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RESEARCH ARTICLE

Silicon accumulation suppresses arbuscular mycorrhizal fungal colonisation in the model grass *Brachypodium distachyon*

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Abstract

Purpose Silicon (Si) accumulation by grasses alleviates diverse biotic and abiotic stresses. Despite this important functional role, we have limited understanding of how root microbial symbionts, such as arbuscular mycorrhizal (AM) fungi, affect Si uptake and even less about how Si supply and accumulation affect AM fungal colonisation. Our objective was to determine the nature of this two–way interaction in the model grass, *Brachypodium distachyon.*

Methods We grew *B. distachyon* with five levels of Si supplementation using wild-type plants and a mutant (*Bdlsi1-1*) that has little capacity for Si uptake. Half of the plants were colonised by AM fungi; half were free of AM fungi. We measured Si accumulation, AM fungal colonisation, leaf carbon (C), nitrogen (N) and phosphorus (P) concentrations.

Results AM fungi did not affect Si accumulation, although small increases occurred when root mass was included as a covariate. Si supplemented soil promoted plant growth and P uptake. Si accumulation suppressed colonisation by AM fungi and C concentrations in wild type but not in *Bdlsi1-1* plants. Si concentrations were negatively correlated with C and N concentrations, with correlations being stronger in wild-type plants than *Bdlsi1-1* plants.

Conclusions Our results indicate that Si accumulation in the plant, rather than Si availability in the soil, underpinned reduced AMF colonisation. We propose that Si accumulation is unlikely to be impacted by AM fungi in plants with inherently high Si accumulation, but Si accumulation may suppress AM fungal colonisation in such plants.

Keywords Arbuscular mycorrhizal fungi · Roots · Silica · Silicification · Soils · Symbiont · Trade-offs

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Introduction

Silicon (Si) accumulation by plants, especially the Poaceae, is now recognised as alleviating a broad range of biotic and abiotic stresses including pathogen

infection, herbivory, drought, salinity and metal toxicity (Coskun et al. 2019; Frew et al. 2018b). In the Poaceae, Si can accumulate up to levels of 10% of dry mass, more than most other inorganic constituents (Epstein 1999). Si is taken up as silicic acid from the soil by the roots and deposited as polymerised silica in tissues (silicification) (Kumar et al. 2017). Si may be accumulated in plants passively via the transpiration stream, but if Si concentrations exceed 1% dry mass, active uptake is required (Deshmukh and Bélanger 2016). This has been best demonstrated in rice; two transporters in the roots, *Lsi1* and *Lsi2* are needed to transport silicic acid into the roots and then into the xylem for transportation to leaves (Ma and Yamaji 2015).

Most terrestrial plants associate with arbuscular mycorrhizal (AM) fungi belonging to the monophyletic group Glomeromycotina (Smith and Read 2008). The symbiosis is frequently mutualistic, whereby plants provide fungi with carbon (C) and AM fungi enhance nutrient (e.g. phosphorus (P) and nitrogen (N) uptake), but the symbiosis can range from being commensal to having aspects of parasitism (Veresoglou et al. 2021). The AM fungi-plant symbiosis is based on the transfer of nutrients and has been extensively studied for essential nutrients. Si is considered a quasi-essential nutrient (Epstein 1999) and there are relatively fewer studies that have considered whether AM fungi affect Si accumulation, although there are a growing number in recent years (de Tombeur et al. 2021b; Vega et al. 2021).

AM fungi have been reported to promote Si accumulation in the tissues of at least eight plant species. These comprise maize (Clark and Zeto 1996; Kothari et al. 1990), chickpea (Garg and Bhandari 2016), banana (Gbongue et al. 2019; Oye Anda et al. 2016), sugarcane (Frew et al. 2017a, 2017b), pigeon pea (Bhalla and Garg 2021; Garg and Kashyap 2017), soybean (Yost and Fox 1982), strawberry (Hajiboland et al. 2018; Moradtalab et al. 2019) and tomato (Ju et al. 2021). The mechanisms for how AM fungi affect Si accumulation are still unclear, but could include AM fungi absorbing Si directly (Vega et al. 2021). For example, Hammer et al. (2011) demonstrated that AM fungi could accumulate Si in their spores and hyphae. Aquaporin transporters, similar to those involved in Si uptake in plants, have been identified in AM fungal species (Deshmukh and Bélanger 2016; Li et al. 2013). While increases in Si accumulation as a result of inoculation with AM fungi are commonly reported, this seems to occur less when bioavailable Si in the soil is more abundant (Frew et al. 2017a, 2017b; Oye Anda et al. 2016). AM fungi may therefore play a more important role in Si accumulation when bioavailable Si in the soil is less available (Frew et al. 2017a). Despite this, no studies to our knowledge have investigated how a gradient of Si supplementation affects how AM fungi influence Si accumulation.

The impact of Si supplementation on AM fungal colonisation is similarly poorly characterised. Si accumulation is usually accompanied by a decrease in plant C concentrations (Cooke and Leishman 2011a). The provision of C resources (i.e. sugars) is an important factor regulating AM colonisation (Kiers et al. 2011), so Si accumulation in the plant has the potential to decrease fungal colonisation. In support of this, AM fungal colonisation of sugarcane roots was higher ($28.2 \pm 1.6\%$) in soils with low bioavailable Si compared to soils with high bioavailable Si ($16.5 \pm 1\%$) (Frew et al. 2017a). In contrast, Oye Anda et al. (2016) observed a modest increase ($+11\%$) in arbuscule colonisation of banana roots with Si supplementation, but no difference in hyphal or spore (vesicle) colonisation. Si supplementation increased AM fungal colonisation (Moradtalab et al. 2019) and effectiveness (Hajiboland et al. 2018) in strawberry. Regardless, all these studies only compared one level of Si supplementation with non-supplemented control plants and it was unclear whether Si in the soil was directly affecting AM fungi or whether Si accumulation in the plants underpinned changes in colonisation.

