is stimulated by activation of the jasmonate pathway ([Ye *et al.*, 2013](#_ENREF_71)). eT often increases transpiration rates in plants and since silicon enters plants via the transpiration stream, silicon uptake may increase under eT ([Hartley & DeGabriel, 2016](#_ENREF_27)), though the link between silicon uptake and transpiration rates is subject to debate ([Quigley & Anderson, 2014](#_ENREF_55); [Hartley, 2015](#_ENREF_26); [Kumar *et al.*, 2017](#_ENREF_35)). Alternatively, eT could also facilitate silicon uptake by higher metabolically driven uptake of nutrients in general ([Hartley & DeGabriel, 2016](#_ENREF_27)).

It is widely held that silicon accumulation may be particularly beneficial to plants growing in seasonally arid environments during periods of drought ([Coughenour, 1985](#_ENREF_10); [Stromberg *et al.*, 2016](#_ENREF_65)), so it conceivable that grasses native to Australia may adjust silicon uptake more than exotic species in response to environmental change. The objective of this study was therefore to determine how widely occurring grass species in Australia, native and exotic, would respond to predicted changes in atmospheric CO2 and temperature, acting individually and in combination. We determined the effects of eCO2 (+240 ppm) and eT (+4ºC) on the silicon and carbon-based (phenolics) defences in eight grass species, relative to plants grown under ambient conditions. We additionally determined changes in nutritional quality (carbon and nitrogen concentrations) and plant growth. We hypothesise that eCO2 causes a decrease in silicon accumulation and an increase in phenolic production because of increased carbon availability and the previously observed trade-off between the two (e.g. [Cooke & Leishman, 2012](#_ENREF_9); [Schaller *et al.*, 2012](#_ENREF_63)). We predict opposing impacts of eT; increased silicon uptake (potentially via increased transpiration) and lower phenolic biosynthesis (e.g. [Zvereva & Kozlov, 2006](#_ENREF_73); [Veteli *et al.*, 2007](#_ENREF_67)). Based on this, we hypothesise that eT diminishes the impacts of eCO2 on silicon and phenolic concentrations. We predict a negative correlation between levels of silicon and phenolic compounds in the plant and also between plant growth (mass) and defence in general. Moreover, we anticipate that Australian native species will show greater plasticity in silicon uptake compared to exotic species partly because they have evolved in a seasonally arid and low nutrient environment.

**Materials and methods**

*Experimental procedure*

Eight grass species were initially grown from seed (Sydney Seeds, Seven Hills, NSW, Australia) in Osmocote Seed Raising & Cutting Mix (Scotts, Bella Vista, NSW, Australia). These comprised 40 replicates of five introduced or exotic species: annual ryegrass (*Lolium rigidum*), cocksfoot (*Dactylis glomerata*), perennial ryegrass (*Lolium perenne*), Rhodes grass (*Chloris gayana*), tall fescue (*Festuca arundinacea*) and three species native to Australia: red grass (*Bothriochloa macra*), weeping meadow grass (*Microlaena stipoides*) and Wallaby grass (*Austrodanthonia bipartia*). Grasses were grown in 70 mm diameter pots filled with coarsely sieved local Hawkesbury soil ([Barton *et al.*, 2010](#_ENREF_4))

Ten plants of each species were transferred after sowing to glasshouse chambers maintained at either ambient CO2 concentrations, aCO2 (400 μmol mol–1), or at eCO2 (640 μmol mol–1) which is broadly anticipated by 2050 ([Garnaut, 2011](#_ENREF_21)). Temperature was maintained at either ambient temperature (aT) (26/18°C day/night on a 15:9 light:dark cycle), or eT (30/22°C day/night on a 15:9 light:dark cycle). aT (26°C) represents the average daily maximum temperature for Richmond, NSW over the last 30 years, and eT was consistent with the predicted maximum temperature increase of 4°C for this region within this century ([CSIRO, 2007-2016](#_ENREF_12)). The environmental conditions within the chambers were logged and monitored continuously throughout the experiment to maintain temperature and CO2 differences between chambers and temperature readings were cross-checked with transportable temperature loggers. To minimise ‘chamber effects’ associated with using four chambers, plants were circulated within each chamber every five days and chambers were swapped every c. 10 days by transferring plants between chambers and adjusting the environmental conditions accordingly. While this does not eliminate pseudoreplication, using this approach in these chambers has provided almost identical empirical results to truly replicated experiments, whether replicated with chambers or experimental run ([Johnson *et al.*, 2016](#_ENREF_32)). Plants were grown for a further 16 weeks before being destructively harvested. Grasses were separated from the soil, washed freeze dried and weighed.

