Dear Prof. Bahn

We summarise in this response the main points raised by reviewer 2 and how we have approached them. They can be grouped into three main issues.

1) Plants do not have an active uptake mechanisms and Si uptake is in principle unspecific and passively taken up and passively transported in plants (Exley et al., 2020; Exley and Guerriero, 2019).

While we retain a neutral position in this debate we note that there are numerous articles that assume a highly selective Si uptake mechanism in Si accumulator plants. Assuming a passive uptake mechanism and passive transport within the plant, the amount of Si incorporated is set by transpiration, provided that the availability of soluble Si in the root space does not differ between species. On the other hand, if we can observe species with higher transpiration rates which incorporate less Si than plant species with a lower transpiration rate (under the same environmental conditions), this demonstrates that plant species must differ in their Si uptake mechanism or in their Si transport within the plant. This is what we observe. In the revised version we however point at several occasions at the controversy surrounding the debate over uptake mechanisms, and in particular to the points made in the papers that question the active uptake (Exley et al., 2020; Exley and Guerriero, 2019).

2) The lack of a control experiment.

The aim of our study is to trace and quantify the uptake of silicon in three different crop species using the naturally occurring shifts (=isotope fractionation) in the abundances of the stable isotopes of silicon and physiological parameters (plant Si amounts and concentrations, transpiration). Performing a control experiment in the absence of plants makes no sense, as Si will not change compartments hence there is no isotope fractionation. However, what we have done can be seen as a variant of a control experiment: We have grown (in triplicate) spring wheat, tomato, and mustard under the ***same*** environmental conditions (humidity, light, temperature, and the initial nutrient composition), and thus the differences observed are controls over differences in plant physiology.

3) Methodological issues and missing information on methods.

One set of questions by reviewer 2 pertains to explanations that were in fact provided elsewhere in the manuscript, and hence no changes were made. The reviewer may not have read these sections. Another set of questions pertains to the isotope analytical sample preparations employed, and to basic isotope data interpretation. All of this is widely available in isotope geochemical literature, and is standard in isotope geochemistry. We believe that providing this background information in this paper would dilute the flow of information and is in any case beyond the scope of this paper.

Still, both reviewers pointed at deficits in the presentations and suggested clarifications. We implemented these. Below we provide a detailed response to all of the points raised by reviewer 2. For many of those points however we rebutted the requests, since the information is already present in the manuscript, or the request is not within the scope of our work.

We hope that our answers together with the (improved) manuscript is now ready for publication in 'Biogeosciences'.

Daniel A. Frick on behalf of all coauthors.

Response to Anonymous Reviewer 2

In the following we respond individually to all the comments:

Title: I appreciate that you changed the title to accomodate another reviewer but the new title really does not make sense in English.

Thanks for bringing this to our attention, after some consultations with native speakers we have changed the title of the manuscript to: *Silicon uptake and isotope fractionation dynamics by crop species*

Line 35: There are excellent papers on the biogeochemical cycle of silicon and silicic acid that could be cited here.

We have added and rearranged citations of excellent papers on the biogeochemical cycle of silicic acid and silica: *"One crucial but poorly understood aspect of terrestrial Si biogeochemistry is biological cycling (Carey and Fulweiler, 2012; Derry et al., 2005; Sommer et al., 2006, 2013)."*

Line 36: What do you mean by this statement? How is silicon 'recycled multiple times' in plants?

The sentence does not claim that Si is recycled *within* plants, but within the ecosystem by plants (e.g. Si from decomposing phytoliths (litter fall) will be available for re-incorporate into plants, before it is lost from ecosystems. We have clarified the statement: *"Si has well documented biological roles, and Si may be recycled multiple through higher plants before being lost from an ecosystem."*

Line 42: Sequestration of heavy metals? Evidence of this? What about Al, this is not a heavy metal.

We have clarified : *"Despite having a disputed biochemical role, Si is considered beneficial for plant growth, including crops: Si increases abiotic stress mediation (aluminium and heavy metal toxicity, salinity), biotic stress resistance (defence against herbivores), and improves the plants' structural stability* (Coskun et al., 2019b; Epstein, 1994, 1999, 2001; Exley and Guerriero, 2019; Ma, 2004; Richmond and Sussman, 2003)*."*

Line 46: Actually plants are classified according to how much 'silica' they deposit in their tissues, see work by Hodson. There is certainly no consensus on their classification according to the parameters you suggest. There is equally good evidence that the movement of silicic acid into and in plants is entirely passive and even if this is not your point of view you should acknowledge this. See for example, most recently; https://link.springer.com/article/10.1007%2Fs12633-019-00360-w

We describe here a very widely used and applied scheme to classify ***the amounts*** of silicon incorporated by higher plant species (see e.g. Coskun et al., 2019b; Guerriero et al., 2020; Handreck and P Jones, 1967; Takahashi et al., 1990). The sentence it is not about ***how*** silicon is taken up. How Si is incorporated is exposed in Line 50/51, see also our next response. No changes were made.

Line 51: The discussion is not simply about the definition of 'active' it is whether or not previous research has identifed active transport of silicic acid.

We made changes to the sentence to include these aspects: *"However, the term "active uptake" is still widely debated. In particular whether the classification as active or passive is justified, as well as the evidence for involvement of an active, metabolically controlled process in some plant species is subject of an intense discussion (Coskun et al., 2019a; Exley, 2015; Exley et al., 2020)."*

Line 59: But these studies have recently been questioned and as yet no unequivocal evidence of active transport of silicic acid has been demonstrated.

We have already highlighted the ongoing dispute regarding the 'active' nature of silicic acid uptake in Line 50. Our study design and results however cannot provide evidence whether the active transport requires the expense of energy, thus we cannot provide answers towards either view of this ongoing discussion. We are thus taking a neutral standpoint in this discussion. Thus, we believe the sentence the reviewer comments on is adequate. No changes were done.

Line 70: But, see Exley et al 2020 why Ge is not suitable as an analogue for movement of silicic acid.

We have added this citation to Line 62ff *"Both techniques impose limitations on growth experiments, either due to safety concerns arising from radioactivity or due to physiological differences between the homologue element Ge and Si (Exley et al., 2020; Takahashi et al., 1990)."*

Line 82: Makes sense since lighter molecules of silicic acid will diffuse more easily than heavier ones.

No change made.

Line 88: No evidence of transporters, better 'channels'.

For the sake of consistency throughout the manuscript we use transporter when talking about the homologues of Lsi1 and Lsi2. See e.g. in the introduction: *"Lsi1, Low Silicon 1 transporter, a thermodynamically passive transporter from the family of aquaporin-like proteins) incorporates Si, whereas a metabolically active efflux transporter (Lsi2, a putative anion-channel transporter)"*

No change made.

Line 99: Na4SiO4

Thanks for bringing this to our attention, we have corrected it with the name (sodium silicate trihydrate) and the corresponding sum formula ($Na_2O_7Si_3 \cdot 3H_2O$).

Line 99: This concentration of sodium orthoscilicate when dissolved in pure water will produce a highly alkaline solution, pH above 12. How did you accomodate this? 1/6th strength MS nutrient soln includes about 0.012 mM silicic acid. What was the Si content of your control?

This information is provided in the supplementary methods S1. The nutrient solution was acidified with HNO_3 to a pH of 6 prior to the start of the experiment and was measured at the end of the experiment and it was on average pH 7.27 ± 0.29. We did not use any 'MS nutrient solution', see the supplement and chapter 2.1 for the composition of the nutrient solution used in our experiment. In terms of the control experiment the strategy of our experiment is to perform growth experiments that compare Si uptake and isotope fractionation between species. Performing a negative control experiment (meaning plants growing in Si free growth solution) makes no sense as Si is not transferred between compartments hence there would be no isotope fractionation.

Line 114: Actually recent research would contend that these aquaporins are barely permeable to silicic acid since their pore size is less than that of silicic acid. See recent research by Guerriero et al and Exley et al. You do not have to agree with this new research but you should include it in your deliberations.

This point is not relevant in the context of the statement made here, as we are discussing the functionality of Lsi1 homologue in tomato and not in *Cannabis sativa*. We have referred to the general controversies regarding the uptake of Si in line 50ff and 56ff, this includes the work by Exley and co-worker and their concerns raised that Lsi1 homologues found in Cannabis sativa have a modelled maximum pore size of 1.77 Å (Exley et al., 2020) whereas the maximum radius of silicic acid is estimated to be 4 Å (Exley et al., 2019).

Line 117: Na4SiO4

See above.

Line 119: Na4SiO4

See above.

Line 123: Can you show how this was achieved as you cannot have a complete lid since the plant has to be accomodated. It is very difficult to make such a seal. A good positive control should be pots without plants sealed in an identical way but using, for example a plastic tube instead of the plant.