The objective of this study was to determine whether AM fungi affected Si accumulation in the model grass *Brachypodium distachyon* when supplied with different levels of Si supplementation, and conversely determine how Si supply affected AM fungal colonisation. *Brachypodium* is used as a model grass because it has a close phylogenetic relationship with temperate grasses (including cereals) and is highly tractable (Brkljacic et al. 2011). We used wild-type plants and a mutant (*Bdlsi1-1*) that has little capacity for Si uptake to answer two research questions. *Bdlsi1-1* plants have impaired channelling function of the Si influx transporter BdLSI1, resulting in substantially less Si accumulation ($>90\%$) than wild-type

plants (see Glazowska et al. 2018 for full details). Our specific questions and hypotheses were:

1. Do AM fungi increase Si accumulation and how does this relate to Si availability? We hypothesised that AM fungi increase Si accumulation, but this effect decreases with increasing Si availability. If we see increased Si accumulation in *Bdlsi1-1* plants with AM fungi, this suggests that AM fungi are playing a direct role in Si acquisition.
2. Do different levels of Si supplementation affect AM fungal colonisation and is this related to Si supply in the soil or Si accumulation in the plant? Based on the concept that Si accumulation could reduce C availability for AM fungi, we hypothesised that Si suppresses AM fungal colonisation, and this is due to increasing levels of Si and decreasing levels of C in the plant rather than direct effects of Si in the soil. Si supply would therefore have no impact on AM fungal colonisation in *Bdlsi1-1* plants because of their incapacity for Si accumulation.

Materials and methods

Soil conditioning

Plants were grown in a 50:50 composite of topsoil and double washed sand that had been fully homogenised with a soil mixer and gamma-irradiated (two doses at 50 kGy) prior to use. Sand was used to lower bioavailable phosphorus (< 21 mg P kg^{-1}) and silicon (< 11 mg Si kg^{-1}) (Table S1). To standardise the microbial community, all soil received a microbial filtrate (300 ml) immediately after irradiation. This filtrate was created by using the extraneous extraction solution (without spores) from the AM fungal inoculant (see below) after two runs of wet sieving to ensure most of the Si-rich inert substrate (calcined diatomaceous earth) was removed, and applied to the soil immediately after irradiation following procedures described by Frew et al. (2018a).

After irradiation, soil was transferred to 200 deep root pots (Johnson et al. 2020), each containing c. 1 kg of soil. The bottom two-thirds of each pot was filled with soil (hydrated with the microbial wash only), while the top third of each pot received soil that was hydrated with microbial wash and one of two AM-treatments; half of the pots received AM fungal inoculation (+AM plants) and half were to remain AM fungi-free (–AM plants).+AM status, assigned at random, was achieved by inoculating soil with a commercial inoculant (Start-up Ultra©, Microbe Smart Pty. Ltd., Melrose DC,, South Australia) that contained spores from four isolates of a *Rhizophagus irregularis* (previously known as *Glomus intraradices*). Prior to inoculation, spores were extracted and separated from the inert substrate (calcined diatomaceous earth) using wet sieving and applied at the recommended rate of 250 g per 2.5 L with hydrating water. The –AM treatment involved the same procedures, however the inoculant (i.e. the extracted spores) was sterilised by autoclaving twice (121ºC) to ensure spores were non-viable.

Experimental design

We grew wild-type and a low Si accumulating mutant (*Bdlsi1-1*) *Brachypodium distachyon* in the deep pots with+AMF and –AMF treatments (50 of each; Fig. 1). *Bdlsi1-1* plants had substantially less Si accumulation (> 90%) than wild-type plants (see Glazowska et al. 2018 for full details). Seeds were supplied by Department of Plant and Environmental Sciences, Faculty of Science, University of Copenhagen (Jan K. Schjoerring) and were originally obtained from Søren K. Rasmussen.

For each plant genotype (wild-type and *Bdlsi1-1*), plants were assigned at random for fertilisation with either no Si supplementation (0.0 mM) or one of four levels of Si supplementation (0.5, 1.0, 2.0 and 3.0 mM), such that there were 10 replicates of each factorial combination of (i) plant genotype, (ii) AM fungal status and (iii) Si supplementation, 200 plants in total (Fig. 1). Si supplementation was achieved by adding potassium silicate (K_2SiO_3; Agsil32, PQ Australia) to irrigation water to give 0.5, 1.0, 2.0 and 3.0 mM SiO_2 equivalent solutions (Table S2). To balance the K+and Cl– in all treatments, we supplemented solutions with KCl according to the level of K_2SiO_3 (Table S2) and adjusted solutions to pH 5.5 using HCl to reduce the polymerization of silicates (Ma and Yamaji 2015). Plants received 60 mL of solution once per week, increasing to twice per week as plants matured; plants were rotated within the glasshouse at random once per week.

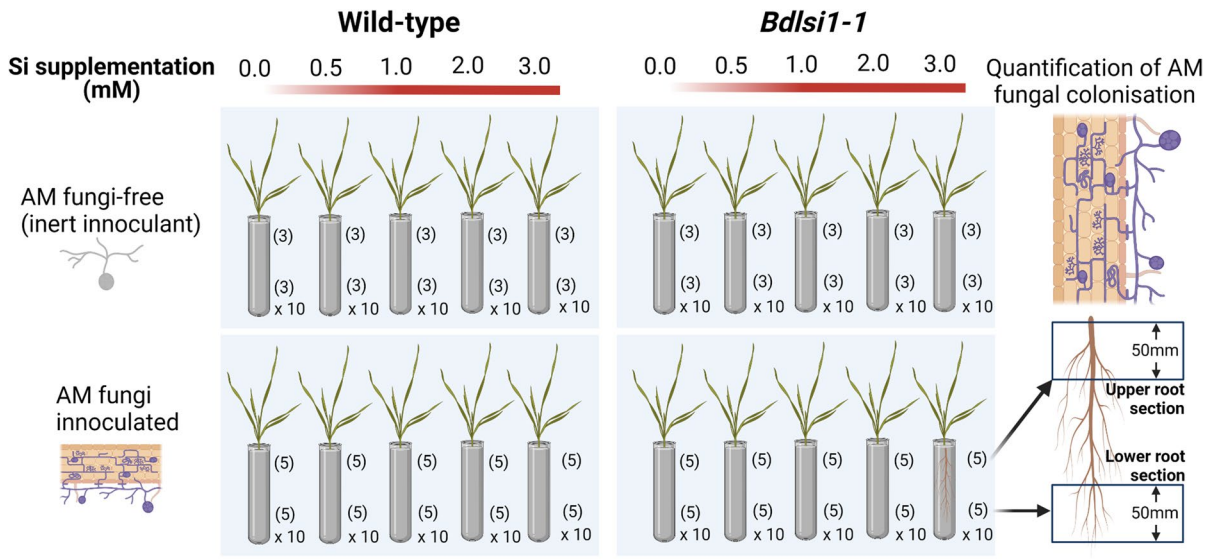

Fig. 1 Schematic of experimental design. Each treatment replicated 10 times (×10). Numbers in parentheses indicate replication for root sections (upper and lower) used to quantify AM fungal colonisation. Figure created with biorender.com