*Chemical analysis*

Dried shoot material was ball-milled. Leaf silicon concentrations were determined by placing approximately 100mg of ground plant material into a small mass holder (PANalytical), and then analysed with an X-ray fluorescence spectrometer (Epsilon 3x, PANalytical), using the procedure and certified reference material described in Hiltpold *et al*. ([2017](#_ENREF_29)). This method was based on the approach of Reidinger *et al.* ([2012](#_ENREF_57)). Concentrations of total phenolics were determined using a Folin–Ciocalteu assay with gallic acid (Sigma–Aldrich, St. Louis, MO, USA) as the quantification standard ([Salminen & Karonen, 2011](#_ENREF_62)). Foliar N and C concentrations were determined using a CHN analyser (LECO TruSpec Micro, LECO Corporation, St. Joseph, MI, USA).

*Statistical analysis*

Three way analysis of variance (ANOVA) tests were conducted on all plant responses with species, CO2 and temperature included as individual and interactive factors. The species term was replaced with origin status (i.e. exotic or native) to test for any patterns between these two groups. Correlations between plant growth, silicon and phenolic concentrations were tested to establish potential basis for trade-offs. Because of the strong interactive effects between species and one or both environmental factors we conducted subsequent statistical analysis for each species separately with two way (CO2 and temperature) ANOVAs. Unless stated in table or figure legends, analysis was conducted on untransformed data. Where appropriate, transformations were chosen to give residual diagnostic plots which best fitted a normal distribution and showed least heteroscedasticity. All analysis was conducted in Genstat (Version 17, VSN International, UK).

**Results**

*Responses of grouped grass species*

All of the plant traits measured varied between species (Table 1). eCO2 increased plant growth, carbon concentrations, C:N ratio and phenolic concentrations, but caused significant declines in silicon accumulation (Table 1). There was a significant negative correlation between concentrations of phenolics and silicon (Fig. 1A) and also between plant growth and concentrations of both phenolics and silicon (Fig. 1B). CO2 and temperature affected native and exotic plant traits similarly except for silicon accumulation which was much more adversely affected by CO2 in natives (-19%) compared to invasive species (< 1%) (F1,279 = 11.36, *P* < 0.001). Conversely, eT increased silicon accumulation in native species (+19%) but decreased silicon accumulation in exotic species (-10 %). Overall, temperature did not impact on any other plant traits but diminished CO2-induced increases in biomass and carbon concentrations, as indicated by significant interaction terms in Table 1.

Because there were significant interactive effects between species and one or more environmental factors (Table 1), analysis was conducted and reported for each species separately (Table 2). A native species, *Microlaena stipoides*, was most affected by eCO2 and eT, whilst an exotic, *Lolium rigidum,* was the least affected.

*Silicon and phenolics*

eCO2 caused significant decreases in foliar silicon concentrations for *M. stipoides* (Fig. 2A) and *A.* *bipartita* (Fig. 2C), but increased concentrations in *C. gayana* (Fig. 2D), though only at elevated temperatures. Warmer temperatures increased silicon concentrations in both *M. stipoides* (Fig. 2A) and *B. macra* (Fig. 2D). eCO2 concentrations increased phenolic concentrations in *C. gayana* (Fig. 3B), *A. bipartita* (Fig. 3C), *B. macra* (Fig. 3D) and *L. perenne* (Fig. 3H), but decreased phenolic concentrations in *M. stipoides* (Fig. 3B). Warmer temperatures caused phenolic concentrations to decline in *C. gayana* (Fig. 3B), and reduced CO2-driven increases in phenolic concentrations observed at ambient temperatures for *L. perenne* (Fig. 3H) and *F. arundinacea* (Fig. 3E), with the interactions operating in the opposite direction for *D. glomerata* (Fig. 3F).