The lids were placed inside the pots on an internal rim. The plants were fixed in their ports with a 3 cm thick foam rubber disc. All unused ports in the lid were sealed with rubber stops (see Figure 1 for the experimental setup). Because of all these measures, the amount of evaporation is extremely low. Even in the unlikely case that evaporation was non-negligible our experiments were made under the same conditions, in the same containers using the same sealing measures to minimise evaporation. A potential bias is thus identical for all three plant species and all triplicates. For this reason, we did not consider it advisable to carry out control tests. Using a plastic tube would raise other methodological questions, the clarification of which is far beyond the focus of our investigations.

We have rephrased it to make this clear: *"The pots were closed with a fixed and completely sealed lid, and thus evaporation is considered very small and, in any case, identical between the plant species and triplicates."*

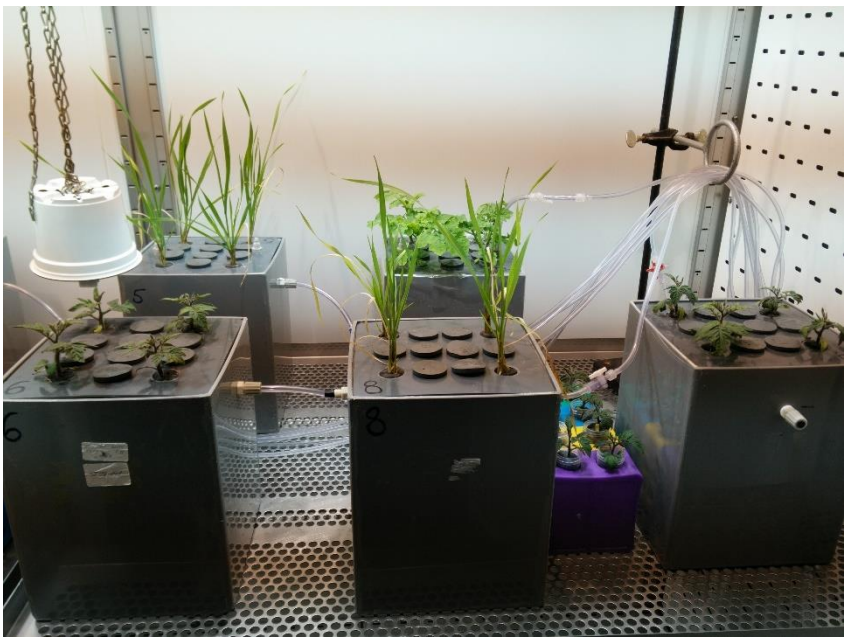

Figure 1: Overview of the arrangements in the growth chamber. The containers were closed with a lid, which was lying on an internal rim, all unused ports were sealed with rubber stoppers and the plants were fixed within a foam disk in their ports to minimise evaporation.

Line 126: I am wondering how the additional 200 mg/L sodium in the Si groups might influence both evaporation and transpiration. Since sodium was not added to the controls then you do really require some other form of control.

Our nutrient solution did not contain 200 mg/L sodium. It is initially approximately 25 µg/g (or 25 mg/L). The sodium concentration of the nutrient solution throughout the experiment is given in

Figure S1. We have not performed any control experiments, neither without sodium nor silicon. As stated above control experiments do not make sense in isotope fractionation studies.

Line 145: What about the high sodium content? Also problematic.

The employed chromatography procedure can handle those large amounts of Na. In fact, in the fusion process an additional 400 mg of NaOH is introduced to dissolve silicates. The Na concentration of the purified solution is verified to be below the detection limits, prior to the stable isotope ratio measurements.

Line 155: an exploratory study? Perhaps this should be included herein? If this preliminary work was integral to method development as seems to be the case then it also requires to be reviewed.

The exploratory study is not an independent study from the here presented results. Rather a test with a subset of the samples to determining the amount of sample needed to obtain. We have adapted the sentence to clarify this: *"50-800 mg of plant material, depending on the Si concentration determined in an exploratory subset of the samples, was weighed into Ag crucibles and combusted overnight (2h at 200 °C, 4h at 600 °C, then cooled to room temperature) in a furnace (LVT 5/11/P330, Nabertherm)."*

Line 158: Why is this added at this point?

This is the chemical reagent needed to perform the dissolution of silicates, see also our explanation in the previous response letter.

Line 161: So, it is a dry digestion? What about the nutrient solutions?

Yes, the fusion is performed dry. The nutrient solution is dried down (see chapter 2.5.1, line 149), prior to the fusion.

Line 162: I don't understand how this is done and how you would know that you have separated 60 micrograms of Si. I do not want to read these papers to know this so a brief explanation would be helpful. What happens to silicon-rich solutions when their pH is adjusted to 1.5? Are these solutions undersaturated wrt silicic acid or will they be solutions of Si4+?

From our previous response: *"The dissolution procedure of silicates, silicon and bio silica is state of the art in geosciences. Si is present in SiO_2 as Si^{4+}, counterbalanced by 2 O^{2-}. Therefore, we do not need to convert Si into a cation. The NaOH accelerates the dissolution of the oxide, and after the addition of water silicon is present as silicic acid (H_4SiO_4 and depending on the pH also in the form of $H_3SiO_4^-$ (see e.g. (Stamm et al., 2019), their Fig. 1 for an aqueous Si species in equilibrium diagram)."* There is no change in the silicon-rich solution when the pH is adjusted to 1.5, this is however needed for the procedure with the anion exchange resin. The solutions (before and after purification) are undersaturated with regard to the solubility of silicic acid to avoid polymerisation and precipitation.

The dissolution procedure of silicates, silicon and bio silica is standard in geosciences and in particular in Si isotope geochemistry. We do not think that they have to be repeated in detail in this paper that should not be burdened with analytical background that geochemists are fully aware of, and others can read in the cited literature. We have clarified some aspects of chapter 2.5.3: *"The crucibles containing the sample (nutrient solution or plant material) and NaOH were placed in a furnace at 750 °C for 15 min to perform the fusion. The fusion cake was dissolved in ultrapure water (for 24h, followed by 30 min ultrasonic bath), the solution was decanted into precleaned PP flask. The remains of the fusion cake were fully dissolved in 0.03 M HCl (for 3h), both solutions were combined and the pH was adjusted to 1.5. The Si concentration was determined by ICP-OES and approximately 60 µg Si (present in the form of silicic acid) was chromatographically separated using cation exchange resin (following a*

procedure outlined by Georg et al., 2006; Zambardi & Poitrasson, 2011; Schuessler & von Blanckenburg, 2014). The Si yield of the fusion procedure and the column chemistry was determined in a 1:10-fold dilution by ICP-OES. Si blanks of the fusion and column separation procedure were in general below 1 µg Si, equivalent to less than 1 % of the total Si processed. See Methods S3 for more details."

Line 163: When I asked you previously about whether or not you were using pH to convert Si to its cation you said no. However you cannot use a cation exchange resin to collect Si unless it is a cation. I have 40 years experience of Si chemistry, you need to explain this procedure clearly since it forms an integral part of your results.

Again, this technique is standard, well-tested, and very well-known in geochemistry. The reviewer may not be aware of this substantial body of analytic-chemical literature. Si is present as a silicic acid in our solution, thus we can purify Si using a cation exchange resin retains unwanted cations (e.g. Ca, Na, Mg etc…) but let neutral silicic acid pass through. No further changes made, see comment before.

Line 185: Where is the Si in stem tissue?

Shoots include the stem and the leaves, we have clarified this on the first occurrence of the term shoots, see also the glossary of Esau's Plant Anatomy for a definition (Evert, 2006).

Line 187: stem?

See the comment before.

Line 189: No mention is made about the health of plants across the different treatments. Did plants grow equally well in each treatment. Did any plants die or show any signs of disease. Were root masses different between treatments as this would influence how much root-associated water was being counted as transpired water. Details, including pictures if possible is needed on plant health throughout the study.

All the requested information is presented in the results chapter:

- Chapter 3.1 reports on the root and shoot dry mass, the individual results ('raw data') are provided in Table S4.

Competition for light (especially for mustard) and nutrients probably led to the different biomass of the plants within the containers, whereas the different temperature requirements of the three plant species may have been responsible for their different biomass formation (see also line 58ff).

- No plants have died during the experiment.
- Regarding the health of the plants we have stated in chapter 3.2 that mustard showed first signs of nutrient deficiency in the form of chlorosis in young and old leaves. This is (likely) caused by the full consumption of the available nutrient elements (Ca and Mg). Other than that, the plants were healthy.

As all this information was already present no changes were made.

Figure 2: Image to support the plant's health between week 4 and week 5.

Line 190: Check written English here, this sentence does not make sense to me.

Thanks for bringing this up, we have improved the section: "We define plant transpiration as the amount of water taken up by the plants via the roots. Transpiration was measured weekly by weighing the remaining growth solution with the lids and plants removed. The difference in mass from the previous week is considered to be the mass of water transpired by the plants. The gravimetrically determined transpiration does not account for the amount of water present in the plants at harvest nor any possible guttation (Joachimsmeier et al., 2012)."