Plants were grown in a sunlit glasshouse chamber maintained at 22/18ºC (day/night) and 60% relative humidity for eight weeks (pre-flowering). Plants were then harvested with shoots being removed and freeze dried. The roots were washed free of soil and weighed. A sub-sample of approximately 600 mg (fresh mass) of roots was taken from the upper (taken within 50 mm from the base of the tiller) and lower (taken within 50 mm from the tip of the root) parts of the root system, weighed and stored in tissue embedding cassettes in 70% ethanol before quantifying AM colonisation following the procedures described by Frew et al. (2018a), with the minor modifications outlined below. The rest of the roots were freeze dried and weighed. We estimated the dry mass of root sections used to quantify of AM fungal colonisation based on their fresh mass and the ratio of fresh:dry root mass observed for the harvested roots. This was combined with the harvested dry root mass to calculate total dry root mass.

AM fungal colonisation

Root samples were rinsed with cold water and cleaned with 10% KOH at room temperature for five days. Samples were then re-rinsed with cold water and stained with 5% ink-vinegar in a water bath at 60ºC for 10 min (Vierheilig et al. 2005). Finally, samples were rinsed with cold water until water ran clear and submerged in lactoglycerol (de-staining solution) overnight. For the+AM treatments, the upper and lower root sections from five plants, selected at random, for each of the Si treatments were used to quantify AM fungal colonisation (Fig. 1). The equivalent root sections from three –AM plants, selected at random, were examined for AM fungal colonisation (Fig. 1). For each plant, ten segments (10 mm in length) were mounted on glass slides with glycerine under a cover slip and scored for presence of AM fungi using the intersect method (McGonigle et al. 1990) for a minimum of 50 intersects per plant. Only hyphae in which there was a visible connection to AM fungal structures (arbuscules, vesicles, spores) were counted. No AM colonisation was detected in the –AM plants.

Elemental analysis

Leaf Si and phosphorus (P) concentrations were determined using approximately 100 mg of ground shoot material placed into a small mass holder, and then analysed with an X-ray fluorescence spectrometer (Epsilon 3^x, Malvern PANalytical, Malvern, UK), using the procedure and certified reference material described in Hiltpold et al. (2017), following the approach of Reidinger et al. (2012). We sub-sampled

50% of replicates (selected at random) to analyse leaf N and C concentrations, which were determined using an elemental analyser (FLASH EA 1112 Series CHN analyser, Thermo-Finnigan, Waltham, MA, USA). We subsequently analysed shoot tissue from *Bdlsi1-1* and wild-type plants treated with either 0 mM or 3.0 mM Si solutions to quantify soluble sugar concentrations using a modified anthrone method (Ebell 1969).

Statistical analysis

Three-way ANOVAs including plant genotype (wild-type or *Bdlsi1-1*), Si supplementation, AM fungal status, and their interactions, were included as fixed effects to analyse shoot and root mass, leaf concentrations of Si, C, N, P and soluble sugars. Two-way ANOVAs including genotype (wild-type or *Bdlsi1-1*), and Si supplementation were used to analyse AM fungal colonisation; AM fungal status was not included in the model because AM fungi were confirmed to be completely absent in plants that were not inoculated with AM fungi. For Si accumulation, root mass was included as a covariate because AM fungi had a marginally significant impact on root mass. All plant responses were significantly affected by plant genotype, so the tests were conducted separately for each plant genotype (results given in Figure panels). Pearson's and Spearman's correlation tests (indicated r and r_s, respectively) were used to explore relationships between leaf Si concentrations, AM fungal colonisation and concentrations of leaf C, N and P. The analysis was conducted in Genstat version 21 (VSN International Ltd, Hemel Hempstead, UK).

Results

All the AM fungi treatment plants were colonised by AM fungi, whereas the AM fungi-free plants were not colonised by AM fungi. Wild type plants had larger shoots (Fig. 2A) but smaller roots (Fig. 2B) than *Bdlsi1-1* plants (Table 1). Shoot and root mass increased with Si supplementation (Table 1), although this plateaued for Si supplementation in the range 1.0–3.0 mM (Fig. 2). Plants colonised by AM fungi had smaller root systems (Table 1), although this was only the case for *Bdlsi1-1* plants (Fig. 2B; Table 1).

As anticipated, Si accumulation was significantly higher in the wild type plants than *Bdlsi1-1* plants, with Si concentrations in leaves increasing with higher levels of Si supplementation (Table 1; Fig. 3). This increase in leaf Si concentrations with Si supply was more pronounced in wild-type plants compared to *Bdlsi1-1* (Fig. 3), reflected in a statistically significant interactive effect of plant genotype and Si supply (Table 1). AM status did not affect Si accumulation when examined for all plants collectively (Table 1) or for each plant genotype separately (Fig. 3). Given that there was a marginally significant effect of AM fungal plants having smaller root systems (Table 1), root mass was included as a co-variate and the analysis (ANCOVA) re-run. AM fungal presence marginally increased Si accumulation under these circumstances (Table 1).

Increasing Si supplementation caused progressive declines in AM fungal colonisation in the upper (Fig. 4A) and lower (Fig. 4B) roots of wild type plants, but this did not occur in *Bdlsi1-1* plants reflected in the statistically significant interaction between plant genotype and Si supply (Table 1). There was a strong negative correlation between leaf Si concentrations and AM fungal colonisation in both the upper and lower root sections of wild type plants (Fig. 5A and 5B, respectively). There was no significant relationship between leaf Si concentrations and AM fungal colonisation in the upper roots of *Bdlsi1-1* plants (Fig. 5C) and a positive correlation between AM fungal colonisation and leaf Si concentrations in the lower roots of these plants (Fig. 5D).

