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Global change biology

Contrasting effects of Miocene and Anthropocene levels of atmospheric CO₂ on silicon accumulation in a model grass

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Grasses are hyper-accumulators of silicon (Si), which they acquire from the soil and deposit in tissues to resist environmental stresses. Given the high metabolic costs of herbivore defensive chemicals and structural constituents (e.g. cellulose), grasses may substitute Si for these components when carbon is limited. Indeed, high Si uptake grasses evolved in the Miocene when atmospheric CO₂ concentration was much lower than present levels. It is, however, unknown how pre-industrial CO₂ concentrations affect Si accumulation in grasses. Using *Brachypodium distachyon*, we hydroponically manipulated Si-supply (0.0, 0.5, 1, 1.5, 2 mM) and grew plants under Miocene (200 ppm) and Anthropocene levels of CO₂ comprising ambient (410 ppm) and elevated (640 ppm) CO₂ concentrations. We showed that regardless of Si treatments, the Miocene CO₂ levels increased foliar Si concentrations by 47% and 56% relative to plants grown under ambient and elevated CO₂, respectively. This is owing to higher accumulation overall, but also the reallocation of Si from the roots into the shoots. Our results suggest that grasses may accumulate high Si concentrations in foliage when carbon is less available (i.e. pre-industrial CO₂ levels) but this is likely to decline under future climate change scenarios, potentially leaving grasses more susceptible to environmental stresses.

1. Introduction

Grasslands account for 20–30% of land mass, one-third of carbon sequestration [1] and provide 70% of human calorie intake either directly or indirectly (via livestock production) [2]. A particular feature of grasses is their ability to accumulate large amounts of silicon (Si, hereafter) from the soil that they deposit in tissues in the form of solid silica (SiO₂) [3]. Si is initially absorbed and transported in the form of monosilicic acid [Si(OH)₄] [4] and relies on passive (i.e. transpiration stream) and active uptake [3]. The latter requires the use of energy derived from the proton gradient [5]. Scientists have debated the importance of Si accumulation in grasses for over 100 years, but there is a consensus that it plays an important role in ameliorating a range of environmental stresses [6]. These include biotic (e.g. pathogens and herbivores) and abiotic (e.g. heavy metal toxicity, extreme temperatures, nutrient deficiency, drought and salt) stresses [3,6].

Another advantage of Si uptake is for carbon substitution, conceivably the reason for the evolution of high Si uptake grasses under low CO₂ [7]. High Si-accumulating Poales evolved during the Miocene, when atmospheric CO₂ was

much lower [3]. In particular, Si may be used in a structural capacity *in lieu* of more energetically costly carbon constituents like cellulose and lignin, which require 10–20 times more energy to incorporate into the cell wall than Si [8]. Moreover, Si may represent a more cost efficient form of plant defence than carbon-based defences such as phenolics and tannins [9]. The expansion of Si-rich grasses in the Miocene (15 million years ago (15 Ma)) [10], coincided with Si phytolith deposition that potentially played a role in resisting the effects of co-evolving herbivores, although the evidence for this is equivocal [11].

Since pre-industrial periods, we have witnessed dramatic increases in atmospheric CO₂ concentrations [12] and this often affects plant chemistry, with carbon (C) and nitrogen (N) being the most uniformly affected [13,14]. In this perspective, increased and decreased C and N concentrations in plant tissues under elevated CO₂ concentration have been reported by Taub & Wang [15]. This is related to N dilution or N re-allocation and increases in structural carbohydrates [16]. Studies on the effects of CO₂ on Si accumulation are scarce and limited to the effects of elevated CO₂. In general, elevated CO₂ causes C concentrations to increase and Si concentrations to decline in several grass species [14,17]. Consequently, Si accumulation is often negatively correlated with C [14].

Brachypodium distachyon (Poaceae) is the model species for grasses, including major cereal crops, because it has phylogenetic and anatomical similarities with maize, wheat and barley [18]. Furthermore, it has a short generation time, a small stature, simple growth requirements and self-fertility [19]. It has comparable levels of shoot Si to major grass crops, which makes it useful for investigating the transport, accumulation and functional properties of Si in crop plants [20].

