The role of silicon on C:N:P stoichiometry and yield of sugar beet under ammonium toxicity

Dilier Olivera Viciado (✉ dilierolvi@gmail.com)

Laboratory of Plant Nutrition, Soils and Fertilizers Sector, Department of Agricultural Production Sciences, São Paulo State University "Júlio de Mesquita Filho" (UNESP), Jaboticabal, São Paulo, Brazil
<https://orcid.org/0000-0002-7975-9508>

Renato de Mello Prado

Laboratory of Plant Nutrition, Soils and Fertilizers Sector, Department of Agricultural Production Sciences, São Paulo State University "Júlio de Mesquita Filho" (UNESP), Jaboticabal, São Paulo, Brazil
<https://orcid.org/0000-0003-1998-6343>

Kolima Peña

Laboratory of Plant Nutrition, Soils and Fertilizers Sector, Department of Agricultural Production Sciences, São Paulo State University "Júlio de Mesquita Filho" (UNESP), Jaboticabal, São Paulo, Brazil
<https://orcid.org/0000-0003-4883-4293>

Alexander Calero-Hurtado

Laboratory of Plant Nutrition, Soils and Fertilizers Sector, Department of Agricultural Production Sciences, São Paulo State University "Júlio de Mesquita Filho" (UNESP), Jaboticabal, São Paulo, Brazil
<https://orcid.org/0000-0001-6536-2908>

Marisa de Cássia Piccolo

University of São Paulo, Center of Nuclear Energy in Agriculture, Piracicaba, São Paulo, Brazil
<https://orcid.org/0000-0003-2163-5630>

Mariana Bomfim Soares

Laboratory of Plant Nutrition, Soils and Fertilizers Sector, Department of Agricultural Production Sciences, São Paulo State University "Júlio de Mesquita Filho" (UNESP), Jaboticabal, São Paulo, Brazil
<https://orcid.org/0000-0001-8705-4578>

Rodolfo Toledo Lizcano

1Universidad de Granada. International Graduate School, University of Granada <https://orcid.org/0000-0003-2797-0237>

Research Article

Keywords: Abiotic stress, Beta vulgaris, nitrogen, plant growth, hydroponic cultivation

Posted Date: February 24th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1393734/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Most plants develop severe toxicity symptoms when grown on NH_4^+ as the sole nitrogen source, resulting in physiological and nutritional disturbances that slow vegetable growth. In this scenario of NH_4^+ stress, it is important to use strategies to mitigate this toxicity, and the use of silicon (Si) has promoted promising results. However, underlying studies on the effects of ammonia toxicity on C:N:P stoichiometric homeostasis and the role of Si in these stoichiometric ratios are lacking. In this study, we investigated the effects of different NH_4^+ concentration on the C:N:P stoichiometry and yield of sugar beet grown under hydroponic conditions, and examined whether the application of Si can alleviate the detrimental effects caused by toxic levels of NH_4^+ . We conducted a hydroponic experiment to evaluate the effects of five concentrations of ammonium (1, 7.5, 15, 22.5, and 30 mmol L^{-1}) on C:N:P stoichiometry and yield of sugar beet cv. Early Wonder in the absence and presence (2 mmol L^{-1}) of Si. The experimental design was a randomized block design based on a 5×2 factorial scheme with four replicates. Our results indicate that in presence of Si both [N] and [P] in shoots and roots increased significantly and reaching its highest value at 15 mmol L^{-1} of NH_4^+ in the nutrient solution, while shoot [C] remained stable, in roots increased with NH_4^+ concentrations of 22.5 and 30 mmol L^{-1} , respectively. In addition, shoot and root [Si] increased as higher levels of NH_4^+ in the nutrient solution. Our findings showed that homeostatic instability under highest levels of NH_4^+ , especially in the plants cultivated without Si in the nutritive solution resulted in decreased shoots and roots dry matter production. In conclusion, both NH_4^+ concentration or Si in presence or absence in the nutrient solution can affect C:N:P stoichiometry of sugar beet.