Averaged across all Si treatments, *Bdlsi1-1* plants had 10.5% higher concentrations of leaf C relative to wild type plants (Fig. 6A). Si supply reduced leaf C concentrations (Table 1), although this was only apparent in wild type plants and Si supply did not affect C concentrations in *Bdlsi1-1* plants (Fig. 6A), again reflected by a significant interaction between plant genotype and Si supply (Table 1). Plants colonised by AM fungi had lower leaf C concentrations, although this effect was stronger in wild type plants than *Bdlsi1-1* plants, the latter being marginally non-significant at the 95% confidence interval ($P = 0.061$).

Leaf N concentrations were higher in *Bdlsi1-1* plants than wild type plants: c. 8.4% when averaged across treatments (Table 1; Fig. 6B). Si supply reduced N concentrations in both plant genotypes, although this effect was slightly stronger in wild

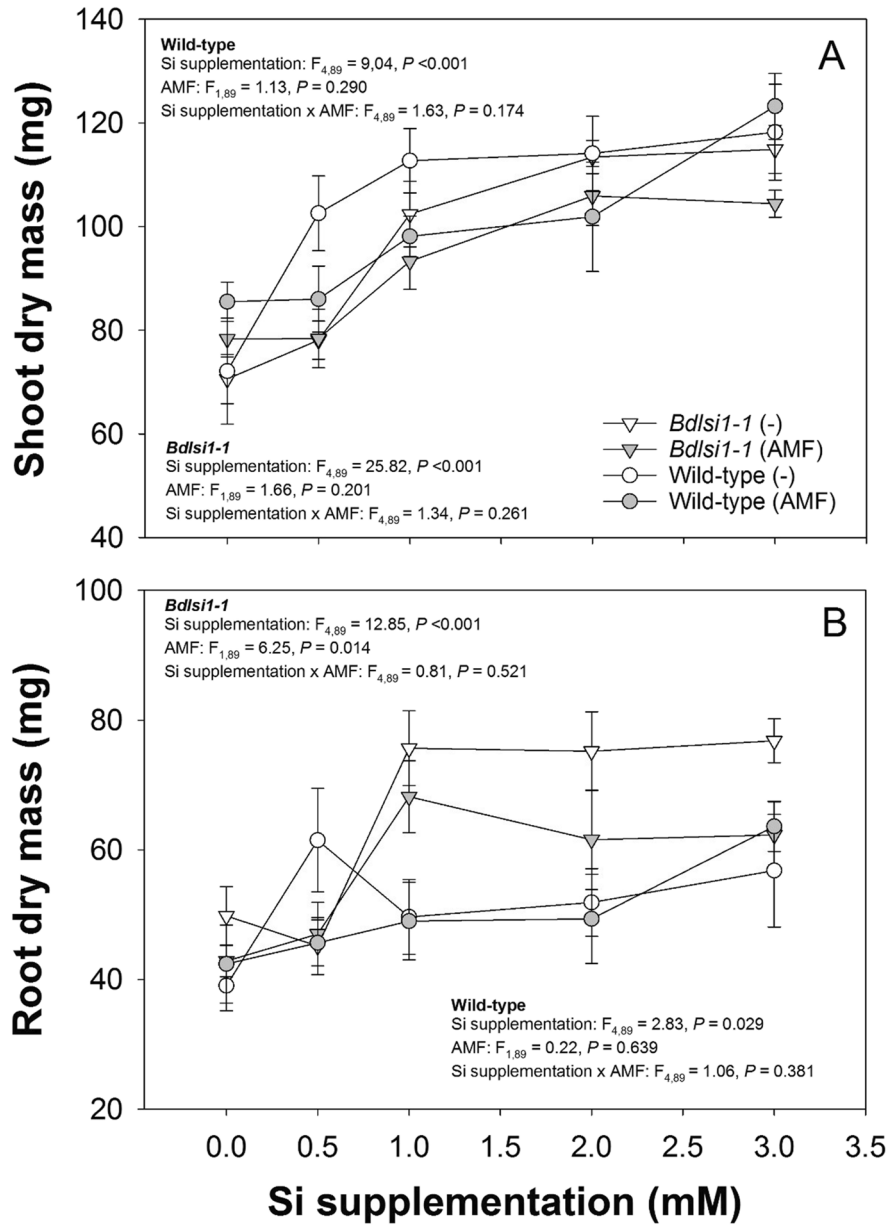

Fig. 2 Impacts of Si supply and AM fungal status (shaded symbols) on (**A**) shoot and (**B**) root mass in wild-type (circles) and *Bdlsi1-1* (triangles) *B. distachyon*. Mean ± standard error shown (N = 10). Results of statistical tests conducted separately for wild type and *Bdlsi1-1* genotypes given within the panels

type plants, which generated a significant interaction between Si supply and plant genotype (Table 1). AM fungi had no discernible impact on leaf N concentrations for either plant genotype (Table 1; Fig. 6B). There was a significant interaction between Si supply and plant genotype for concentrations of soluble sugars (Table 1), whereby Si significantly increased concentrations in *Bdlsi1-1* but concentrations were unaffected, or slightly reduced, in the Si-accumulating wild-type plants (Fig. 7). *Bdlsi1-1* plants had higher concentrations of leaf P than wild-type plants; both Si supply and AM fungi promoted P concentrations in leaves (Table 1; Fig. 8). The AM fungi had weaker (marginally non-significant at the 95% confidence interval) effects on P concentrations in wild-type plants compared to *Bdlsi1-1* plants (Fig. 8).