*Plant growth*

Elevated temperature caused significant decreases in plant growth for *M. stipoides*, *F. arundinacea*, *L. rigidum* (Figs. S1 A, E, G, respectively) but increased growth of *C. gayna* (Fig. S1B). eCO2 promoted growth of *F. arundinacea* (Fig. S1E) and *A. bipartita* (Fig. S1C). Elevated temperature and CO2 had interactive effects on several occasions. Higher temperatures reduced CO2 driven increases in growth for *M. stipoides* (Fig. S1A), *C. gayana* (Fig. S1B), *A. bipartita* (Fig. S1C), *D. glomerata* (Fig. S1F), *L. rigidum* (Fig. S1G) and *L. perenne* (Fig. S1H).

*Primary chemistry*

Elevated temperature caused a small decline in foliar carbon concentrations for *M. stipoides* (Fig. S2A), and interacted with elevated CO2 to reduce the non-significant increase in carbon concentrations in *L. perenne* seen under elevated CO2 (Fig. S2H). eT increased foliar nitrogen in *M. stipoides* (Fig. S3A) and *A. bipartita* (Fig. S3D) but caused declines in in *C. gayana* (Fig. S3B). eCO2 caused foliar nitrogen concentrations to decline for both *B. macra* (Fig. S3D), *F. arundinacea* (Fig. S3E) *D. glomerata* (Fig. S3F). eT and eCO2 interacted on three occasions, with eCO2 slightly increasing nitrogen concentrations at ambient temperatures but decreasing concentrations at higher temperatures in *M. stipoides* (Fig. S3A). eCO2 had exactly the opposite effect for *A. bipartita* (Fig. S3C) and *D. glomerata* (Fig. S3F); it caused concentration declines under ambient temperature conditions which were no longer manifested when temperature increased. Changing nitrogen concentrations, in response to elevated CO2, altered foliar C:N ratio in *F. arundinacea* (Fig. S4E) and *L. rigidum* (Fig. S4G), whereas temperature effects on nitrogen concentrations caused C:N shifts in *M. stipoides* (Fig. S4A), *C. gayana* (Fig. S4B) and *A. bipartita* (Fig. S4C). Some *C. gayana* plants contained particularly low levels of nitrogen, which had a disproportionately large effect on foliar C:N.

The overall effects of eT and eCO2 and their interactive effects are shown in Fig. 4 (C:N ratios not shown).

**Discussion**

This study has shown widespread changes in the primary and secondary chemistry of both native and exotic grass species common to Australia under predicted climate and atmospheric change conditions. Hovenden & Williams ([2010](#_ENREF_30)) reported that Australian native grasses seemed less responsive to increases in atmospheric concentrations of CO2, at least in terms of growth and physiology, than other plant taxa but we have shown that changes in their chemical composition in response to these factors are common. In particular, silicon accumulation in Australian native species showed significant responses to both eCO2 (declines) and eT (increases).

Silicon ameliorates abiotic stresses including heavy metal toxicity, salinity, nutrient imbalances, heat and drought ([Liang *et al.*, 2007](#_ENREF_38)), some of which are likely to themselves be exacerbated under future climate change ([Newman *et al.*, 2011](#_ENREF_51)), as well as defending plants against both pathogens ([Van Bockhaven *et al.*, 2013](#_ENREF_66)) herbivores ([Reynolds *et al.*, 2009](#_ENREF_59); [Hartley & DeGabriel, 2016](#_ENREF_27)). The fact that eCO2 caused silicon accumulation to decline overall, and very strongly in native species, may compromise the alibility of these grasses to cope with the biotic and abiotic stresses in the future. This seems especially true for the Australian native *M. stipoides*, which showed decreased levels of both silicon and phenolic-based defences under eCO2, while nitrogen concentrations increased under eT. Since nitrogen is often limiting in insect herbivore diets ([Mattson, 1980](#_ENREF_46)), this could make *M. stipoides* both more nutritious and less defended to herbivores under future climates. At the same time, all of the invasive species either maintained or increased concentrations of defensive compounds under these scenarios. Under such circumstances, it is conceivable that *M. stipoides* would suffer increased rates of herbivory, giving a selective advantage to the less nutritious and better defended invasive species. Plant community dynamics can be strongly driven by selective herbivory ([Olff & Ritchie, 1998](#_ENREF_53); [Lakeman-Fraser & Ewers, 2013](#_ENREF_37)), so changes in host quality arising from climate and atmospheric change may alter the competitive balance between species.