Introduction

Nitrogen (N) availability is one of the most important factors that determine plant growth in a wide range of environments ^{1,2}. Ammonium (NH_4^+) and nitrate (NO_3^-) are the main forms of N absorbed by plants ³. Unlike NO_3^- , NH_4^+ is directly incorporated into organic compounds without the need for energy-dependent enzymatic reduction. Thus, urea, the worldwide leading nitrogen-fertilizer is converted to ammonium by urease enzymes of soil microorganisms and plants ⁴. Consequently, the lower metabolic energy expenditure associated with ammoniacal nutrition can be converted into higher plant growth, and thus higher production ^{1,5}.

Despite its value as a fertilizer, it is well established that an excess of NH_4^+ can promote the production of reactive oxygen species (ROS), which can cause reductions in cytoplasmic pH, photosynthetic rate, transpiration, and stomatal conductance ¹. Depending on concentration, the exclusive supply of N as NH_4^+ is harmful to many plants and may cause poor biomass accumulation and ion imbalance ^{1,5,6}. Toxic levels of NH_4^+ in plant tissues accumulate when the overall rate of the conversion of NH_4^+ into

amino acids and amides becomes lower than the rate of its uptake and cellular production by amino acid catabolism, NO_3^- reduction, phenylpropanoid metabolism, and photorespiration ⁷.

A known strategy to mitigate nutritional disorders of NH_4^+ is to utilize Silicon (Si) in the nutrient solutions ^{1,5}. Si is the second most abundant element, after oxygen in the Earth's crust, is absorbed by plants as monosilicic acid (H_4SiO_4) ^{8,9}, with concentrations varying between 0.1 and 0.6 mM, which is about two orders of magnitude higher than the concentrations of phosphorus (P) ¹⁰. The beneficial effects of Si have been observed in various plant species, including Si-accumulating and non-accumulating species ^{1,5,11,12}. Seven out of the ten most produced crops in the world (ranked by quantity) are accumulators, see ¹³.

All land plants contain Si in their tissues and although its concentration varies considerably with the species of plant, oscillating between 0.1 and 10% of its dry weight, its beneficial effects are more notable under environmental stress, caused by both biotic and abiotic sources ¹⁴⁻¹⁶. In plants, a relatively higher quantity of Si is found than many other essential macronutrients such as calcium, magnesium and phosphorus. For example, grasses even may contain higher levels of Si than any of the other inorganic mineral nutrients ¹¹. Application of Si positively impact almost all aspects of N nutrition, i.e., uptake, assimilation, and remobilization ^{8,17,18}. This beneficial effect of Si on overall plant performance has been established under low, optimal, and excessive N supply. In addition to a direct effect on N metabolism, Si supply induces changes in carbon (C) and P stoichiometry in shoots ^{19,20} and increases nutrient acquisition by roots ^{21,22}, which in turn can contribute to improving N utilization within plant tissues.

Sugar beet (*Beta vulgaris* L.) is one of the more important commercial crops, which is around 20% of the world sugar demand with supplies 36 million mt of raw sugar yearly ²³. In Brazil, this crops production constitutes one of the main cultivated plants and is the 13th most economically valuable and the estimated planted area in the country is approximately 10,000 ha, with average production rates varying between 20 and 35 t ha⁻¹ ^{15,24}. Nitrogen is the most important element that is applied to sugar beet, because it is difficult finding soils that contain sufficient amounts of this element in any of its available forms. Insufficient supply of N can reduce plant N concentration and rate of photosynthesis ¹ and simultaneously reduce plant growth and quality of harvestable materials ⁵. The optimal application of N fertilizers positively impacts production. However, oversupply of N does not always lead to increased yield, and it might actually result in reduced growth and yield, especially for crops where roots and tubers are harvested ²⁵.

Elemental stoichiometry has been suggested as a new approach for studying the interaction between plants and soils, as well as the elemental cycling in plant-soil systems in a changing world ²⁶. The concept of stoichiometric homeostasis refers to the ability of an organism to maintain relatively constant the concentrations or ratios of elements within its tissues under variable environmental conditions ²⁷. Numerous studies have concluded that carbon (C), N, and phosphorus (P) stoichiometry in plants tends

to be decoupled under global change^{28,29}, but studies involving ammonia toxicity are lacking. Therefore, stoichiometric homeostasis can be used to predict the strategies used by different plant species under biotic and abiotic stresses to cope with limited resources in their surroundings. We hypothesized that plants of sugar beet will adjust nutrient conservation strategies to balance the altered elemental stoichiometric homeostasis (C:N:P) and dry matter production accordingly to NH_4^+ concentrations in presence of Si. While with absence of Si in the nutrient solution may exacerbate NH_4^+ toxicity due to disturbance in elementary stoichiometric ratios. Thus, the aim of this study was to evaluate the combined and isolated effects of different NH_4^+ concentration in presence or absence of Si on C:N:P stoichiometry and yield of sugar beet.