Line 191: How do you account for water associated with the roots when you lift the plants from their pots?

The roots are lifted such that the roots drip their excess water into the nutrient container. Water which is adhered or associated with the roots will be counted as a transpiration loss. When the roots are immersed back into the nutrient solution the adhered / associated water will be mixed again with the nutrient solution. No changes made.

Figure 3: Exemplary tomato plants (container 3) lifted from the nutrient solution to measure the transpired water mass during the previous week. Roots were at the time of taking the picture still dripping excess water back into the container, the reading of the balance was made after the roots have stopped dripping and the balance was stable.

Line 193: Guttation is not negligible and it varies considerably between plants. For example, anyone who has grown cucumber plants will see guttation visibly as the tips of leaves. Find data on guttation for all your plants and include it in your calculations. The reference you cite is not an authoritative example.

We have grown tomato, mustard, and spring wheat, and not cucumber and can thus only comment regarding our experiments: we did not observe guttation. This is not to a surprise, since under the conditions of the climate chamber 65% humidity (day and night) and permanent air flow, any possible guttation liquid would evaporate, which means that escaping Si would remain on the shoot surface and counted towards the shoot Si content at the end of the experiment. According to Singh, *"high atmospheric humidity is an essential prerequisite for guttation fluid to appear. As stated earlier, guttation is ample and continues for a relatively longer period when the soil and atmosphere are saturated with water"* (Singh, 2016).

We have clarified that guttation was not observed, but if it were present that the Si would not have been lost but counted towards the amounts of Si present in the shoots. This can also be seen in the high retrieval rate (Si pool present at the start of the experiment vs the Si pool present at the end of the experiment) of up to 100% (see Table 3). In Chapter 4.1 we added: *"Guttation (Joachimsmeier et al., 2012; Yamaji et al., 2008) and litter fall were not observed during the experiment. Even if guttation were present no Si would be lost since under the experimental conditions the fluid would evaporate leaving amorphous silica on the shoots. Thus, silicic acid excreted by guttation is counted towards the Si amounts in the shoots."*

Line 196: Do you mean the weight of whole plants prior to sampling?

No, this is the dry weight of the biomass after harvest and drying to constant weight. No change made.

Line 199: Do you mean the sum of Si in roots, stem and shoot?

Yes, the total Si mass in the roots, shoots (which include the steam and leaves). No change made.

Line 200: By transfer you mean from stem to shoot?

Shoots include stems, see also the comment before. No change made.

Line 204: You mean the amount of silicic acid entering plants with transpired water? I think some example calculations are required here since the culture medium silicic acid content fell continuously over the growth period both due to transpired water being replaced with pure water but also sampled water being likewise replaced. I do wonder why you needed to replace water lost as this greatly complicates things. Just use a high volume of culture medium.

Yes, this is the amount of silicic acid entering the plants with the transpired water. We have extended the paragraph and provided the formula to calculate the expected concentration of Si in the plants, assuming a purely passive uptake: *"We also calculated an "expected Si uptake" defined to represent exactly the mass of Si contained in the water utilised. This value was calculated from on the amount of transpired water and the nutrient solution Si concentration determined in the week prior:*

$$Expected\ Si\ Uptake = \sum_{Week=1}^{Week=6} [Si]_{week\ i-1} \cdot m_{transpired\ water,week\ i}$$

where $[Si]_{week\ i-1}$ *is the Si concentration in the nutrient solution the week prior, and* $m_{transpired\ water,\ week\ i}$ *the mass of water transpired during past week."*

Not replenishing the water transpired by the plants would eventually expose the roots to air instead of the nutrient solution, thereby changing the *effective* root biomass. This would complicate the study even more.

Line 206: I am confused. If you use the change in silicic acid concentration of nutrient media combined with water loss due to transpiration and sampling over the culture period then you will have a figure for how much silicic acid WAS taken up over this period. Si loss from nutrient media = Si movement into plant. Si deposited in plant cannot be higher than the amount taken up. It can be lower due to silicic acid taken up not being deposited and being lost by guttation. This why you need to show your calculations for this 'expected' value. It is not an expected value it is an actual value based upon loss of Si in nutrient media.

The measured and the expected Si uptake are two independent measures. The measured Si uptake is the determined by the biomass and the Si concentration in the roots and shoots. The expected Si uptake is determined by the mass of transpired water and the Si concentration of the nutrient solution. These two measures can be equal in the case of a purely passive uptake. A rejective Si incorporation is when the measured Si uptake is smaller than the expected Si uptake by transpiration and vice versa for an active incorporation. We have clarified the section: *"The plant Si uptake characteristics can be classified based on the ratio between the measured (based on the biomass and the Si concentration measured therein) and the expected Si uptake. A ratio of greater than 1 indicates an active uptake mechanism, a ratio much smaller than 1 a rejective strategy, and a ratio of 1 indicates passive uptake."*

Si deposited in the plants is not higher than the amount lost from the nutrient solution, this information can be found in chapter 3.3 and the accompanying Table 3.

Line 208: These need to take account of all water loss being replaced by pure water?

As described in ch. 2.4 we have taken account for the water loss, both in the element and the isotopic budgets. The weekly sampling has taken place after the replenishing the nutrient solution with ultra-pure water (which contained no significant amounts of the investigated nutrients) and arrogating for a prolonged time. No changes made.

Line 214: The isotopes of Si in nutrient media are not changing over time?

Of course, the composition of Si in the nutrient solution changes over time (see Figure 2 in our manuscript) if there is isotope fractionation during uptake! This is the point of the entire experiment, and basic stable isotope chemical mass balance. In detail mass balance requires that the overall pool of Si in the experiment (Si in the nutrient solution, plants) shall be invariant. We calculate the mass balance at the start and at the end of the experiment to assure that this requirement is fulfilled. The results (in ch. 4.1) show this: *"There is no significant difference between the isotopic composition of the starting solution and the weighted average of the isotopic compositions of the different compartments at the end (see Table 4)."*

Line 223: A complicated way to collect silica from plant tissue. Just follow the digestion method of Law and Exley 2011, much simpler.

Thanks for providing information on an alternative method for the extraction for plant silica.

Line 224: revealed?

Changed.

Line 224: This is a Result of this study? Put in Results not here.

We have removed this sentence from the materials and method section.

Line 233: Where are the transpiration data for all plants including the controls? These are critical data and should be presented.

The data is shown in Figure 1, panel A. We realised that the raw data were not provided, and have added the weekly measured mass of transpiration in the supplement tables (Table S6).

Line 238: What about differences between control and treatment plants?

As remarked in the introduction to this response letter, we have performed a growth experiment in which three crop species with known differences in their Si uptake capabilities were grown under the same environmental conditions. In such a study, there are no control experiments in a *classical sense*. We have performed all experiments in triplicate, with four plants in each pot and have discussed possible systematic differences between the triplicates and within the pots and cause thereof in our manuscript (see e.g. ch. 3.1, and the individual results ('raw data') are provided in Table S4).

Line 240: There are no data for the control plants?

There were no control experiments performed, see the explanation in the introduction of this response letter. No change made.

Line 245: Larger plants had higher transpiration, not unexpected!

No change made

Line 245: What is water use efficiency? How is it calculated and why?

This is explained in ch. 2.6.1. We added a link to the chapter.

Line 248: This is where much clearer information is needed as to what exactly was measured. You have Si concentrations for each nutrient solution for each weekly time point. You can use these data to measure exactly how much Si has gone from each of them over the culture period. You also have bulk Si measurements for each plant and you can then compare Si in a plant with Si that was lost from the culture solution in which the plant was growing. Clearly the amount in the plant cannot exceed the amount lost from the nutrient solution. What do these data look like?

These results are already presented in Table 3. m_{start} is the mass of the element in the nutrient solution before the start of the experiment, m_{End} is the remaining mass of the element in the nutrient solution after the harvest of the plants. m_{Plants} is the mass of the element found in the plants. The amount of Si found in the plants has not exceeded the amount lost from the nutrient solution. The retrieval rate for Si is between 79 – 100 % and corroborate the reliability of our results. No change made.

Line 249: Do you mean based upon how much Si disappeared from culture media? Where are these data in Table 1?

Yes, the data is shown in Figure 1, panel B and in the supplement Figure S1 for the other nutrient elements. The results were however not provided in tabulated form. We have supplied these data and added to the supplement the concentration of the nutrient Ca, Fe, K, Mg, P, S, and Si and silicon isotope composition in tabulated form (see our new Supplement Table S5).

Line 250: So the question is (i) what was the Si content of tissues at the start of the experiment or in the control plants at the end of the culture period. (ii) What is the difference in Si tissue content in the Si groups at the end of the culture period and (iii) how does this 'difference' compare with how much Si was actually lost from nutrient media over the culture period. These are the critical data at this point. Where are they? Once these data are established one might ask whether there is any relationship with transpired water.