Given the functional importance of Si in grasses, the objective of this study was to investigate the impact of pre-industrial, current and future levels of atmospheric CO₂ on Si uptake in this model grass and how this relates to stoichiometry (C and N). Therefore, we hypothesized that Si uptake is greatest under lower CO₂ concentrations and lowest under eCO₂ concentrations.

2. Material and methods

(a) Plant growth conditions

Seeds of *B. distachyon* were grown in three laboratory plant growth chambers (TPG-1260TH, Thermoline Scientific), maintained at pre-industrial (reduced) (rCO₂), ambient (aCO₂) and elevated (eCO₂) CO₂ concentrations; 200, 410 and 640 ppm (predicted for 2050 [21]), respectively. Chambers were illuminated with five 400 W Sunmaster Dual Spectrum High-Pressure Sodium globes generating 350 µmol m⁻² s⁻¹ at the plant canopy level. Air temperatures within each chamber were maintained at 26/18°C day/night on a 15 L:9D photoperiod cycle. Humidity was controlled at 50% (±6%). Carbon dioxide within each chamber was monitored by CO₂ probe (GMP222; Vaisala, Vantaa, Finland), with CO₂ (food grade, Air Liquide, Australia) injected from pressurized cylinders through solenoid valves.

(b) Experimental procedure

B. distachyon (accession B21-3) seeds were obtained from French Institute for Agricultural Research and grown hydroponically. Briefly, seeds were soaked in water for 2 h to soften the lamella and palea, which were then removed using forceps. Subsequently, seeds were sterilized in a solution of 0.9% sodium hypochlorite and 0.1% Triton X-100 for 30 min, followed by washing several

times with water before being inserted into perlite irrigated with water. Seeds were stratified at 4 °C for 3 days, and plants were grown in the glasshouse for 18 days to achieve uniform seedling growth and transplanted to hydroponics with one plant per cup. The hydroponics set-up consisted of two nested disposable plastic cups as previously described [22]. Each cup was filled with approximately 350 ml of the full strength standard hydroponic solutions as previously described [23], with the exception that Fe(III) EDTA concentration was doubled owing to iron deficiencies previously detected in *B. distachyon* [22].

A total of 180 cups were randomly assigned to one of eight treatments in a factorial combination of CO₂ (200, 410 or 640 ppm) and Si concentrations (0.0, 0.5, 1, 1.5 and 2 mM). For Si treatments (+Si), liquid potassium silicate (K₂SiO₃) (Agsil32, PQ Australia, SA, Australia) at five different concentrations was added to the nutrient solution by adjusting at pH 5.5 using hydrochloric acid (HCl) to reduce polymerization of silicate, since silicic acid polymerizes when its concentration exceeds 2 mM [24]. Potassium chloride was added to the control (–Si) cups to balance additional K⁺ in +Si treatments. Solutions were replaced weekly for the first two weeks and then twice a week until harvesting. Cups were rotated and chambers were swapped weekly to minimize chamber effects and pseudo-replication, as previously described [25].

(c) Elemental analysis

All *B. distachyon* shoots and roots were harvested separately, dried at 60°C for 3 days and weighed. Above and below ground biomass was recorded. Weighed samples were ball-milled to powder, and a sub-sample of approximately 7 mg was analysed for C and N concentrations using an elemental analyser (FLASH EA 1112 Series CHN analyser, Thermo-Finnigan, Waltham, MA, USA). Foliar and root Si concentrations were analysed by pressing ca 80 mg of sample powder into a 3 mm thick, 13 mm diameter pellet. Si concentration (expressed as percentage dry mass (DM)) was analysed from the pellets with an X-ray fluorescence spectrometer (Epsilon 3^x, PANalytical, EA Almelo, The Netherlands), as previously described by Reidingier *et al.* [23]. Si was calibrated against a certified plant reference material of known Si concentrations (NCS ZC73018 Citrus leaves) [26].

(d) Statistical analysis

Minitab software (v. 18.1) was used for statistical analyses and graphs were produced in SigmaPlot (v. 14.0). Spearman's rank correlation test was used to analyse the relationship between Si and C concentrations, as well as biomass and Si concentrations. To test the main and interaction effect of Si-supply and CO₂ levels on plant biomass, foliar C and N concentrations, two-way analysis of variance (ANOVA) was performed and significant differences were separated using Bonferroni test for least-squares means separation adjusted for multiple comparisons. Foliar and root Si concentrations were square-root transformed and analysed similarly, with control plants excluded from analysis because control plants have a lower detection limit [23].