Materials And Methods

2.1. Installation and conduction of the experiment

This study was performed out in a greenhouse at the Faculty of Agricultural and Veterinary Sciences (FCAV), UNESP Jaboticabal, SP, Brazil (21°15'22"S and 48°18'58"W), to evaluate the impact of silicon on C:N:P stoichiometry and yield parameters of sugar beet plants (*Beta vulgaris* L.) cv. Early Wonder under different ammonium concentration. Plants were cultivated in controlled conditions in a plant growth chamber with a light/dark cycle of 13/11 h, temperature regime of 35/25 °C, and relative humidity of approximately 50%. Every experimental unit was composed of a plastic pot with a capacity of 3.8 L, in which was a mixture of the coarse vermiculite substrate (90 to 100% of particles between 1.19 and 1.50 mm) in a 1:1 proportion (v/v) with super fine particles (90 to 100% of particles between 0.21 and 0.33 mm), to allow for adequate porosity and aeration (Sarasketa et al. 2014).

2.2. Growth condition

In each pot, 6 seeds were placed at a depth of 2 cm, which were thinned 5 days after their emergence, leaving only 2 plants for every pot to allow for an adequate distance for root growth. Initially 100 ml of distilled water per pot was used for the first 7 days, to maintain the required humidity of the seedlings. Distilled water was replaced by 200 ml of nutritive solution for every pot (Hoagland and Arnon 1950), with modification in the source of iron alternating between Fe-EDTA and Fe-EDDHMA. The pH of nutrient solution was monitored daily and maintained at 5.4 ± 0.3 using 0.01 mol L^{-1} sodium hydroxide (NaOH) and/or hydrochloric acid (HCl).

2.3. Experimental design

The experimental design used was completely randomized, in a factorial scheme of 5×2 , corresponding with five concentrations of NH_4^+ (1.0, 7.5, 15, 22.5, and 30 mmol L^{-1}) and the absence and presence of Si (2 mmol L^{-1}), with four replicates. Two mmol L^{-1} Si concentration used here was based on the experiments carried out by our research group (GENPLANT)^{5, 9, 11} and the fact that the solubility of monosilicic acid (H_4SiO_4) in nutrient solution is close to this concentration³⁰. The source of N used was NH_4Cl , with Si in the form of H_4SiO_4 (Si = 28.5 g L^{-1}).

2.4. Determination of dry matter

After harvest, all the plants were collected and washed with detergent solution (0.2%), hydrochloric acid solution (0.1%), and finally deionized water. All plant material was placed in paper sacks and dried in a forced air circulation oven ($65 \pm 5 \text{ °C}$) until they reached a constant weight, and then the total dry mass was measured on a digital precision scale. Later, it was pulverized in an electric grinder (Wiley).

2.5. Carbon, nitrogen, phosphorus, and silicon analysis

For the nutrients (N, P, and C) the material collected at every replication (n=4) per pot was mixed to form a composite sample. Using the powdered plant material, we measured the total C, and N concentration in shoots and root using the method of dry combustion (at 1000 °C) in an elemental analyzer (LECO Truspec CHNS) calibrated with the pattern LECO 502-278 of wheat (C = 45.00% and N = 2.68%). Si concentration was determined according to (Korndörfer et al., 2004), meanwhile, the P concentration (of acid digests) was measured by spectrophotometer (model SP-1105) using the molybdenum antimony colorimetric method and N following the methods described by Bataglia et al. (1983). Using the C, N, and P concentrations in different plant organs, we calculated the C:P, C:N, and N:P ratios ²⁹.

2.6. Statistical analysis

Data were subjected to a two-way analysis of variance (ANOVA) after checking the homogeneity of variance of Levene, *F* test. The Shapiro-Wilks *W* test was used to test the normality of the data. The mean values were compared using Tukey's test (*p* < 0.05). In this study, we tested the main effects of five ammonium concentrations (NH₄⁺) in absence and presence of Silicon (Si), as well as their interactions (Si×N). All analyses were performed using the statistical software Agroestat ³¹.