We are somewhat perplexed by these questions: these questions are answered in our manuscript and the results are presented (see Table S4 and ch. 3.3 and 4.1)

(i) The plants have been germinated on half strength nutrient media containing no Si, thus the amount Si at the start of the experiment is equal to that of the seeds.
There were no control experiments performed, see also the introduction to this response.

(ii) The Si content of the shoots (which includes the stems!) and the roots are presented in Table S4. The only source of Si which could be present at the start of the experiment originates from the seed. In an approximation, the difference between start and the end of the experiments is equal to the amounts at the end, neglecting the initial Si amounts in the seeds.

(iii) This is the retrieval rate, see results in 3.3 and discussion in 4.1.

Line 251: Where are the data for stems? They should be included. They are not included anywhere in figures or tables.

See our comment before. The definition of shoots include stems and leaves.

Response to Prof. Hodson

Line 46 regulates
Line 212 revealed
Line 219 3 Results should be a subheading and on the next line
Line 237 wheat as an Si
Line 416 researchers
Line 438 experiments

We thank Prof. Hodson and made the suggested corrections.

Additional changes made to the manuscript

We added in ch. 3.2 a short discussion of the expected Si uptake and the ratio of measured and expected Si uptake results: "*The expected Si uptake (see ch. 2.6.1 and Eq. 3 for definition) traces the passive uptake of Si contained in the water utilised by the plants. The dynamics throughout the experiment is shown in Figure 1c (closed symbols) together with the ratio of measured and expected Si uptake (open symbols) at the end of the experiment. The measured and expected Si uptake ratios for all three species deviate significantly from 1 (see Table 2). The means of the measured and expected Si uptake for mustard ($57.2^{a} \pm 1.3$ vs $457.9^{b} \pm 16.4$), wheat ($337.0^{b} \pm 67.9$ vs $177.3^{a} \pm 40.7$) and tomato ($15.5^{a} \pm 4.9$ vs $141.1^{b} \pm 27.0$) are significantly different (based on t-test at 5% significance level, denoted). This indicates that Si uptake and/or transport in the three plant species investigated under the given environmental conditions differ from unspecific passive uptake and/or unspecific passive transport within the plants.*"

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Silicon ~~stable~~uptake and isotope fractionation ~~and uptake~~ dynamics ~~of~~by crop species

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Abstract. That silicon is an important element in global biogeochemical cycles is widely recognized. Recently, its relevance for global crop production gained increasing attention too. Silicon is beneficial for plant growth and is taken up in considerable amounts by crops like rice or wheat. However, plants differ in the way they take up silicic acid from soil solution. Correspondingly species encompass a broad spectrum, from varieties that reject silicic acid to species that actively incorporate it. Yet these classifications are subject to intense debate. To forge a new perspective on the processes involved, we investigated the dependence of silicon stable isotope fractionation on silicon uptake strategy, transpiration, water use, and Si transfer efficiency. Crop plants with rejective (tomato, Solanum lycopersicum and mustard, Sinapis alba) and active (spring wheat, Triticum aestivum) uptake were hydroponically grown for 6 weeks. Using inductively coupled plasma mass spectrometry, the silicon amounts and the isotopic composition of the nutrient solution, the roots, and the shoots were determined. Wheat revealed the highest Si transfer efficiency from root to shoot followed by tomato and mustard. All three species preferentially incorporated light ^{28}Si, with a fractionation factor $1000 \cdot \ln(\alpha)$ of -0.33 ‰ (tomato), -0.55 ‰ (mustard) and -0.43 ‰ (wheat) between growth medium and bulk plant. Even though the rates of active and passive Si root uptake differ, the physico-chemical processes governing Si uptake and stable isotope fractionation do not. We assume that isotope fractionation during root uptake is governed by a diffusion process. In contrast, the transport of silicic acid from the roots to the shoots depends on the amount of silicon previously precipitated in the roots and the presence of active transporters in the roots. Plants with a significant biogenic silica precipitation in roots (mustard, and wheat), preferentially transport silicon enriched in ^{30}Si into their shoots, whereas the transport in tomato is dominated by a diffusion process in the absence of precipitation of biogenic silica and hence preferentially transports light silicon ^{28}Si into the shoots.

1 Introduction

Silicon (Si) is the second-most abundant element in the Earth's crust and occurs in a wide variety of silicate minerals. Weathering of these minerals mobilises Si and represents the starting point of Si biogeochemical cycling in terrestrial

ecosystems – an often complex web of Si transfers and transformations. One crucial but poorly understood aspect of terrestrial Si biogeochemistry is biological cycling~~. Si has well documented biological roles, and Si may be recycled multiple times through higher plants before being lost from the system~~ (Carey and Fulweiler, 2012; Derry et al., 2005; Sommer et al., 2006, 2013). Si has well documented biological roles, and Si may be recycled multiple times through higher plants before being lost from an ecosystem. Developing and validating geochemical tools to trace plant Si uptake will improve our ability to address questions on weathering, ecosystem nutrition strategies, and geo-pedo-biosphere interactions.

Despite having a disputed biochemical role, Si is considered beneficial for plant growth, including crops: Si increases abiotic stress mediation (aluminium and heavy metal ~~sequestration~~toxicity, salinity), biotic stress resistance (defence against herbivores), and improves the plants' structural stability (Coskun et al., 2019b; Epstein, 1994, 1999, 2001; Exley and Guerriero, 2019; Ma, 2004; Richmond and Sussman, 2003). Higher plant species form a continuous spectrum in the extent to which Si is incorporated. According to the amount of Si taken up they are grouped into three categories: active, passive and rejective (Marschner and Marschner, 2012). Crop plants with an active incorporation mechanism (e.g. rice, and wheat) take up Si with a higher silicon / water ratio than that in the soil solution, thus enriching Si relative to transpired water. Passive uptake plants (most dicotyledons) neither enrich nor deplete the Si relative to the transpired water. Rejective Si uptake plants (e.g. tomato, mustard, and soybean) actively discriminate against Si during uptake (Epstein, 1999; Hodson et al., 2005; Ma et al., 2001; Takahashi et al., 1990). However, the ~~process that is meant by the~~ term "active uptake" is still widely debated. In particular whether the classification as active or passive is justified, as well as the evidence for involvement of an active, metabolically controlled process in some plant species is subject to an intense discussion (Coskun et al., 2019a; Exley, 2015; Exley et al., 2020). Genome sequencing has disclosed the transporter and mechanism that ~~regulate~~regulates Si uptake (Ma & Yamaji, 2006; Ma *et al.*, 2006, 2007; Mitani *et al.*, 2009, see also Ma & Yamaji, 2015; YAN *et al.*, 2018 for an overview). In rice, a cooperative system of Si-permeable channels at the root epidermis (called Lsi1, Low Silicon 1 transporter, a thermodynamically passive transporter from the family of aquaporin-like proteins) incorporates Si, whereas a metabolically active efflux transporter (Lsi2, a putative anion-channel transporter) loads Si into the xylem (Broadley et al., 2012). These observations are predictive in nature, and only recently have empirical studies demonstrated the simultaneous operation of passive and active uptake mechanisms (Sun et al., 2016b; YAN et al., 2018). Yet other researchers have suggested that the low permeability of Lsi1 does not permit the transfer of silicic acid at all (Exley et al., 2020). Thus h~~H~~ow these different Si transporter and passive Si pathways and the resulting magnitude of Si uptake affect the mobility of silicic acid within plants ~~remains~~ is still not fully ~~however un~~known.

Conventional approaches employed in the study of uptake, translocation, and accumulation of Si in living organisms include either radioactive tracers (e.g. ^{31}Si, ^{32}Si) or homologue elements (e.g. Germanium and the radionuclide ^{68}Ge). Both techniques impose limitations on growth experiments, either due to safety concerns arising from radioactivity or due to physiological differences between the homologue element Ge and Si ~~(Takahashi et al., 1990)~~(Exley et al., 2020; Takahashi et al., 1990). As

a homologue element, Ge is taken up in the same form as Si, $Ge(OH)_4^0$. In the absence of Si, plants seem to incorporate $Ge(OH)_4$ at a higher rate than in its presence (Takahashi et al., 1990). Several studies have shown that plants fractionate Si relative to Ge, resulting in a lowered Ge/Si ratio in the phytoliths formed (Blecker et al., 2007; Cornelis et al., 2010; Derry et al., 2005; Opfergelt et al., 2010). There is also evidence that Ge interacts differently with organic molecules than Si (Pokrovski and Schott, 1998; Sparks et al., 2011; Wiche et al., 2018). In some cases, Ge also appears to be toxic to organisms (Marron et al., 2016). Thus, Ge or Ge/Si ratios are problematic tracers of plant Si uptake and translocation processes.