*Microlaena stipoides* is an important native species in Australia, much valued for its forage quality and year round growth, to the extent that there is a concerted effort to domesticate this species as a food crop ([Shapter *et al.*, 2013](#_ENREF_64)). In addition to having a large grain size and beneficial architectural traits, it is well adapted to a range of marginal and variable environments ([Mitchell *et al.*, 2014](#_ENREF_48)). The extent to which silicon allows *M. stipoides* to cope with these environmental conditions is unknown, though it was one of the highest accumulators of silicon we tested, so presumably silicon has a functional role for this species. Lower silicon accumulation under future climates could only diminish its capacity to cope with unfavourable environmental conditions. Moreover, *M. stipoides* has hitherto been seen as fairly competitive against invasive grass species ([Badgery *et al.*, 2005](#_ENREF_3)), but this prediction may be less robust with reduced silicon accumulation, particularly since the exotic species we tested maintained silicon accumulation under eCO2. We previously reported that root herbivory of *M. stipoides* was more pronounced under eCO2 ([Johnson *et al.*, 2014](#_ENREF_33)), which may in part be due to reductions in silicon uptake reported here. Given that invasive root-feeding scarabs can outweigh the mass of grazing mammals aboveground in Australian pastures ([Britton, 1978](#_ENREF_6)), increased levels of root herbivory could therefore be highly detrimental to a less well defended *M. stipoides*.

While eCO2 frequently increases defences such as phenolics ([Robinson *et al.*, 2012](#_ENREF_60)), negative correlations between phenolics and silicon-based defences reported here and previously ([Cooke & Leishman, 2012](#_ENREF_9); [Schaller *et al.*, 2012](#_ENREF_63); [Frew *et al.*, 2016](#_ENREF_20)) could make some plants more susceptible to herbivory. Cooke & Leishman ([2012](#_ENREF_9)) demonstrated that silicon was negatively correlated with the abundance of Coleoptera (including many herbivores), whereas phenolics and tannins were unrelated to insect abundance. Moreover, phenolic defences are often ineffective against root herbivores ([see examples in Johnson & Nielsen, 2012](#_ENREF_34)). Canegrub performance was even positively correlated with phenolic concentrations in sugarcane because of the trade-off with the more effective silicon defences ([Frew *et al.*, 2016](#_ENREF_20)). These examples suggest that silicon is potentially a more effective anti-herbivore defence in grasses than some carbon-based defences, particularly against root herbivores, although there are examples of grass phenolics being effective against both foliar and root herbivores ([Vicari & Bazely, 1993](#_ENREF_68); [Moore & Johnson, 2017](#_ENREF_49)). Moreover, we found negative correlations between plant dry mass (and implicitly growth rates) and both defences. This is at least compatible with a growth-defence trade-off in plants; defence acquisition comes at the cost of plant growth. Interestingly, silicon accumulation would appear to be the less costly defence since it was acquired at almost four times the rate as phenolic biosynthesis per unit of dry mass decrease.