Results

Our data revealed interactive effects between N × Si for C concentration [C], N concentration [N], P concentration [P], and Si concentration [Si] in shoots (Fig. 1a-d). While [C] remained stable for the shoots of *B. vulgaris*, in the presence of Si, the [N] increased, reaching its highest value in the concentration of 15 mmol L⁻¹ in the presence of silicon, showing significant differences between the treatments (Fig.1a-b). Similarly, to the [N], was observed for [P] and [Si] in the shoots (Fig. 1c-d). The concentrations of P increased in the presence of Si up to a concentration of 15 mmol L⁻¹ of NH₄⁺ and after they decreased, but without significant differences in relation to the same concentrations in the absence of Si.

The ANOVA revealed a significant interaction between [Si] and [N] (Si × N) in the roots of sugar beet plants depending on the treatment. In the absence of Si, the [C] decreases linearly as NH₄⁺ concentration increased up to 30 mmol L⁻¹ NH₄⁺. In an opposite direction, the presence of Si in the nutrient solution increases the [C] (Fig. 2a). On the other hand, an increase in the concentration of NH₄⁺ decreased [N], especially in the plants cultivated without the incorporation of Si in the nutritive solution in the last NH₄⁺ concentration (Fig. 2b). Both [P] and [Si] in root increased in the presence of Si in the nutrient solution. Specifically, [P] increased up to 15 mmol L⁻¹ NH₄⁺, meanwhile, the addition of Si in the nutrient solution resulted in higher levels of Si in the root of the sugar beet, relative to plants without Si supplementation, across in all concentrations of NH₄⁺ (Figure 2c-d).

Shoot C:N ratio increased in absence of Si, however, an interactive effect between Si × N decreased C:N ratio in the presence of Si for all NH₄⁺ concentrations (Fig. 3a). Shoot C:P ratio show an interaction Si × N and increased under higher NH₄⁺ concentration conditions in the nutrient solution, independently of presence or absence of Si (Fig. 3b). The highest N: P ratio of the shoot was found in 15 mmol L⁻¹ NH₄⁺ concentration in the absence of Si (Fig 3c). Conversely, the N: P ratio increased with the higher NH₄⁺

concentration in the presence of Si in the nutrient solution (Fig 3c). We found a growing increase in the dry matter production of the shoots up to the ammonium concentration of 15 mmol L⁻¹ (Fig. 3d). From this concentration, the tendency was to gradually decrease, but it was always greater in the presence of Si, when compared to plants without the addition of Si. Shoot dry matter was significantly influenced by the interactive effects between Si × N.

Root C:N, C:P, and N:P ratios were significantly influenced by the interactive effects of silicon and ammonium (Fig. 4a-c). In comparison with the plants that growing in the presence of Si, the concentration of 15 mmol NH₄⁺ had a significant positive effect on the C:N ratio of the roots in the absence of Si, while the opposite effect was observed at a higher concentration of 22.5 mmol NH₄⁺ (Fig. 4a). C:P ratio decreased in plants supplied with Si under the first three concentrations of NH₄⁺ (from 1 mmol L⁻¹ to 15 mmol L⁻¹) in the nutrient solution (Fig. 4b). However, Si increased C:P ratio under last two concentrations of NH₄⁺ (from 22.5 mmol L⁻¹ to 30 mmol L⁻¹) in the nutrient solution. Except for the first and last concentration of NH₄⁺ in the nutrient solution, C:N ratio in the root increased significantly in plants in the absence of silicon (Fig 4c). In contrast, root dry matter increased for all treatment in the presence of Si, regardless of the concentration of NH₄⁺ in the nutrient solution, compared with plants the absence of silicon (Fig. 4d).

Discussion

The use of N supply based on the NH₄⁺ form is used in cheap fertilizers as urea, the use of which can reduce the costs of production and increases farmers' incomes ¹. Unlike NO₃⁻, NH₄⁺ is directly incorporated into organic compounds without the need for energy-dependent enzymatic reduction ⁴. Consequently, the lower metabolic energy expenditure associated with ammoniacal nutrition can be converted into higher plant growth, and thus higher production. However, despite its value as a fertilizer, it is well established that an excess of NH₄⁺ can promote the production of reactive oxygen species (ROS), which can cause reductions in cytoplasmic pH, photosynthetic rate, transpiration, and stomatal conductance ^{1,5,32}. In addition, an excess NH₄⁺ can inhibit root growth, and subsequently vegetative shoot growth and yield ³³.