Si stable isotope ratios provide a powerful alternative approach. When combined with measurements of plant physiological properties, they allow exploration of Si cycling in organisms. Each physico-chemical transport process (e.g. absorption, uptake, diffusion, and precipitation) may be accompanied by a shift in an element's stable isotope ratios - so-called mass-dependent isotope fractionation (Poitrasson, 2017). This isotope fractionation either entails an equilibrium isotope effect, where the isotopes are partitioned between compounds according to bond strength, or a kinetic isotope effect, where the isotope fractionation depends on the relative rate constants of reactions involving the different isotopologues. For stable Si isotope fractionation in aqueous media, both equilibrium effects (He et al., 2016; Stamm et al., 2019) and kinetic effects (Geilert et al., 2014; Oelze et al., 2015; Poitrasson, 2017; Roerdink et al., 2015) have been observed. Previous studies on stable Si fractionation in higher plants focused on accumulator plants, namely rice (Ding et al., 2008a; Köster et al., 2009; Sun et al., 2008, 2016b, 2016a), banana (Delvigne et al., 2009; Opfergelt et al., 2006, 2010), bamboo (Ding et al., 2008b) and cucumber (Sun et al., 2016b) and most of these studies show the preferential incorporation of lighter Si isotopes. Importantly, in most of these studies, Si concentrations in the growth media were held constant by frequently replenishing the nutrient solution. This imparts the disadvantage that the dynamics (temporal evolution) of the Si isotope fractionation during uptake cannot be derived from the isotope shift recorded by the nutrient solution over the course of the experiment, nor does the provision of constant Si amounts allow additional constraints to be placed on Si uptake mechanisms employed by plants.

In this study we elucidated the mechanisms of Si uptake using crop species that differ significantly in their Si uptake capacity ~~and~~, the presence of specific Si transporters and their transpiration rate. To do so, we combined the measurement of physiological plant performance ratios with observations of the shifts in the Si isotope ratios due to mass dependent isotope fractionation. Three crops - tomato, mustard, and wheat - were grown in a hydroponic system under the same environmental conditions, with nutrients being supplied only once, during the onset of the experiment, allowing direct quantification of the dynamics of isotopic fractionation from the temporal evolution of the nutrient solutions' isotopic composition. With the combination of the physiological plant performance ratios and isotope chemical parameters we developed new insights to the mechanisms underlying the different Si uptake and translocation strategies.

2 Materials and methods

2.1 Nutrient solution

The nutrient solution was prepared from technical grade salts following the recipe after Schilling *et al.*, 1982; and Mühling & Sattelmacher, 1995. Silicon was added in the form of ~~$NaSiO_4$~~sodium silicate trihydrate ($Na_2O_7Si_3 \cdot 3H_2O$) to an initial Si starting concentration of 49.5 $\mu g \cdot g^{-1}$ (1.76 mM). Detail composition can be found in supplementary methods S1. Ultrapure water (resistivity 18.2 MΩ·cm) was used to prepare the nutrient solutions and to weekly restock water transpired by the plants.

2.2 Plant species

Three species were chosen based on their silicon uptake characteristics, the ability to grow in hydroponic environments, and previous knowledge about their Si transporter. Tomato (*Solanum lycopersicum* cultivar MICRO TOM) and mustard (*Sinapis alba*) are both rejective of Si, while spring wheat (*Triticum aestivum* cultivar SW KADRILJ) actively takes up Si (Hodson et al., 2005; Takahashi et al., 1990). The two Si excluder species differ in the presence of the NOD26-like-instrinsic proteins (orthologues of Lsi1, homologous gene sequence of low-silicon rice 1) which are associated with the transport of Si. In the family of Brassicaceae (mustard) these are absent (Sonah et al., 2017), whereas for tomato the Lsi1 homologue seems to be present but inactive (Deshmukh et al., 2016, 2015). Conversely, the alleged active Si efflux transporter (Lsi2-like) are present in the family of Brassicacea (Sonah et al., 2017), but not in tomato (Sun et al., 2020). An ongoing controversy surrounds the significance of the Lsi1 homologue in tomato. Whereas Deshmukh et al., 2015 used Si uptake studies to infer the transporter to be non-functional, Sun et al., 2020 observed the contrary using Ge as homologue element. Sun and co-workers concluded that the low Si uptake is caused by the lack of a functional Si efflux transporter Lsi2 at the root endodermis.

2.3 Plant germination and growth conditions

Plant seeds were germinated in Petri dishes with half-strength nutrient solution used for the later growth experiment that contained no added ~~$NaSiO_4$.~~sodium silicate trihydrate. After cotyledons formed, seedlings were transferred into a foam ~~block~~disk and grown for a further two weeks in the same half-strength nutrient solution. Four plants each were then transferred into one experimental container that was filled with fresh nutrient solution including ~~$NaSiO_4$~~sodium silicate trihydrate, and each species was replicated in three containers. Plants were germinated and grown in a growth chamber under controlled climate conditions. Each week the pots were weighed without the lid and the plants, and the mass of transpired water was replenished with ultrapure water (18.2 MΩ·cm). The weight difference to the previous week is considered to quantify the mass of water transpired by the plants. The pots were closed with a fixed and completely sealed lid, and ~~we~~ thus ~~neglect~~ evaporation is considered to be very small and, in any case, identical between the plant species and triplicates. The temperature in the growth chamber during the day and night was maintained at 18 °C for 14 h and at 15 °C for 10 h, respectively, and the daylight intensity at the top of the container was adjusted to 350 $\mu E \cdot m^{-2} \cdot s^{-1}$) at the start of the experiment. The relative humidity was

maintained at approximately 65 %. Details of the plant germination and growth conditions are provided in supplementary methods S2.

2.4 Sampling

The nutrient solutions were sampled at the start of the experiment and then every seven days until harvesting. For sampling, 40 mL were taken after replenishing water loss via transpiration loss and mixing of the solution. All sampled nutrient solutions were stored until analysis in precleaned PP vials in darkness at 4 °C. The 280 mL sample taken over the course of 6 weeks corresponds to 3.5 % of the initial nutrient solution. After 6 weeks the plants were harvested, and shoots (stem and leaves) were separated from the roots. The roots were immersed multiple times in ultrapure water to remove potential extracellular Si deposits and attached nutrients. The plant parts were dried at 104 °C to constant weight.

2.5 Determination of concentrations and isotope ratios

The chemical compositions of the growth solution and the digested plant samples (see section 2.5.2 for the digestion procedure) were measured using an axial inductively coupled plasma optical emission spectroscopy (ICP-OES, Varian 720-ES, instrument settings are reported in Table S1). Samples and standard were analysed following a procedure by Schuessler *et al.*, 2016. Briefly, the samples and standards were doped with an excess of $CsNO_3$ (1 mg g^{-1}) to reduce matrix effects in the ICP source that are likely to be caused from the high nitrogen content of the samples and quantified applying an external calibration. The relative analytical uncertainties are estimated to be below 10% and agreed with the nominal concentration of the starting solutions.

2.5.1 Nutrient solution purification

The high nutrient content and the organic acids in the nutrient solution potentially impair the chromatographic purification of Si. Thus the nutrient solution was digested following the "Sample preparation of water samples" by Steinhoefel et al., 2017 without employing an additional step for the removal of dissolved organic carbon. Briefly, based on the concentration measured, an aliquot of each nutrient solution containing approximately 1000 µg Si was dried down in silver crucibles on a hotplate at 80-95 °C. The crucibles were then filled with 400 mg NaOH (Merck pellets, p.a. grade, previously checked for low Si blank levels) and ultrapure water to the initial fill level and dried down. This step ensured that Si attached to the crucible walls was also immersed in NaOH. A blank containing ultrapure water and NaOH was processed in parallel to the samples to check for contamination of Si and other elements introduced in the procedure.

2.5.2 Plant samples digestion

The oven-dried samples were homogenised by milling the plant parts in a tungsten carbide planetary ball mill (Pulversiette 7, Fritsch). 50-800 mg of plant material, depending on the Si concentration determined in an exploratory ~~study~~subset of the samples, was weighed into Ag crucibles and combusted overnight (2h at 200 °C, 4h at 600 °C, then cooled to room

temperature) in a furnace (LVT 5/11/P330, Nabertherm). A blank (empty crucible) was processed together with the samples. After cooling 400 mg NaOH (TraceSELECT, Sigma-Aldrich, checked for low Si blank levels) was added.

2.5.3 Fusion and chromatography

The crucibles containing the sample (nutrient solution or plant material) and NaOH were placed in a furnace at 750 °C for 15 min~~. The fusion cake was dissolved in ultrapure water and 0.03 M HCl, and the pH was adjusted to 1.5. Approximately 60 µg Si was chromatographically separated using cation exchange resin (Georg *et al.*, 2006; Zambardi & Poitrasson, 2011; Schuessler & von Blanckenburg, 2014). The purity and Si yield of the fusion procedure and the column chemistry was determined by ICP-OES. Si blanks of the fusion and column separation procedure were in general below 1 µg Si, equivalent to less than 1 % of the total Si processed. See Methods S3 for a detailed account of the procedural steps.~~ to perform the fusion. The fusion cake was dissolved in ultrapure water (for 24h, followed by 30 min ultrasonic bath), the solution was decanted into precleaned PP flask. The remains of the fusion cake were fully dissolved in 0.03 M HCl (for 3h), and both solutions were combined and the pH was adjusted to 1.5. The Si concentration was determined by ICP-OES and approximately 60 µg Si (present in the form of silicic acid) was chromatographically separated using cation exchange resin (following a procedure outlined by Georg *et al.*, 2006; Zambardi & Poitrasson, 2011; Schuessler & von Blanckenburg, 2014). The Si yield of the fusion procedure and the column chemistry was determined in a 1:10-fold dilution by ICP-OES. Si blanks of the fusion and column separation procedure were in general below 1 µg Si, equivalent to less than 1 % of the total Si processed. See Methods S3 for more details.