3. Results

(a) Silicon concentrations

Foliar Si concentrations were significantly increased with Si-supply (table 1). This increase, however, plateaued when Si-supply exceeded 1 mM for lower and ambient CO₂ but 1.5 mM for eCO₂ (figure 1*a* and table 1). rCO₂ caused significant increases in foliar Si concentrations, while eCO₂ caused the opposite effect by reducing foliar Si concentrations. Specifically, rCO₂ increased foliar Si concentrations by 47% and 56% relative to plants grown under aCO₂ and eCO₂, respectively. Increasing

Figure 1. Effect of Si-supply and the three CO₂ regimes (reduced, rCO₂; ambient, aCO₂; and elevated, eCO₂) on (a) foliar and (b) root Si concentrations (% dry mass) together with the (c) ratio of foliar to root Si concentrations. Mean $\pm$ s.e. ($N = 8$). For (a), (b) and (c), uppercase letters indicate significant differences between CO₂ concentrations and lowercase letters indicate significant differences between Si-supply under each CO₂ level. (d) Relationship between foliar C and Si-supply. For (d), the solid line represents linear regression through all data points.

Table 1. Summary of ANOVA results showing the impacts of CO₂ and Si-supply on plant traits. Statistically significant effects are indicated in bold at p -values < 0.05 .

response variable	CO ₂			Si-supply			CO ₂ $\times$ Si-supply		
	<i>F</i>	d.f.	<i>p</i>	<i>F</i>	d.f.	<i>p</i>	<i>F</i>	d.f.	<i>p</i>
foliar Si (figure 1a)	143.56	2,84	<0.001	37.95	3,84	<0.001	1.34	6,84	0.249
root Si (figure 1b)	3.45	2,84	0.036	86.71	3,84	<0.001	1.65	6,84	0.143
foliar Si:root Si (figure 1c)	61.59	2,84	<0.001	14.59	3,84	<0.001	0.36	6,84	0.923
foliar C	0.09	2,45	0.917	0.29	4,45	0.880	0.09	8,45	0.999
root C	22.02	2,45	<0.001	5.81	4,45	0.001	3.91	8,45	0.001
foliar N	19.98	2,45	<0.001	2.66	4,45	<0.045	1.34	8,45	0.249
root N	5.27	2,45	<0.009	86.71	4,45	<0.001	4.03	8,45	0.001
shoot biomass (figure 2a)	86.46	2,144	<0.001	5.58	4,144	<0.001	0.97	8,144	0.465
root biomass (figure 2b)	13.53	2,144	<0.001	2.79	4,144	0.029	0.57	8,144	0.805

Si-supply significantly increased root Si concentrations in a linear manner (figure 1b and table 1). However, root Si concentrations were higher under aCO₂ compared with rCO₂ and eCO₂. The ratio of foliar to root Si concentrations was significantly higher under rCO₂ relative to atmospheric and elevated CO₂ (figure 1c and table 1). There was no significant interaction effect between CO₂ and Si-supply on foliar and root Si concentrations (table 1).

(b) Primary chemistry

Si-supply significantly increased root C concentrations under ambient CO₂ but had no significant effect on foliar C concentrations (table 1) (data not shown). By contrast, there was

significantly higher root C concentration in control plants compared with Si-supplemented plants under rCO₂. Both CO₂- and Si-supply interactively increased root C concentrations (table 1). This relationship, however, was not observed for foliar C concentrations. There was a strong negative correlation between foliar C and Si-supply: $r_{46} = -0.29$, $p = 0.029$ under eCO₂ (figure 1d). However, no such relationship was observed under rCO₂ and aCO₂ concentrations.