Several experiments have focused on the relationships between nutrient stoichiometry in plant communities and geographical variations or climatic factors ^{28,29,34}. However, to the best of our knowledge, few studies have focused on the effects of NH₄⁺ toxicity on C:N:P ratio in horticultural crops.

A strategy that has proven its effectiveness in mitigating the damages caused by excess of NH₄⁺ is the use of Si. Although Si is not an essential nutrient, its application is beneficial. Si application can mitigate the harmful effects of oxidative stress in the photosynthetic apparatus of different crops ^{1,5}, improve antioxidant responses ²², increasing dry matter by enhancing ionic homeostasis in roots and shoots of different plant species ³⁵. The addition of Si induces changes in the C:N:P stoichiometry and enhances

stoichiometric homeostasis of sorghum and sunflower plants under salt stress³⁴. However, the patterns of plant C:N:P stoichiometry are species-specific and dependent of various factors and ambient conditions^{29,36}.

Plant exposed to high concentrations of NH_4^+ typically show symptoms of NH_4^+ toxicity. At low concentrations ($<3 \text{ mmol L}^{-1}$), NH_4^+ is typically the N source preferred by plants, but above a certain threshold e.g., 15 mmol L^{-1} in sugar beet, 30 mmol L^{-1} in radish, NH_4^+ becomes toxic^{1,5}. This threshold depends on plant species and on variety or cultivars of the same species^{1,5,37}. Recently, (Olivera-Viciedo et al., 2020) reported that a concentration of 30 mmol L^{-1} , NH_4^+ was shown to have negative effects on the total dry biomass, photosynthesis, transpiration, and stomatal conductance of radish seedlings, even in the presence of Si in the nutrient solution, whereas instantaneous water-use efficiency and the green color index increased in the presence of Si.

In this study, was observed that the highest levels of NH_4^+ affected stoichiometric homeostasis and reduced the dry matter by decreased the shoots and roots [N] and [P], especially in the plants cultivated without Si in the nutritive solution corroborating our main hypothesis. These findings indicate that application of Si to the nutrient solution used in hydroponic cultivation could provide an effective means of alleviating the unfavorable effects induced by NH_4^+ toxicity in agreement with ours previous experiment^{1,5}. Although plants are able to survive with very low Si availability under greenhouse or some controlled laboratory conditions, several studies have shown that plants fertilized with Si increase photosynthesis, water use efficiency, and consequently have higher biomass production compared with non-Si-fertilized plants^{1,5,11,22}.

Higher dry matter of shoots and roots (Fig 3d, and Fig 4d) in the presence of Si may be given by a higher [N] and [P] in both plant-organs (Fig 1b, c; Fig 2b, c). However, it should be noted that there was a more severe effect of NH_4^+ toxicity in the roots than shoot when NH_4^+ concentrations were higher than 15 mmol L^{-1} , probably because roots constitute the first NH_4^+ sensor, and the initial signals of NH_4^+ toxicity appear at root level with a severe modification of the root architecture that result in a decreases root/shoot ratio, as reported in previous studies^{4,38}. Therefore, it was concluded that the role of Si on C:N:P stoichiometry and dry matter production of sugar beet could be related to growth conditions, NH_4^+ concentrations, and the ability of plants to maintain a relatively stable composition of nutrients (C, N, P), and remain in homeostasis.