2.5.4 Silicon isotope ratio measurements

The purified solutions were acidified to 0.1 M HCl and diluted to a concentration of 0.6 µg·g^{-1}. Sample and standard were both doped with 0.6 µg·g^{-1} Mg and the $^{25}Mg/^{24}Mg$ ratio used as a monitor of mass bias drift and to ensure stable measurement conditions during the analysis (Oelze et al., 2016). The solutions were introduced using an ESI ApexHF desolvator and a PFA nebuliser (measured uptake 140 µL min^{-1}) into the MC-ICP-MS (Neptune, equipped with the Neptune Plus Jet Interface, Thermo Fisher Scientific; instrument settings are given in Table S1). Measurements were made in dynamic mode (magnet jump) alternating between Si and Mg isotopes, each for 30 cycles with 4 s integration time. ERM-CD281 and BHVO-2 were analysed together with the nutrient and plant samples to ensure complete fusion, dissolution, and chromatographic separation. ERM-CD281 resulted in $\delta^{30}Si$ = -0.34 ± 0.20 ‰, 2s, n=13 and BHVO-2 in $\delta^{30}Si$ = -0.29 ± 0.09 ‰, 2s, n=40, in line with literature values (Jochum et al., 2005 for BHVO-2 and Delvigne et al., 2019 for ERM-CD281). The results of reference materials are reported in the supplementary information in Table S2, and the results of growth solutions and plants in Table S3 and Table S4. All $\delta^{29/28}Si$ and $\delta^{30/28}Si$ are reported in delta notation relative to NBS28 (NIST SRM8546) unless stated otherwise (Coplen et al., 2002; Poitrasson, 2017). An isotopic difference between two compartments is expressed as $\Delta^{30}Si$, calculated following Eq. (1):

$$\Delta^{30}Si_{a-b} = \delta^{30}Si_a - \delta^{30}Si_b \quad (1)$$

where $\delta^{30}Si_a$ is the Si isotopic composition of the compartment a and $\delta^{30}Si_b$ the composition of compartment b. The silicon isotopic composition of a bulk plant is calculated from the mass weighted Si isotopic composition of separate plant parts and expressed as $\delta^{30}Si_{plant}$:

$$\delta^{30}Si_{plant} = \frac{\delta^{30}Si_{root} \cdot M_{root} + \delta^{30}Si_{shoot} \cdot M_{shoot}}{M_{root} + M_{shoot}} \quad (2)$$

where the subscripts plant, root and shoot refer to the bulk plant, and roots and shoots, respectively, and M is the mass of silicon incorporated into the roots or shoots of the plant.

2.6 Plant performance ratios, elemental and isotopic budgets

2.6.1 Plant performance ratios

We define the plant transpiration as the amount of water taken up by the plants ~~followed by transpiration. The transpiration is~~via the roots. Transpiration was measured weekly by weighing the ~~pots without~~remaining growth solution with the lids and plants removed. The difference in mass ~~to~~from the previous week is considered to be the mass of water transpired by the plants. The gravimetrically determined transpiration does not account for the amount of water present in the plants at harvest ~~and the negligible amount of~~nor any possible guttation (Joachimsmeier et al., 2012). ~~To compare the plant species with respect to their transpiration, Si uptake, and Si transfer~~At the end of the experiment, the following plant performance ratios were calculated ~~at the end of the experiments:~~:

1. Water use efficiency: total dried phytomass (g) divided by the amount of transpired water (L), calculated separately for each pot.
2. Si uptake efficiency: total Si mass (mg) in plants divided by the amount of transpired water (L), calculated separately for each pot.
3. Si transfer efficiency: Si mass (mg) in plant shoots divided by the amount of transpired water (L), calculated separately for each pot.

We also calculated an "expected Si uptake" defined to represent exactly the mass of Si contained in the water utilised. This value was calculated from on the amount of transpired water and the nutrient solution Si concentration determined in the week prior~~.~~:

$$Expected\ Si\ Uptake = \sum_{Week=1}^{Week=6} [Si]_{week\ i-1} \cdot m_{transpired\ water,week\ i} \quad (3)$$

where $[Si]_{week\ i-1}$ is the Si concentration in the nutrient solution the week prior, and $m_{transpired\ water,\ week\ i}$ the mass of water transpired during past week. The plant Si uptake characteristics can be classified based on the ratio between the measured (based on the biomass and the Si concentration measured therein) and the expected Si uptake. A ratio of greater than 1 indicates an active uptake mechanism, a ratio much smaller than 1 a rejective strategy, and a ratio of 1 indicates passive uptake.

2.6.2 Element budgets

The digested plant samples and nutrient solutions were analysed prior to the column purification by ICP-OES, and the concentrations of major elements (Ca, Fe, K, Mg, P, S and Si) and the retrieval was determined using Eq. (~~3~~4):

$$Retrieval^{X} = \frac{M^{X}_{Solution,end} + M^{X}_{Plants}}{M^{X}_{Solution,start}} \; in \; [\%] \quad (~~3~~4)$$

where $M_{solution,\ end}$ is the mass of the element X in the solution at the end of the experiments, M_{Plants} is the mass of the element X in the plants, and $M_{Solution,\ start}$ the mass of the element X in the solution at the beginning of the experiment.

2.6.2 Silicon isotope budget

A simple test of whether incomplete recovery of Si or analytical artefacts in the Si isotope composition measurements are affecting the results is offered by an isotope budget. The concept is that the summed Si isotope composition of the remaining growth solution at the end of the experiment and the Si taken up by plants should be identical to the Si isotope composition of the initial growth solution. The Si total isotope composition at harvest is estimated using Eq. (~~4~~5):

$$\delta_{Total} = \frac{M^{Si}_{solution}\delta^{30}Si_{solution} \cdot M^{Si}_{plants}\delta^{30}Si_{plants}}{M^{Si}_{solution} + M^{Si}_{plants}} \quad (~~4~~5)$$

where $M^{Si}_{solution}$ and M^{Si}_{plants} are the Si amounts in the remaining nutrient solution and the plant parts at harvest, respectively, and $\delta^{30}Si_{solution}$ and $\delta^{30}Si_{plants}$ the Si isotope composition of the remaining nutrient solution and plants parts at the end of the experiment, respectively.

2.7 SEM-EDX analysis of mustard root phytoliths

~~Mustard roots reviled remarkably high Si concentration and amounts in comparison to the other two crop species.~~ To explore the form of silica in mustard roots, phytoliths were extracted and visualised using SEM-EDX. One gram of dried mustard roots was taken for analysis. Removal of organic matter was conducted by igniting the samples in a muffle furnace at 500°C for 5h. The residue was subjected to additional oxidation using 30% H_2O_2 for 0.5h. Ca oxalates were dissolved by 80°C in HCl (10Vol.%) for 10 min. The residue was washed with water, and dried at 105°C. SEM-EDX analysis was performed with a ZEISS EVO MA10 (HV, LV, LaB6 cathode) equipped with a Bruker QUANTAX EDS system including a liquid nitrogen free XFlash R 5010 Detector (energy resolution of 123 eV for MNKa at 100,000cps). The SEM operated at 20keV, with an average working distance of 10.5 mm. Software: Esprit 2.1.1., incl Qmap.

3 Results

3.1 Plant dry mass and transpiration

Substantial differences are apparent in the growth rate between and within all three plant species. During the six-week period mustard formed the greatest amount of dry biomass, with an average of 7 g per plant (range: 0.7 - 16.6 g). Spring wheat produced on average 4 g (range: 1.9 - 5.6 g), and tomato produced the lowest amount of biomass per plant with an average of 3 g (range: 0.2 – 8.7 g, see Table 1 and Table S4 for the individual results). No dependence of replicated growth experiments on pot placement or proximity to the venting system was apparent. The amount of water transpired by the plants during the growth period is correlated with the biomass formed ($r_{Spearman\ Rank}$ = 0.95, p-value <0.001). In contrast, no differences between plant species were observed in terms of the shoot-root ratios (5.4 – 6.5 $g \cdot g^{-1}$, Table 2).