Considering all plants collectively, there was a strong negative correlation between Si concentrations and concentrations of both C ($r = -0.88$, $P < 0.001$) and N ($r = -0.43$, $P < 0.001$). These correlations were

Table 1 ANOVA of plant responses to experimental treatments: plant genotype (wildtype / *Bdsli1-1*), Si supply (0, 0.5, 1.0, 2.0 or 3.0), AMF presence (+/–) and interactions between treatments. Statistically significant effects indicated in **bold**

Plant response variable	Plant genotype		Si supply		AMF		Plant genotype × Si supply		Plant genotype × AMF		Si supply × AMF		Plant genotype × Si supply × AMF	
	F	*P*	F	*P*	F	*P*	F	*P*	F	*P*	F	*P*	F	*P*
Shoot mass	**7.24**	**0.008**	**36.50**	**< 0.001**	2.52	0.114	1.16	0.329	0.04	0.833	2.11	0.081	0.98	0.421
Root mass	**14.78**	**< 0.001**	**9.88**	**< 0.001**	**3.98**	**0.048**	**4.40**	**0.002**	1.64	0.201	0.21	0.933	1.70	0.152
Leaf Si concentration	**5683.6**	**< 0.001**	**88.14**	**< 0.001**	2.33	0.129	**77.46**	**< 0.001**	1.90	0.169	0.28	0.891	0.250	0.909
Leaf Si concentration (covariate: root mass)	**6494.8**	**< 0.001**	**78.29**	**< 0.001**	**3.89**	**0.050**	**94.05**	**< 0.001**	0.95	0.331	1.00	0.932	0.99	0.997
AMF colonisation (upper roots)	**18.89**	**< 0.001**	**31.75**	**< 0.001**	–	–	**28.63**	**< 0.001**	–	–	–	–	–	–
AMF colonisation (lower roots)	**26.80**	**< 0.001**	**13.80**	**< 0.001**	–	–	**7.50**	**< 0.001**	–	–	–	–	–	–
Leaf C concentration	**2281.29**	**< 0.001**	**44.59**	**< 0.001**	**9.53**	**0.002**	**28.31**	**< 0.001**	0.23	0.630	0.82	0.511	0.08	0.989
Leaf N concentration	**24.34**	**< 0.001**	**20.12**	**< 0.001**	0.06	0.805	**2.57**	**0.040**	0.54	0.465	0.37	0.833	1.16	0.330
Leaf soluble sugars[1]	2.84	0.096	2.88	0.094	0.10	0.756	**8.47**	**0.005**	0.15	0.704	0.81	0.370	1.33	0.252
Leaf P concentration[1]	**132.86**	**< 0.001**	**24.26**	**< 0.001**	**8.06**	**0.005**	0.43	0.785	0.08	0.780	1.63	0.169	1.04	0.390

[1]Log_{10} transformation applied prior to analysis to meet assumptions of normality and homogeneity of variances

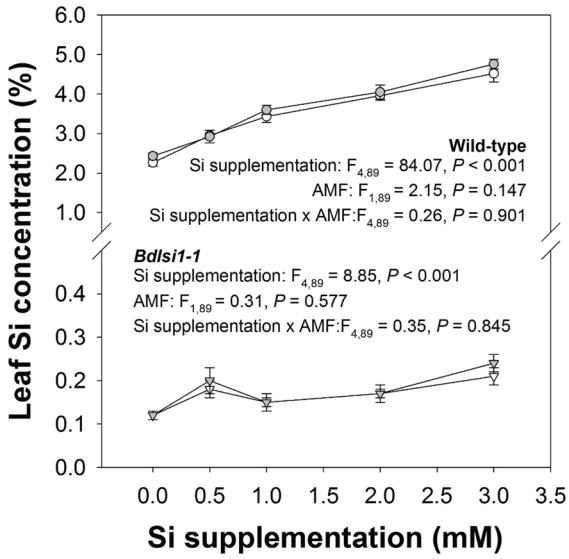

Fig. 3 Leaf Si concentrations as affected by Si supplementation and AM fungal presence (shaded symbols) and absence (open symbols) in wild type (circles) and *Bdlsi1-1* (triangles) *B. distachyon*. Mean standard ± error shown (N = 10). Results of statistical tests conducted separately for wild type and *Bdlsi1-1* given within the panel

substantially stronger in wild-type plants (r = –0.87, $P < 0.001$ and r = –0.75, $P < 0.001$, respectively) than *Bdlsi1-1* plants (r = –0.29, $P = 0.003$ and r = –0.25, $P = 0.013$). AM fungal colonisation in the lower portion of roots was positively correlated with leaf C (r = 0.58, $P < 0.001$) and leaf N (r = 0.44, $P < 0.001$). Leaf P was negatively correlated with leaf Si for all plants collectively ($r_s = –0.334$, $P < 0.001$), but this appeared to be driven by the very low or very high concentrations of Si in *Bdlsi1-1* and wild-type plants, respectively, since P concentrations were positively correlated with Si concentrations when examined for each genotype (wild-type $r_s = 0.516$, $P < 0.001$; *Bdlsi1-1* $r_s = 0.408$, $P < 0.001$). Leaf P was negatively correlated with leaf C in wild-type plants ($r_s = –0.503$, $P < 0.001$) but not in *Bdlsi1-1* plants ($r_s = –0.049$, $P = 0.635$). There was no significant correlation between AM fungal colonisation and leaf P concentrations.

Discussion

In this study, we demonstrated that increasing Si supply to *B. distachyon* resulted in considerable reductions in AM fungal colonisation. This was not due to direct effects of Si in the soil but was associated with Si accumulation in plant tissues. In support of this, these changes did not occur in the low Si accumulating mutant *Bdlsi1-1* plants and Si supply did not negatively impact AM fungal colonisation or affect C concentrations in *Bdlsi1-1* plants.

Si supply promoted plant growth and P uptake

Supplementing the soil with Si strongly promoted plant growth to similar levels in the *Bdlsi1-1* mutant, which accumulated little Si, and wild-type plants which accumulated large amounts. We also observed that Si supply increased P concentrations in both plant genotypes, but to a greater extent in *Bdlsi1-1* plants. Given that *Bdlsi1-1* plants do not accumulate much Si, this suggests that improved growth and P uptake was influenced by interactions in the soil. One possible explanation is that Si in the soil is mobilising and releasing P facilitating greater plant growth (de Tombeur et al. 2021a). Moreover, AM fungi also increased P concentrations in the leaves, although this was less apparent in wild-type plants with high Si concentrations and low colonisation rates.