Declarations

Conflicts of Interest

The authors declare that they have no conflicts of interest

References

- 1 Olivera-Viciedo D, Mello Prado R, Lizcano Toledo R, Salas Aguilar D, Claudio Nascimento dos Santos L, Calero Hurtado A, Peña Calzada K, and Betancourt Aguilar C. Physiological role of silicon in radish seedlings under ammonium toxicity. *J Sci Food Agric* :jsfa.10587 (2020).
- 2 Prado R de M. Introduction to Plant Nutrition. *Miner Nutr Trop plants* Springer, Cham; :1–38 (2021).
- 3 Hachiya T and Noguchi K. Integrative response of plant mitochondrial electron transport chain to nitrogen source. *Plant Cell Rep* Springer; **30**:195–204 (2011).
- 4 Bittsánszky A, Pilinszky K, Gyulai G, and Komives T. Overcoming ammonium toxicity. *Plant Sci* Elsevier Ireland Ltd; **231**:184–190 (2015).
- 5 Olivera-Viciedo D, Mello Prado R de, Lizcano Toledo R, Santos LCN dos, Calero Hurtado A, Nedd LLT, and Castellanos Gonzalez L. Silicon Supplementation Alleviates Ammonium Toxicity in Sugar Beet (*Beta vulgaris* L.). *J Soil Sci Plant Nutr* Springer; **19**:413–419 (2019).
- 6 Domínguez-Valdivia MD, Aparicio-Tejo PM, Lamsfus C, Cruz C, Martins-Loução MA, and Moran JF. Nitrogen nutrition and antioxidant metabolism in ammonium-tolerant and -sensitive plants. *Physiol Plant* John Wiley & Sons, Ltd; **132**:359–369 (2008).
- 7 Hirel B, Gouis J Le, Ney B, and Gallais A. The challenge of improving nitrogen use efficiency in crop plants: Towards a more central role for genetic variability and quantitative genetics within integrated approaches. *Journal of Experimental Botany* Oxford Academic; p. 2369–2387 2007.
- 8 Pavlovic J, Kostic L, Bosnic P, Kirkby EA, and Nikolic M. Interactions of Silicon With Essential and Beneficial Elements in Plants. *Front Plant Sci* Frontiers; **0**:1224 (2021).
- 9 Epstein E. The anomaly of silicon in plant biology. *Proc. Natl. Acad. Sci. U. S. A.* National Academy of Sciences; p. 11–17 1994.
- 10 Yan G chao, Nikolic M, Ye M jun, Xiao Z xi, and Liang Y chao. Silicon acquisition and accumulation in plant and its significance for agriculture. *J Integr Agric* Elsevier; **17**:2138–2150 (2018).
- 11 Bakhat HF, Bibi N, Zia Z, Abbas S, Hammad HM, Fahad S, Ashraf MR, Shah GM, Rabbani F, and Saeed S. Silicon mitigates biotic stresses in crop plants: A review. *Crop Prot* Elsevier; **104**:21–34 (2018).
- 12 Rocha JR, Mello Prado R de, and Cássia Piccolo M de. New outcomes on how silicon enables the cultivation of *Panicum maximum* in soil with water restriction. *Sci Reports 2022 121* Nature Publishing Group; **12**:1–13 (2022).