3.2 Dynamics of water, Si and other nutritive elements uptake

The three plant species revealed quite different transpiration dynamics during the 6 weeks of plant growth. After a lag phase of two weeks, differences in transpiration between mustard and the other two species became apparent. Figure 1a shows the cumulative transpiration for the three replicate growth experiments and species. (see also Table S6). Mustard showed the highest, wheat intermediate and tomatoes the lowest cumulative transpiration. The water use efficiency (see ch. 2.6.1) of tomato was significantly higher (3.8 $g \cdot L^{-1}$) than that of the other two plant species (2.4 - 2.6 $g \cdot L^{-1}$, Table 2).

Based on the temporal evolution of Si concentrations in the nutrient solutions (Figure 1b) spring wheat exhibited the highest total Si uptake, mustard an intermediate amount, and tomato the lowest total Si uptake and the Si contents of bulk plants reflect this sequence (Table 1): spring wheat as an Si accumulator took up the most Si (448 mg), followed by mustard (150 mg). Tomato took up the least amount (95 mg). Considering only roots, the highest Si concentrations and Si amounts were found in mustard, while spring wheat and tomato were significantly lower. In contrast, considering only plant shoots, the highest Si mass were found in wheat while Si concentrations in mustard and tomato were similar, but more than an order of magnitude lower (Table 1). Spring wheat also showed a much higher Si uptake efficiency than the other two plant species, which resemble each other (Table 2 and Figure 1). The same trend holds for the Si mass ratio between roots and shoots (Table 2). Moreover, wheat shows a much higher efficiency of Si transport into the shoot per mass of transpired water than the other two plant species. In contrast to the Si uptake efficiency, the Si mass ratio between root and shoot for mustard was lower than for tomato (Table 2). For the calculation of Si uptake rates, we assume there is no back diffusion or efflux of Si out of the plant roots. Such a process has not been reported in the literature and would be driven against the concentration difference between the root and the nutrient solution Si concentration and against the water flow direction (Raven, 2001).

The expected Si uptake (see ch. 2.6.1 and Eq. 3 for definition) traces the passive uptake of Si contained in the water utilised by the plants. The dynamics throughout the experiment is shown in Figure 1c (closed symbols) together with the ratio of

measured and expected Si uptake (open symbols) at the end of the experiment. The measured and expected Si uptake ratios for all three species deviate significantly from 1 (see Table 2). The means of the measured and expected Si uptake for mustard ($57.2^{a} \pm 1.3$ mg vs $457.9^{b} \pm 16.4$ mg), wheat ($337.0^{b} \pm 67.9$ mg vs $177.3^{a} \pm 40.7$ mg) and tomato ($15.5^{a} \pm 4.9$ mg vs $141.1^{b} \pm 27.0$ mg) are significantly different (denoted $^{a/b}$, based on t-test at 5% significance level,). This indicates that Si uptake ~~and/~~or transport in the three plant species investigated under the given environmental conditions differs from unspecific passive uptake ~~and/~~or unspecific passive transport within the plants.

After 6 weeks of growth, some nutrients were fully consumed, and the first mustard plants showed signs of deficiency in the form of chlorosis in young and old leaves. Mustard, forming the largest biomass, had also the largest demand for Ca (mean ~644 mg per container), Mg (~140 mg), P (~205 mg) and S (~209 mg). Fig. S1 in the supplement shows the temporal evolution of the other nutrient concentrations for the three plant species.

3.3 Element and Si isotope budgets

The biomass amounts, concentrations, and isotope compositions used to calculate element and Si isotope budgets are reported in the supporting information Table S4. The element retrievals are shown in Table 3. All three plant species showed less than complete retrieval, with variable deficits between elements. For Si the retrieval amounted to between 83% (mustard) and 90% (wheat). For the other nutrients (Ca, Fe, K, Mg, P and S, see Table 3) the retrievals were between 70% and 110%. Sulphur in mustard was an exception, with a retrieval of only 50%, which we attribute to the loss of volatile S species during drying and charring, leading to the low retrieval (Blanck et al., 1938). The results for the Si isotope budget are shown in Table 4. Within uncertainty, there is no significant difference between the isotopic composition of the starting solution and the weighted average isotopic composition of the different compartments at the end of the experiment. Thus, we conclude that all significant pathways that fractionate Si isotopes are accounted for.

3.4 Dynamics of isotope fractionation between the nutrient solution and plants

The average initial $\delta^{30}Si$ composition of the nutrient solution is -0.21 ± 0.07 ‰ (2 s, relative to NBS28; individual results are reported in Table S3). The temporal evolution of the nutrient solution and the individual Si isotopic composition of the roots, shoots and the entire plants are shown in Figure 2 (reported as $\Delta^{30}Si$ relative to the nutrient solution). All three plant species preferentially incorporated the lighter silicon isotope (^{28}Si), leaving the nutrient solution enriched in heavier silicon (^{30}Si). After an initial lag phase for all three species, in which the nutrient solutions' Si isotope composition does not vary, its isotopic composition becomes increasingly enriched in ^{30}Si. Tomato and mustard, as rejective Si taxa, took up only about 10% of the Si predicted by water transpiration rates over the course of the experiment (Fig. 1; Table 2), such that the enrichment of the nutrient solution in ^{30}Si was relatively small ($^{Tomato}\Delta^{30}Si_{Solution:End-Start}$=+0.13 ‰, $^{Mustard}\Delta^{30}Si_{Solution:End-Start}$=+0.19 ‰, calculated using Eq. (1)). As an Si accumulator, wheat incorporated almost all available Si within six weeks. The remaining Si is strongly

enriched in ^{30}Si ($^{Wheat}\Delta^{30}Si_{Solution:End-Start}$=+0.83 ‰). In week six one growth solution was so strongly depleted in Si that Si isotope ratios could not be determined.

Tomato plants incorporate light Si, where the bulk plant Si isotope composition, expressed as $^{Tomato}\Delta^{30}Si_{plants}$ averaged -0.27 ±0.06 ‰ ($^{Species}\Delta^{30}Si_{parts}$ are relative to the nutrient solution at the beginning, calculated using Eq. (2), and uncertainties are 95% CI). The Si present in the roots is isotopically indistinguishable from the nutrient solution ($^{Tomato}\Delta^{30}Si_{roots}$ = 0.01 ± 0.16 ‰), whereas the tomato shoots contain lighter Si ($^{Tomato}\Delta^{30}Si_{shoots}$ = -0.36 ±0.12 ‰). In contrast, mustard roots are lighter in their Si isotope composition ($^{Mustard}\Delta^{30}Si_{roots}$ = -0.77 ± 0.15 ‰) than the above-ground parts ($^{Mustard}\Delta^{30}Si_{shoots}$ = -0.05 ± 0.11 ‰). Nevertheless, mustard plants incorporated overall light Si ($^{Mustard}\Delta^{30}Si_{plants}$ = -0.45 ± 0.09 ‰). Since wheat consumed almost all available Si no significant fractionation between the plant and solution was observable ($^{Wheat}\Delta^{30}Si_{plants}$ = -0.07 ± 0.26 ‰). Most of the Si was deposited in the shoots, with an isotopic composition close to the composition of the starting solution ($^{Wheat}\Delta^{30}Si_{shoots}$ = -0.06 ± 0.26 ‰). The roots, however, preferentially stored light Si ($^{Wheat}\Delta^{30}Si_{roots}$ = -1.04 ± 0.34 ‰), similar to the mustard roots.

Our experimental setup allows us to determine the Si isotope fractionation factors into bulk plants directly from the temporal evolution of the Si isotope composition of the nutrient solution. This approach differs from previous studies of Si isotope fractionation by plants, in which the Si pool in the nutrient solution was frequently replenished (Ding et al., 2008a; Sun et al., 2008, 2016b). Evaluating the temporal evolution of wheat nutrient solution (Figure 3) and assuming no back-diffusion, a Rayleigh like fractionation can be fitted using Eq. (~~5~~6) (Mariotti et al., 1981):

$$\frac{R}{R_0} = f_{solution}^{\alpha-1} \quad (~~5~~6)$$

where $f_{solution}$ is the fraction of Si in the remaining solution, R_0 the initial $^{30}Si/^{28}Si$ isotope ratio, R the $^{30}Si/^{28}Si$ isotope ratio of the product, and α the fractionation factor. A best fit to the data, minimising the root-mean-square-deviation, results in $\alpha_{Plant-solution}$ for tomato of 0.99970 (1000·ln(α) = -0.33 ‰), for mustard an $\alpha_{Plant-solution}$ of 0.99945 (1000·ln(α) = -0.55 ‰), and for wheat an $\alpha_{Plant-solution}$ of 0.99957 (1000·ln(α) = -0.43 ‰), respectively (Figure 3). We use a Monte Carlo approach to estimate uncertainty on $\alpha_{Plant-solution}$, by calculating $\alpha_{Plant-solution}$ on 500 permutations of the dataset in which values for $\delta^{30}Si$ and Si concentration were randomly drawn from a normal distribution with means and standard deviations provided by the measurement (Table 5). Within uncertainty, there is no significant difference in the bulk fractionation factor between active and rejective uptake species. The best fit through all results, across the three plant species from this study, results in a fractionation factor 1000·ln(α) of -0.41 ± 0.09 ‰ (1 s) at an initial Si concentration of 49.5 $\mu g \cdot g^{-1}$ (ca. 1.76 mM).