AM fungi did not significantly affect Si accumulation

Our results do not support our first hypothesis that AM fungi promote Si accumulation, particularly at low levels of Si supply, as AM fungi did not affect Si accumulation at any level of Si supply. When root mass was included as a covariate, AM fungi had a marginally significant positive effect on Si concentrations. AM fungi often cause plants to have smaller root systems because they increase the efficiency of nutrient uptake (Veresoglou et al. 2012), which is consistent with our observations that AM fungal plants had smaller root systems but were achieving similar levels of Si accumulation in leaves as non-AM plants that had bigger root systems. In essence, AM fungal roots were marginally more efficient at Si uptake than non-AM plants.

Si accumulation in the leaves suppressed AM fungal colonisation in the roots

The negative correlation between Si and C in plants may arise from a ‘stoichiometric dilution effect’

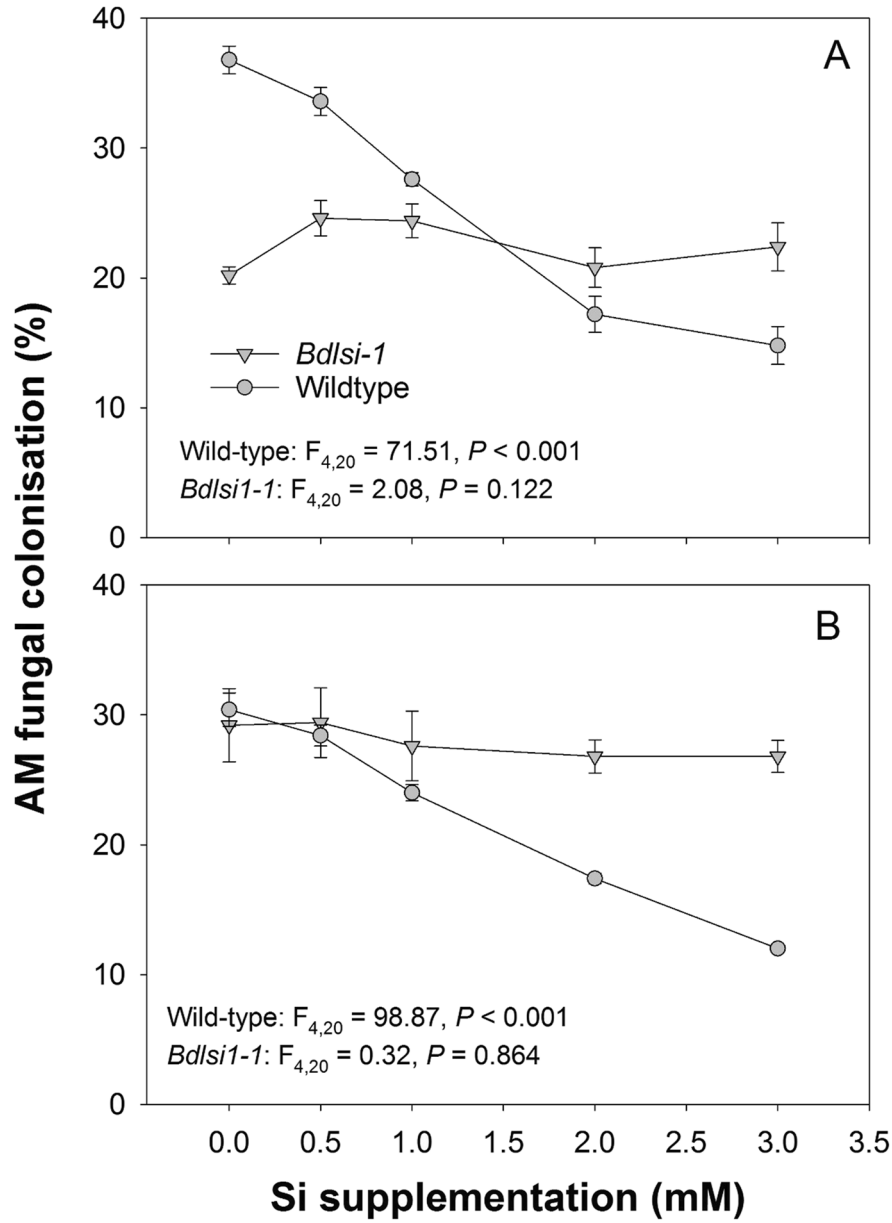

Fig. 4 AM fungal colonisation in (**A**) upper and (**B**) lower sections of the roots with different levels of Si supplementation in wild type (circles) and *Bdlsi1-1* (triangles) *B. distachyon*. Mean standard ± error shown (N = 5). Results of statistical tests conducted separately for wild type and *Bdlsi1-1* given within the panels

whereby an increase in Si, by definition, necessitates lower levels of other constituents, with C being the most abundant and therefore most likely to decline (Quigley et al. 2020). Alternatively, there could be a trade-off between Si and C because Si may be used as a metabolically cheaper substitute for C (Raven 1983), either in a structural or a defensive capacity (Cooke and Leishman 2011b; 2012; McNaughton et al. 1985). The mechanisms are not mutually exclusive and may operate in tandem.

We consider that the negative correlation between Si accumulation and C concentrations is a feasible mechanism for diminished AM fungal colonisation if plants were less able to allocate C assimilates to the AM fungal partner. Quantification of soluble sugars provided partial, but not clear cut, support for this; Si supply increased concentrations of soluble sugars in the non-Si accumulating *Bdlsi1-1* genotype, potentially because of increased growth and P uptake, but this increase was not seen in Si accumulating