(c) Plant biomass

Increasing Si-supply to 1 mM significantly increased shoot biomass, but this plateaued around 1 mM under lower and

Figure 2. Effect of Si-supply on shoot (a) and root (b) biomass of plants grown under three CO₂ regimes (as figure 1). Mean $\pm$ s.e. ($N = 8$). For (a) and (b), uppercase letters indicate significant differences between CO₂ concentrations and lowercase letters indicate significant differences between Si-supply under each CO₂ level. (c) relationship between biomass and Si concentrations. For (c), the solid lines represent linear regression through all data points.

elevated CO₂ (figure 2a and table 1). Additionally, root biomass significantly increased with increasing Si-supply up to 1.5 mM under lower CO₂ (figure 2b and table 1). However, Si-supply had no significant effect on root biomass under ambient and elevated CO₂. eCO₂ caused significant increases in both shoot and root biomass. Si accumulation is positively correlated with plant biomass under rCO₂ ($r_{146} = -0.52$, $p = 0.002$), whereas it is negatively correlated under eCO₂ ($r_{146} = -0.59$, $p = 0.004$; figure 2c).

4. Discussion

In the present study, we examined the impact of pre-industrial (Miocene), ambient and elevated (Anthropocene) CO₂ concentrations on Si uptake on *B. distachyon*. While several other studies [14,17,27] have reported that eCO₂ can cause declines in Si concentrations in plants, to our knowledge this is the first study to demonstrate that lower CO₂ causes Si concentrations to increase. This is owing to higher accumulation of Si overall, but also the reallocation of Si from the roots into the shoots. Si reallocation was reflected in the higher ratio of foliar to root Si concentrations under rCO₂ conditions. Moreover, while Si accumulation was expectedly driven by Si-supply, eCO₂ negated the positive effect of Si-supply. Given that Si uptake is driven by both active and passive mechanisms—the latter driven by the transpiration stream—the well-documented decreases in transpiration rates under eCO₂ [28,29] may partly explain decreased Si uptake. Conversely, higher transpiration rates observed under rCO₂ [30,31] may result in greater Si uptake. Increasing foliar concentrations greater than 1% [32], however, relies on

active uptake so it seems plausible that active uptake is involved under rCO₂, in addition to passive transpiration-based uptake. Expressed as a proportion of root DM, foliar Si accumulation was 10.96 mg g⁻¹ under eCO₂ and 36.4 mg g⁻¹ under rCO₂, which provides further support for this.

Si has been hypothesized to act as a structural substitute for C at lower metabolic cost [7], particularly at times when atmospheric CO₂ concentrations were less abundant, such as during the Miocene [7]. Therefore, it is conceivable that C substitution with Si may have driven the evolution of high Si-accumulating grass species during this era [3,7]. In sharp contrast, CO₂ concentrations are predicted to double by the end of the twenty-first century [12], potentially resulting in higher C and reduced Si accumulation within plants.

Our results indicated that eCO₂ reduced grass Si accumulation, while limited to just four studies, to our knowledge, is consistent with other studies in grasses [14,17,27], suggesting that it is a common response among the grasses. Working with several tree species Fulweiler *et al.* [33] reported that eCO₂ did not alter foliar Si concentrations, although they acknowledge that concentrations were unexpectedly high compared to high-accumulating species. In the present study, accumulating Si reduced plant biomass under eCO₂, which was reflected in the strong negative correlation between Si accumulation and overall biomass. This is compatible with the proposed growth–defence trade-off in plants [14,34]. Conversely, Si accumulation was positively correlated with biomass under rCO₂, supporting the hypothesized Si substitution for C under limited CO₂ [7], possibly to maintain growth and defence [3].

In addition to offering support to the hypotheses about the evolution of Si hyper-accumulation in grasses under lower CO₂ conditions [7], this study provides further evidence that grasses

may accumulate less Si as global atmospheric CO₂ concentration rises. Given the importance of Si in alleviating abiotic stresses such as salinity and drought, as well as providing protection against biotic pathogen infection and herbivore feeding [3,6], lower accumulation of Si in grasses could impact agricultural crop productivity. We still have limited information about the impacts of rising CO₂ on Si accumulation in grasses, limited to a few species [14,17,27], but it could affect functional groups differently. C3 grasses, for instance, are generally more responsive to eCO₂ than C4 grasses [35], so may be more affected (but see [36]). Further work to determine the effects of eCO₂ on Si accumulation, particularly considering the C3/C4 pathway, is warranted [37]. Moreover, the declines in Si accumulation could have implications for the global Si cycle, its links to global primary productivity, and the C cycle [38].

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Data accessibility. Data available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.ncjsxkssv> [39].

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