The extent of silicon accumulation in plants appears to be related to the presence of silicon transporter genes, NIP2s in particular, which are present in plants accumulating silicon in excess of 1% of dry mass (termed ‘silicon competent’) ([Deshmukh & Bélanger, 2016](#_ENREF_14)). Plants lacking functional NIP2s typically accumulate <1% silicon dry mass ([Deshmukh & Bélanger, 2016](#_ENREF_14)). All of the native species in this study contained >1% silicon, whereas all of the exotic species contained less than 1%. It is still uncertain how and why plants have acquired and lost silicon competence ([Stromberg *et al.*, 2016](#_ENREF_65)), but we can conclude that the natives in the current study possessed this trait and that they showed greatest changes in silicon accumulation in response to eCO2 and eT. It could be speculated that absorbing relatively more silicon, with greater plasticity (i.e. regulating active transporters as needed), would be a selective advantage to plants evolving in low nutrient soils and seasonally arid climates ([Stromberg *et al.*, 2016](#_ENREF_65)) such as Australia. As previously discussed, silicon may act as substitute for nutrient costly structural compounds ([Raven, 1983](#_ENREF_56); [McNaughton *et al.*, 1985](#_ENREF_47)) and has been widely shown to alleviate the effects of heat and drought ([Liang *et al.*, 2007](#_ENREF_38)). The fact that Australian natives in this study responded to warmer temperatures by increasing silicon uptake is at least compatible with this hypothesis.

Given the importance that silicon plays in the Poaceae, particularly in terms of stress mitigation, we suggest silicon should be included in the suite of chemical responses that experimenters measure when assessing the potential impacts of global change on plant communities. Here we provide empirical support for this suggestion and our findings demonstrate that diminished silicon accumulation in a high CO2 world could leave some grass species more susceptible to herbivores and potentially other forms of plant stress.

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**Table 1.** Plant responses to varying CO2 and temperature across all eight grass species. Mean values ± S.E. shown. F values (maximum degrees of freedom shown in parentheses) shown with statistical significance indicated \* *P* < 0.05, \*\* P < 0.01 or \*\*\* *P* < 0.001 in **bold**. Spp. = grass species, CO2 = carbon dioxide and T = temperature.

|  |  |
| --- | --- |
| **Environmental Factors** | **Plant responses** (mean ± S.E.) |
| Temperature | CO2 | Silicon (%) | Phenolics (GAE mg g-1) | Biomass (g)1 | Carbon (%)2 | Nitrogen (%)2 | C:N3 |
| 26ºC | aCO2 | 0.93 ± 0.06 | 8.05 ± 0.40 | 2.69 ± 0.32 | 41.61 ± 0.20 | 0.55 ± 0.33 | 89.24 ± 4.66 |
| eCO2 | 0.83 ± 0.05 | 8.87 ± 0.29 | 5.04 ± 0.68 | 42.35 ± 0.21 | 0.50 ± 0.20 | 100.95 ± 6.34 |
| 30ºC | aCO2 | 1.00 ± 0.09 | 7.88 ± 0.40 | 5.01 ± 0.93 | 41.99 ± 0.24 | 0.54 ± 0.03 | 118.10 ± 13.52 |
| eCO2 | 0.88 ± 0.05 | 8.78 ± 0.45 | 3.03 ± 0.41 | 41.93 ± 0.16 | 0.52 ± 0.03 | 102.98 ± 6.72 |
| **Statistical analysis** | F |  | F | F | F | F |
| Spp (7,288) | **65.39\*\*\*** | **34.07\*\*\*** | **242.86\*\*\*** | **16.43\*\*\*** | **63.09\*\*\*** | **66.43\*\*\*** |
| CO2 (1,288) | **11.04\*\*\*** | **9.67\*\*** | **5.05\*** | **5.34\*** | 3.20 | **4.06\*** |
| T (1,288) | 2.13 | 0.14 | 1.91 | 0.01 | 1.38 | 1.35 |
| Spp x CO2 (7,288) | **5.32\*\*\*** | **2.60\*** | 1.47 | 0.31 | **3.58\*\*\*** | 3.58 |
| Spp x T (7,288) | **3.74\*\*\*** | **2.12\*** | **8.22\*\*\*** | 0.99 | **10.23\*\*\*** | **9.99\*\*\*** |
| CO2 x T (1,288) | 0.13 | 0.01 | **70.99\*\*\*** | **8.10\*\*** | 2.62 | 3.68 |
| Spp x CO2 x T (7,288) | **2.87\*\*** | **4.20\*** | **6.85\*\*\*** | **5.06\*\*\*** | **4.65\*\*\*** | 3.96 |