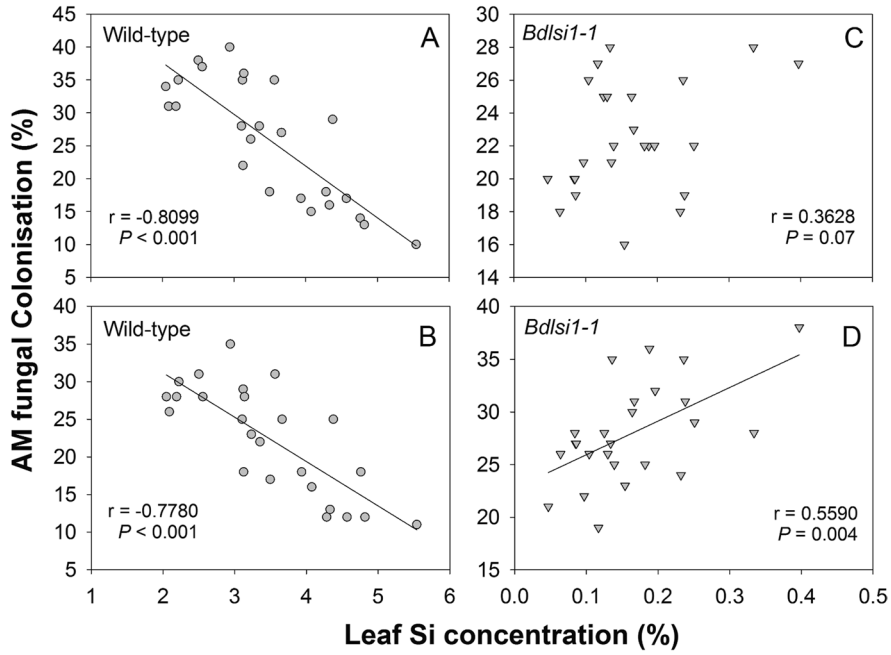

Fig. 5 Relationship between leaf Si concentrations and AM fungal colonisation in wild type (**A** and **B**, upper and lower roots, respectively) and *Bdlsi1-1* (**C** and **D**, upper and lower roots, respectively) *B. distachyon* (N = 25–26). Correlation result shown with regression lines included where $P < 0.05$

wild-type plants. In other words, Si accumulation had an inhibitory effect on the enhanced soluble sugar production seen in plants growing in Si supplemented conditions. Nonetheless, we should note that much of the reduced C probably occurs in cell walls and it remains possible that changes in cell wall composition reduced AM fungal colonisation.

Poaceae versus non-Poaceae

On the face of it our results stand in contrast to the other studies which almost universally report that AM fungi promote Si accumulation. Six of the eight plant species where AM fungi are reported to promote Si accumulation are not Poaceae and from families that accumulate comparatively little Si (Hodson et al. 2005). de Tombeur et al. (2021b) provide a very useful quantitative summary of changes in Si accumulation arising from AM fungal colonisation for most of these studies. It is noticeable that the two Poaceae (maize and sugarcane) show relatively smaller increases in Si as a result of AM fungal colonisation than the non-Poaceae species, sometimes being negligible or even reduced Si concentrations under certain conditions such as alkaline (Clark and Zeto 1996) or high Si (Frew et al. 2017a, 2017b) soils. Moreover, Kothari et al. (1990) found that AM fungi promoted Si accumulation in maize roots but caused Si decreases in the shoots. When they took root mass into account (Si uptake per unit dry root mass / unit root length), Si accumulation in the shoots increased with AM fungi compared to non-AM plants, although this was only statistically significant for root length. This aligns with our observation that roots colonised by AM fungi were marginally more efficient at Si uptake.

We suggest that AM fungal promotion of Si accumulation is probably much weaker and more context dependent in the Poaceae than for other plant families because many Poaceae species are inherently high accumulators of Si (Hodson et al. 2005). This is born out when we compare the minimum and maximum Si leaf concentrations for wild-type *B. distachyon* in the current study (2.26–4.76%) and those reported for chickpea (0.22–0.38%; Garg and Bhandari 2016), banana (0.32–0.37%; Oye Anda et al. 2016), pigeon pea (0.35–0.45%; Bhalla and Garg 2021), soybean (0.27–0.66%; Yost and Fox 1982) strawberry (0.03–0.19%; Hajiboland et al. 2018) and tomato (0.17–0.21%; Ju et al. 2021). The Poaceae studies, which found context-dependent evidence of AM fungi promoting Si accumulation, reported Si concentrations above those seen in the non-Poaceae plant studies: maize (0.72–1.66%; Clark