- 13 Guntzer F, Keller C, and Meunier J-D. Benefits of plant silicon for crops: a review. *Agron Sustain Dev* 2011 321 Springer; **32**:201–213 (2011).
- 14 Frew A, Weston LA, Reynolds OL, and Gurr GM. The role of silicon in plant biology: a paradigm shift in research approach. *Ann Bot Oxford Academic*; **121**:1265–1273 (2018).
- 15 Camargo MS de, Bezerra BKL, Vitti AC, Silva MA, and Oliveira A. Silicon fertilization reduces the deleterious effects of water deficit in sugarcane. *J soil Sci plant Nutr Chilean Society of Soil Science*; **17**:99–111 (2017).
- 16 Olivera-Viciedo D, Mello Prado R de, Lizcano Toledo R, Nascimento dos Santos LC, and Peña Calzada K. Respuesta de las plántulas del rábano (*Raphanus sativus* L.) a diferentes concentraciones de nitrógeno amoniacal en ausencia y presencia de silicio. *Agron Colomb* Universidad Nacional de Colombia1; **35**:198–204 (2017).
- 17 Gou T, Yang L, Hu W, Chen X, Zhu Y, Guo J, and Gong H. Silicon improves the growth of cucumber under excess nitrate stress by enhancing nitrogen assimilation and chlorophyll synthesis. *Plant Physiol Biochem Elsevier Masson*; **152**:53–61 (2020).
- 18 Prado R de M. Introduction to Plant Nutrition. Miner. Nutr. Trop. plants. Springer, Cham; 2021.
- 19 Teixeira GCM, Prado R de M, Rocha AMS, and Piccolo M de C. Root- and foliar-applied silicon modifies C: N: P ratio and increases the nutritional efficiency of pre-sprouted sugarcane seedlings under water deficit. *PLoS One Public Library of Science*; **15**:e0240847 (2020).
- 20 Schaller J, Brackhage C, Gessner MO, Bäuker E, and Dudel EG. Silicon supply modifies C:N:P stoichiometry and growth of *Phragmites australis*. *Plant Biol John Wiley & Sons, Ltd*; **14**:392–396 (2012).
- 21 Calero-Hurtado A, Aparecida Chiconato D, Mello Prado R de, Silveira Sousa Junior G da, and Felisberto G. Silicon attenuates sodium toxicity by improving nutritional efficiency in sorghum and sunflower plants. *Plant Physiol Biochem Elsevier Masson SAS*; **142**:224–233 (2019).
- 22 Calero-Hurtado A, Chiconato DA, Prado R de M, Sousa Junior G da S, Gratão PL, Felisberto G, Olivera Viciedo D, and Mathias dos Santos DM. Different methods of silicon application attenuate salt stress in sorghum and sunflower by modifying the antioxidative defense mechanism. *Ecotoxicol Environ Saf* **203**:110964 (2020).
- 23 Lucchi C De, Biancardi E, Skaracis G, Biaggi M De, Pavli O, Ravi S, Chiodi C, Broccanello C, and Stevanato P. Sugar Beet (*Beta vulgaris* ssp. *vulgaris* L.) Improvement with Next-Generation Breeding Technology. *Adv Plant Breed Strateg Veg Crop Springer, Cham*; :305–343 (2021).
- 24 Souza R, Fontanetti A, Fiorini C, and Almeida K. Cultura da beterraba: Cultivo convencional e cultivo orgânico. 2003.

- 25 Sinta Z and Garo G. Influence of Plant Density and Nitrogen Fertilizer Rates on Yield and Yield Components of Beetroot (*Beta vulgaris* L.). Serrano M, ed. *Int J Agron* **2021**:1–7 (2021).
- 26 Sterner RW and Elser JJ. Ecological stoichiometry: the biology of elements from molecules to the biosphere. Princeton University Press; 2002.
- 27 Elser JJ, Fagan WF, Kerkhoff AJ, Swenson NG, and Enquist BJ. Biological stoichiometry of plant production: metabolism, scaling and ecological response to global change. *New Phytol* **186**:593–608 (2010).
- 28 Olivera-Viciedo D, Mello Prado R de, Martínez CA, Habermann E, and Cássia Piccolo M de. Short-term warming and water stress affect *Panicum maximum* Jacq. stoichiometric homeostasis and biomass production. *Sci Total Environ* **681**:267–274 (2019).
- 29 Olivera-Viciedo D, Mello Prado R de, Martinez CA, Habermann E, Cássia Piccolo M de, Calero Hurtado A, Barreto RF, and Peña Calzada K. Changes in soil water availability and air-temperature impact biomass allocation and C:N:P stoichiometry in different organs of *Stylosanthes capitata* Vogel. *J Environ Manage* **278** (2021).
- 30 Gao X, Zou C, Wang L, and Zhang F. Silicon Improves Water Use Efficiency in Maize Plants. *J Plant Nutr* Taylor & Francis Group ; **27**:1457–1470 (2005).
- 31 Barbosa J and Maldonado J. Experimentação agronômica e AgroEstat: sistema para análises estatísticas de ensaios agronômicos. Jaboticabal: Departamento de Ciências Exatas–Universidade Estadual Paulista (UNESP); 2015.
- 32 Hu L, Yu J, Liao W, Zhang G, Xie J, Lv J, Xiao X, Yang B, Zhou R, and Bu R. Moderate ammonium: Nitrate alleviates low light intensity stress in mini Chinese cabbage seedling by regulating root architecture and photosynthesis. *Sci Hortic (Amsterdam)* Elsevier; **186**:143–153 (2015).
- 33 Di DW, Sun L, Zhang X, Li G, Kronzucker HJ, and Shi W. Involvement of auxin in the regulation of ammonium tolerance in rice (*Oryza sativa* L.). *Plant Soil* Springer International Publishing; **432**:373–387 (2018).
- 34 Calero-Hurtado A, Chiconato DA, Prado R de M, Sousa Junior G da S, Olivera Viciedo D, and Piccolo M de C. Silicon application induces changes C:N:P stoichiometry and enhances stoichiometric homeostasis of sorghum and sunflower plants under salt stress. *Saudi J Biol Sci* **27**:3711–3719 (2020).
- 35 Calero-Hurtado A, Chiconato DA, Mello Prado R de, Silveira Sousa Junior G da, Viciedo DO, Díaz YP, Calzada KP, and Gratão PL. Silicon Alleviates Sodium Toxicity in Sorghum and Sunflower Plants by Enhancing Ionic Homeostasis in Roots and Shoots and Increasing Dry Matter Accumulation. *Silicon* Springer; **13**:475–486 (2021).