1Log+1 transformed, 2Logit transformed, 3Log transformed

**Table 2**. Results of ANOVA tests of plant responses to CO2 and temperature (and interactions of the two). F1,36 values shown with statistical significance indicated **in bold**: \**P* < 0.05, \*\**P* < 0.01 or \*\*\**P* <0.001.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Species** | **Response** | **CO2** | **T** | **CO2 x T** |  | **Species** | **Response** | **CO2** | **T** | **CO2 x T** |
|  |  | F | *P* | F | *P* | F | *P* |  |  |  | F | *P* | F | *P* | F | *P* |
| Weeping meadow grass*Microlaena stipoides* | Silicon2 | **6.25** | **\*** | **4.27** | **\*** | 0.72 |  |  | Tall fescue*Festuca arundinacea* | Silicon2 | 0.02 |  | 0.12 |  | 1.16 |  |
| Phenolics | **4.03** | **\*** | 0.35 |  | 0.84 |  |  | Phenolics | 0.84 |  | 1.55 |  | **4.35** | \* |
| Mass | 0.24 |  | **7.75** | **\*\*** | **8.14** | **\*\*** |  | Mass | **12.96** | **\*\*\*** | 12.85 | **\*\*\*** | 0.27 |  |
| C2  | 3.25 |  | **6.34** | **\*** | 0.23 |  |  | C2 | 0.16 |  | 2.26 |  | 2.81 |  |
| N2  | 0.20 |  | **9.89** | **\*\*** | **8.91** | **\*\*** |  | N2 | **6.25** | **\*** | 2.11 |  | 0.35 |  |
| C:N3 | 0.39 |  | **10.81** | **\*\*** | **7.96** | **\*\*** |  | C:N | **6.31** | **\*** | 1.88 |  | 1.10 |  |
| Rhodes grass*Chloris gayana* | Silicon | **4.90** | **\*** | **12.72** | **\*\*\*** | **22.92** | **\*\*\*** |  | Cocksfoot*Dactylis glomerata* | Silicon | 2.16 |  | 0.20 |  | 1.18 |  |
| Phenolics | **4.31** | **\*** | **17.97** | **\*\*\*** | 1.75 |  |  | Phenolics2 | 0.09 |  | 1.76 |  | **7.46** | **\*\*** |
| Mass1 | 0.08 |  | **9.82** | **\*\*** | **18.47** | **\*\*\*** |  | Mass1 | **0.72** |  | 3.63 |  | **16.08** | **\*\*\*** |
| C2 | 0.08 |  | 0.01 |  | 3.55 |  |  | C2 | 2.88 |  | 2.61 |  | 2.21 |  |
| N2 | 3.17 |  | **24.33** | **\*\*\*** | 0.34 |  |  | N | **9.80** | **\*\*** | 1.03 |  | **14.81** | **\*\*\*** |
| C:N3 | 2.94 |  | **23.06** | **\*\*\*\*** | 0.52 |  |  | C:N | 1.37 |  | 1.10 |  | **11.88** | **\*\*** |
| Wallaby grass*Austrodanthonia* *bipartita* | Silicon2 | **14.00** | **\*\*\*** | 0.18 |  | 2.21 |  |  | Annual ryegrass*Lolium rigidum* | Silicon2 | 1.31 |  | 0.21 |  | 0.31 |  |
| Phenolics | **8.64** | **\*\*** | 0.71 |  | 2.89 |  |  | Phenolics2 | 3.51 |  | 0.01 |  | 0.76 |  |
| Mass1 | **6.33** | **\*** | 0.17 |  | **4.53** | **\*** |  | Mass1 | 1.99 |  | **13.79** | **\*\*\*** | **10.83** | **\*\*** |
| C | 0.32 |  | 0.11 |  | 2.41 |  |  | C2 | 2.31 |  | 0.06 |  | 1.45 |  |
| N | 0.71 |  | **11.67** | **\*\*** | **10.92** | **\*\*** |  | N2 | 3.71 |  | 1.06 |  | 2.96 |  |
| C:N3 | 2.12 |  | **11.96** | **\*\*** | **11.54** | **\*\*** |  | C:N3 | **4.29** | **\*** | 0.95 |  | 2.32 |  |