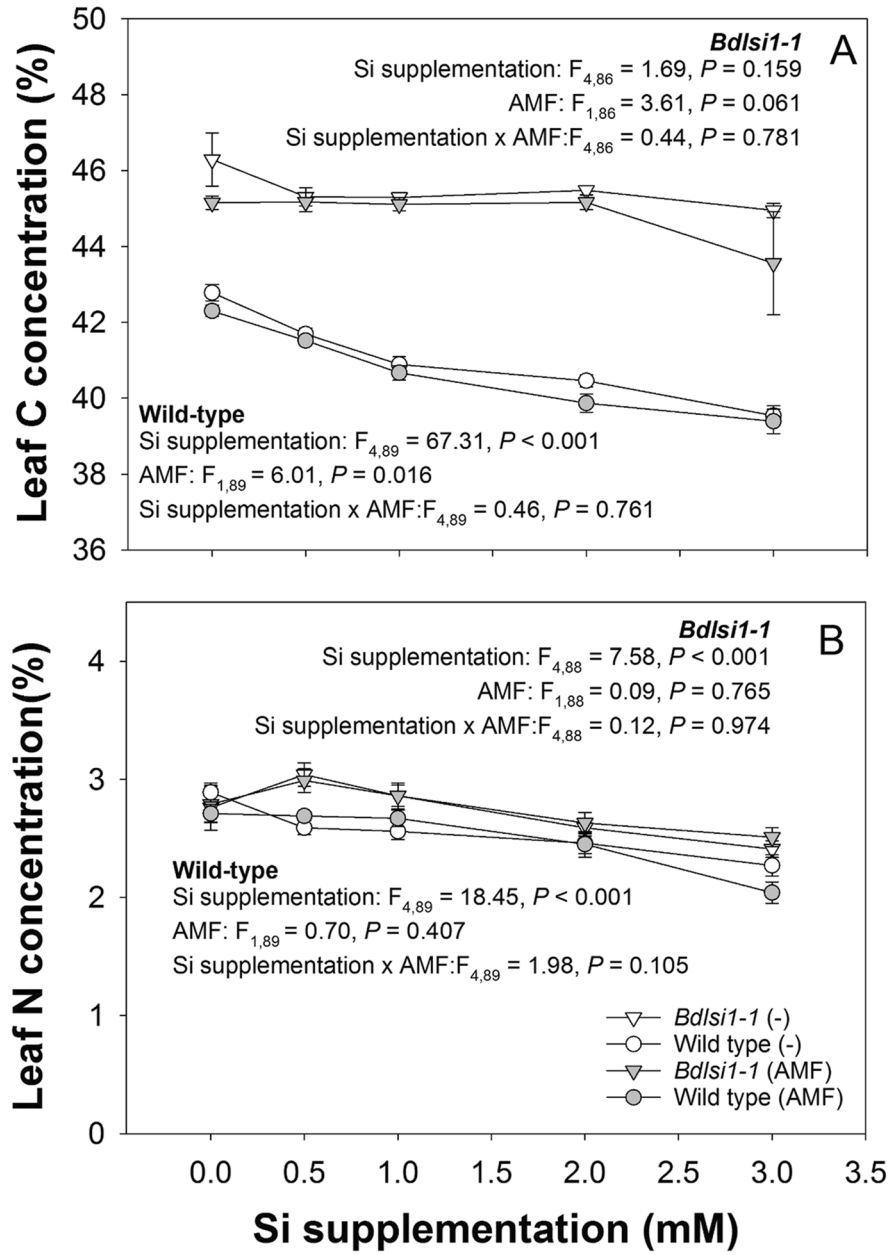

Fig. 6 Leaf (**A**) C and (**B**) N concentrations in wild type (circles) and *Bdlsi1-1* (triangles) supplemented with different levels of Si with (shaded symbols) and without (open symbols) AM fungi (N = 10)

and Zeto 1996) and sugarcane (0.93–1.37%; Frew et al. 2017a), but these were lower than the current study. It is also notable that most studies considering the effects of AM fungi on Si accumulation use *Rhizophagus irregularis* (de Tombeur et al. 2021b), and as with many other functions (e.g. P uptake, defence), AM fungal effects on Si accumulation are likely to vary with fungal identity (Chagnon et al. 2013).

Conclusions

We did not find evidence for AM fungi promoting Si accumulation in *B. distachyon* in a substantive manner and conclude that this is because this species is already well adapted for high levels of Si uptake. AM fungi may, however, play a role for Si accumulation for plant species that are low Si accumulators such as those listed above and reviewed by de Tombeur et al.

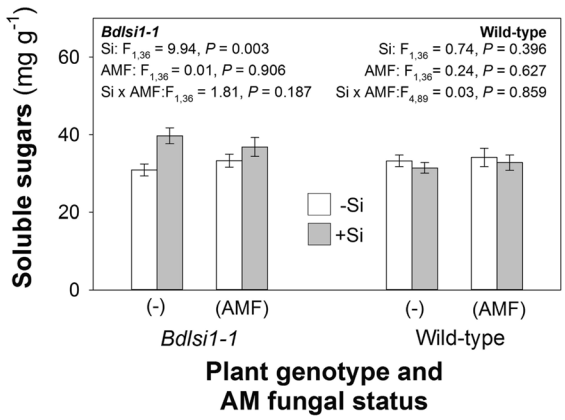

Fig. 7 Leaf soluble sugar concentrations in wild-type and *Bdlsi1-1* plants supplied with 0.0 mM (–Si) or 3.0 mM (+Si) Si solutions with and without AM fungi. Mean standard ± error shown (N = 10)

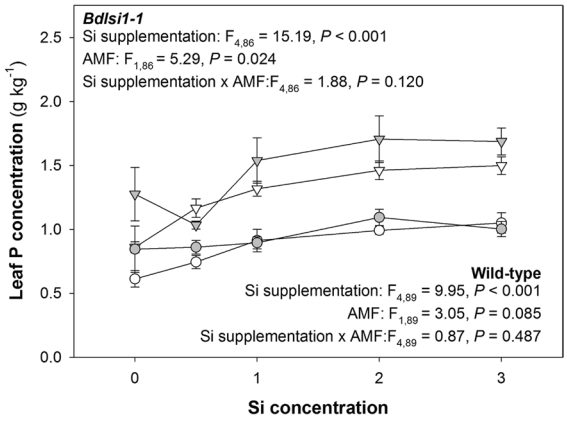

Fig. 8 Leaf P concentrations in wild type (circles) and *Bdlsi1-1* (triangles) supplemented with different levels of Si with (shaded symbols) and without (open symbols) AM fungi (N = 9–10). Symbols as displayed in Fig. 2A

(2021b). In support of this, there was a positive correlation between AM fungal colonisation in the lower parts of the roots of the *Bdlsi1-1 B. distachyon* plants and the (comparatively low) concentrations of leaf Si, with a similar but non-significant trend for the upper roots. Alternatively, AM fungal colonisation may be limited by Si availability at very low levels *in planta*, possibly due to Si influencing biochemical interactions during colonisation or AM fungi requiring plant uptake of Si to alleviate Si-related limitation of fungal growth. Further work is required to resolve this.

We found strong evidence that Si supplementation suppressed AM fungal colonisation in wildtype *B. distachyon*, which is possibly influenced by reduced C resources as more Si accumulates in the tissues. This is more likely to occur in plant species that are high Si accumulators (Hodson et al. 2005) than plant species with low Si accumulation. The fact that Si supply did not negatively affect AM fungal colonisation in *Bdlsi1-1 B. distachyon*, which had low levels of Si accumulation (0.12–0.24%), indicates that Si effects on AM fungi were plant-mediated. Results from the current study suggest that synergies between Si application and microbial symbionts may show promise for improving plant resilience to environmental stresses (Johnson et al. 2016; Putra et al. 2020; Vega et al. 2021), but the degree of synergy may be lower or even antagonistic in high Si accumulating plant species such as *B. distachyon*.

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Author contributions Scott Johnson (SNJ) conceived the work with experimental design input from Jeff Powell (JRP) and Ximena Cibils-Stewart (XCS). Material preparation, data collection and analysis were performed by XCS with technical assistance from Rhiannon Rowe. Interpretation and analysis of data was conducted by JRP and SNJ with inputs from XCS and Adam Frew (AF). The first draft of the manuscript was written by SNJ; all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability The datasets generated during the current study are available in the Fig Share repository: https://doi.org/10.6084/m9.figshare.19770970s.

Declarations

Competing interests The authors have no relevant financial or non-financial interests to disclose.

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