- 36 Huang J, Wang P, Niu Y, Yu H, Ma F, Xiao G, and Xu X. Changes in C:N:P stoichiometry modify N and P conservation strategies of a desert steppe species *Glycyrrhiza uralensis*. *Sci Rep* Nature Publishing Group; **8**:12668 (2018).
- 37 Cruz C, Domínguez-Valdivia MD, Aparicio-Tejo PM, Lamsfus C, Bio A, Martins-Loução MA, and Moran JF. Intra-specific variation in pea responses to ammonium nutrition leads to different degrees of tolerance. *Environ Exp Bot* Elsevier; **70**:233–243 (2011).
- 38 Esteban R, Ariz I, Cruz C, and Moran JF. Review: Mechanisms of ammonium toxicity and the quest for tolerance. *Plant Sci* Elsevier Ireland Ltd; **248**:92–101 (2016).

Figures

Figure 1

Effects of NH_4^+ concentrations, in the presence (+Si) and absence of Si (-Si) on shoot C concentration (**a**), shoot N concentration (**b**), shoot P concentration (**c**), and shoot Si concentration (**d**) of sugar beet plants. Different uppercase letters indicate significant differences between different NH_4^+ concentration at the same Si concentration, while Different lowercase letters indicate significant differences between different Si concentration within the same NH_4^+ concentration, according to the F test. Error bars show standard errors based on the average values of four replicates (n=4). * $p \leq 0.05$; ** $p \leq 0.01$; ns, not significant; Si $\times$ N, Si- NH_4^+ interaction.

Figure 2

Effects of NH_4^+ concentrations, in the presence (+Si) and absence of Si (-Si) on root C concentration (**a**), root N concentration (**b**), root P concentration (**c**), and root Si concentration (**d**) of sugar beet plants. Different uppercase letters indicate significant differences between different NH_4^+ concentration at the same Si concentration, while Different lowercase letters indicate significant differences between different Si concentration within the same NH_4^+ concentration, according to the F test. Error bars show standard errors based on the average values of four replicates (n=4). * $p \leq 0.05$; ** $p \leq 0.01$; Si $\times$ N, Si- NH_4^+ interaction.

Figure 3

Effects of NH_4^+ concentrations, in the presence (+Si) and absence of Si (-Si) on shoot C:N ratio (**a**), shoot C:P ratio (**b**), shoot N:P ratio (**c**), and shoot dry matter (**d**) of sugar beet plants. Different uppercase letters indicate significant differences between different NH_4^+ concentration at the same Si concentration, while Different lowercase letters indicate significant differences between different Si concentration within the same NH_4^+ concentration, according to the F test. Error bars show standard errors based on the average values of four replicates (n=4). * $p \leq 0.05$; ** $p \leq 0.01$; ns, not significant; Si $\times$ N, Si- NH_4^+ interaction.

Figure 4

Effects of NH_4^+ concentrations, in the presence (+Si) and absence of Si (-Si) on root C:N ratio (**a**), root C:P ratio (**b**), root N:P ratio (**c**), and root dry matter (**d**) of sugar beet plants. Different uppercase letters indicate significant differences between different NH_4^+ concentration at the same Si concentration, while Different lowercase letters indicate significant differences between different Si concentration within the same NH_4^+ concentration, according to the F test. Error bars show standard errors based on the average values of four replicates (n=4). * $p \leq 0.05$; ** $p \leq 0.01$; ns, not significant; Si $\times$ N, Si- NH_4^+ interaction.