*Continued overleaf*

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| *Continued* | **Response** | **CO2** | **T** | **CO2 x T** |  | **Species** | **Response** | **CO2** | **T** | **CO2 x T** |
| Red grass*Bothriochloa macra* | Silicon2 | 0.01 |  | **22.71** | **\*\*\*** | 1.80 |  |  | Perennial ryegrass*Lolium perenne* | Silicon2 | 0.04 |  | 2.83 |  | 0.01 |  |
| Phenolics | **5.59** | **\*** | 0.75 |  | 0.56 |  |  | Phenolics2 | **4.29** | **\*** | 0.06 |  | **9.47** | **\*\*** |
| Mass1 | 0.02 |  | 0.08 |  | 1.42 |  |  | Mass1 | 0.61 |  | 0.40 |  | **41.09** | **\*\*\*** |
| C2 | 0.15 |  | 2.00 |  | 0.56 |  |  | C2 | 0.48 |  | 0.12 |  | **35.23** | **\*\*\*** |
| N2 | **4.08** | **\*** | 0.90 |  | 2.82 |  |  | N2 | 2.63 |  | 0.07 |  | 1.11 |  |
| C:N3 | 4.03 |  | 1.24 |  | 2.28 |  |  | C:N | 2.26 |  | 0.08 |  | 0.17 |  |

1Log+1 transformed, 2Logit transformed, 3Log transformed

**Figure Legends**

**Figure 1.** Negative correlations between (A) silicon and phenolic concentrations and (B) plant dry mass and both silicon (Si) and phenolic (Ph) concentrations. 95% confidence intervals displayed as dashed lines.

**Figure 2**. Impacts of eCO2 and eT on shoot Si concentrations. Statistically significant (*P* < 0.05) impacts and interactions indicated in each panel. Mean values ± standard error shown. N = 10. Statistically significant terms and interactions (temperature, T, and carbon dioxide, CO2) indicated in panels (see Table 2 for statistical analysis).

**Figure 3**. Impacts of eCO2 and eT on shoot phenolic concentrations. Statistically significant (*P* < 0.05) impacts and interactions indicated in each panel. Mean values ± standard error shown. N = 10. Statistically significant terms and interactions (temperature, T, and carbon dioxide, CO2) indicated in panels (see Table 2 for statistical analysis).

**Figure 4**. Summary of impacts of eCO2 and eT on grass traits. Native (N) and exotic (E) species indicated. Downward red arrows indicate significant declines and upward green arrows indicate increases. Significant interactions between factors shown in scaled illustrated panels.

*Fig. 1.*



*Fig. 2.*

*Fig. 3.*

*Fig. 4*

**Supporting information**

**Figure S1**. Impacts of eCO2 and eT on shoot biomass. Statistically significant (*P* < 0.05) impacts and interactions indicated in each panel. Mean values ± standard error shown. N = 10. Statistically significant terms and interactions (temperature, T, and carbon dioxide, CO2) indicated in panels (see Table 2 for statistical analysis).

**Figure S2**. Impacts of eCO2 and eT on plant carbon concentration. Statistically significant (*P* < 0.05) impacts and interactions indicated in each panel. Mean values ± standard error shown. N = 10. Statistically significant terms and interactions (temperature, T, and carbon dioxide, CO2) indicated in panels (see Table 2 for statistical analysis).

**Figure S3**. Impacts of eCO2 and eT on plant nitrogen concentration. Statistically significant (*P* < 0.05) impacts and interactions indicated in each panel. Mean values ± standard error shown. N = 10. Statistically significant terms and interactions (temperature, T, and carbon dioxide, CO2) indicated in panels (see Table 2 for statistical analysis).

**Figure S4**. Impacts of eCO2 and eT on plant C:N ratio. Statistically significant (*P* < 0.05) impacts and interactions indicated in each panel. Mean values ± standard error shown. N = 10. Statistically significant terms and interactions (temperature, T, and carbon dioxide, CO2) indicated in panels (see Table 2 for statistical analysis).

*Fig. S1*

*Fig. S2*

*Fig. S3*

*Fig. S4*