If we assume the uptake of Si to be governed by diffusion through cell membranes and Si permeable transporters (Ma et al., 2006, 2007; Ma and Yamaji, 2015; Mitani et al., 2009; Zangi and Filella, 2012) and the diffusion of Si is non-quantitative, the lighter isotopes will be enriched in the target compartment (Sun et al., 2008; Weiss et al., 2004). To a first approximation, the difference between the diffusion coefficient of isotopologues $^{28}Si(OH)_4$ and $^{30}Si(OH)_4$ sets the theoretical upper limit of

observable isotopic fractionation in a system dominated by diffusion. The diffusion coefficient ratio approximated by Eq. (~~6~~7) corresponds to the fractionation factor in an idealised system consisting of pure water and silicic acid only (Mills and Harris, 1976; Richter et al., 2006).

$$\frac{D_{^{28}Si(OH)_4}}{D_{^{30}Si(OH)_4}} = \sqrt{\frac{\left(\frac{m_{^{30}Si(OH)_4} \times m_{H_2O}}{m_{^{30}Si(OH)_4} + m_{H_2O}}\right)}{\left(\frac{m_{^{28}Si(OH)_4} \times m_{H_2O}}{m_{^{28}Si(OH)_4} + m_{H_2O}}\right)}} \quad (~~6~~7)$$

where D is the diffusion coefficient of a given Si molecule, and $m_{H_2O}, m_{^{28}Si(OH)_4}, m_{^{30}Si(OH)_4}$ are the molecular masses of the solvent (assuming pure water), $^{28}Si(OH)_4$ and $^{30}Si(OH)_4$, respectively. For $^{28}Si(OH)_4$ and $^{30}Si(OH)_4$ in pure water this results in a ratio of 0.99839 (1000·ln(α) = -1.61 ‰). The observed α_{Plant} is about four times smaller with 1000·ln(α) of -0.33 to -0.55‰. The theoretical diffusion coefficient exceeding the measured coefficient has been observed in other systems (e.g. O'Leary, 1984).

3.5 SEM-EDX analysis of mustard root phytoliths

Phytolith extraction revealed that considerable amounts of Si in the mustard roots are stored as phytoliths. The phytoliths observed were of elongated shape and consisted mainly of SiO_2 with some minor fraction carbon (~16 %), potassium (~4 %) and iron (~1 %) (see Fig. S2). The mechanisms of precipitation of the silicic acid in the mustard root remains unclear. The finding offers however an explanation for the isotopic difference between mustard, wheat, and tomato roots. Here we have shown that in mustard is precipitated in the roots, a process shown previously for wheat too (Hodson and Sangster, 1989). Precipitation favours the incorporation of light ^{28}Si, whereas tomato does not form root phytoliths.

4 Discussion

4.1 Reliability of the combined element and isotope ratio approach

In contrast to previous studies, we added a finite nutrient amount to growth solutions and replenished only the transpired water. The combination of plant physiological ratios (water use efficiency, element budgets and biomass production) with stable isotope ratio measurements allows us to explore the temporal evolution of Si uptake and translocation. Several aspects of our data attest to the reliability of our approach and results. Concerning Si uptake dynamics, Si recovery rates of >80% (see Table 3) corroborate the reliability of our results. The same is observed for the isotope budgets. There is no significant difference between the isotopic composition of the starting solution and the weighted average of the isotopic compositions of the different compartments at the end (see Table 4). This implies all significant pathways that fractionate Si isotopes have been accounted for. The Si retrieval rate between 83 and 90% is likely not caused by a single systematic analytical uncertainty or unaccounted sink of Si, but rather a combination of container wall absorption (up to 0.1%), root washing procedure (up to 1%), the weekly sampling (up to 3.5%) and analytical uncertainties (up to 10%). As the initial concentration of Si at the onset of the experiment

(49.5 µg/g) was slightly above the solubility limits of amorphous silica at 15-18 °C (44.2 – 47.1 µg/g), a fraction of the silicon could also have been lost to polymerisation and precipitation. Guttation (Joachimsmeier et al., 2012; Yamaji et al., 2008) and litter fall were not observed during the experiment. Even if guttation were present, no Si would be lost since under the experimental conditions the fluid would evaporate leaving amorphous silica on the shoots. Thus, silicic acid excreted by guttation is counted towards the Si amounts in the shoots.

4.2 Si uptake strategies

The ratio between measured Si uptake and the expected Si amount that would have entered the plant in a purely passive uptake mechanism (see section 2.6.1, plant performance ratios), shows that wheat accumulates Si and mustard and tomato both reject Si (Figure 1 and Table 2). The accumulation of Si in wheat can be explained by the cooperation of an influx transporter (Lsi1-like) into the roots and the presumed presence of an efflux transporter (Lsi2-like) from the roots into the xylem. As closely related cereals have such transporters, we expect them to be present in wheat too (Ma and Yamaji, 2015). In rice, mutants with either defective Lsi1 or Lsi2 transporter lead to significantly lower Si accumulation (Köster et al., 2009). The direct comparison between both mutants revealed that Lsi1 carries a larger share of Si incorporation, thus a defective Lsi2 can partially be compensated (Köster et al., 2009). Our results show clear evidence that active, metabolism-driven processes or mechanisms must have been involved for wheat. The 2-fold excess of the expected amount of Si taken up cannot be explained by a passive mechanisms (e.g. Exley, 2015).

Our experiments show a striking similarity in Si uptake characteristics between mustard and tomato. Considering the differences in ontogenesis between the plant species, this may be a fortuitous coincidence. In particular, the relatively low temperatures may have inhibited the growth of the more thermophilic tomato, while the conditions were closer to optimal for mustard and summer wheat. Tomatoes have the genetic capacity to accumulate Si, since an orthologue of Lsi1 is present in the genes. An insertion in the amino acid sequence however, lead to a loss of the Si uptake functionality (Deshmukh et al., 2016, 2015), and thus tomato like mustard, rejects Si.

With our experimental approach we also detect significant differences between the crop species in Si transfer from the root to the shoot (Table 2). Wheat, which probably has a metabolically active efflux transporter (Lsi2-like) at the root-xylem interface, has the highest Si transfer efficiency per water mass (49.3 ± 8.4 mg shoot $Si \cdot L^{-1}$). The transfer efficiency for tomato is significantly higher than mustard (3.5 ± 0.4, and 2.4 ± 0.3 mg shoot $Si \cdot L^{-1}$, respectively), which is not readily explainable by differences in root Si efflux pathways since tomato does not contain the active efflux transporter orthologue Lsi2 while mustard does (Ma & Yamaji, 2015; Sonah *et al.*, 2017). The remarkably high Si concentration and amounts in mustard roots, and thus the lower Si transfer efficiency of mustard can be explained by phytolith formation (see Fig. S2). A similar immobilization of silica in roots has already been observed in wheat (Hodson and Sangster, 1989) and other grasses (Paolicchi et al., 2019). Other possible reasons for this phenomenon will be discussed based on the results on Si isotope fractionation.

4.3 Dynamics of Si isotope fractionation during uptake

The plant performance parameters disclose two distinctly different Si uptake mechanisms: an active strategy in wheat, and a rejective strategy in tomato and mustard. Despite these different Si uptake mechanisms, we find preferential uptake of light Si isotopes observed in all three species with the average $1000 \cdot \ln(\alpha)$ of -0.41 ± 0.09 ‰ (1 s). We can only speculate on the reasons for the plants preferring ^{28}Si over ^{30}Si. Si is taken up (actively facilitated) through Si permeable channels (orthologues of Lsi1 in rice, maize and barley) and passively with the water flow. Nowhere along these pathways does a change in the coordination sphere of silicic acid occur (Ma et al., 2006, 2007; Mitani et al., 2009) which could lead to the preferential incorporation of the heavy Si isotope in the fraction taken up. Thus we speculate that both pathways favour the light isotopologue because of its greater diffusion coefficient (Sun et al., 2008; Weiss et al., 2004), a process for which a predicted maximum isotope fractionation of -1.6‰ (based on Eq. (~~6~~7)) is expected. While the processes of active and rejective Si uptake differ in the amounts of Si (per time, and root mass) taken up into the plants, we speculate that the physico-chemical processes governing Si uptake, which induce the stable isotope fractionation, are identical at a given initial concentration in the nutrient solution.

Our new Si fractionation factors (tomato -0.33 ‰, and mustard -0.55 ‰) are the first to be reported for non-Si accumulator plants and together with wheat (-0.43 ‰) are similar to those measured in other Si accumulator species. These include rice: -0.30 ‰ (Sun et al., 2008), -1.02 ± 0.33 ‰* (* indicates results recalculated from $^{29/28}Si$ to $^{30/28}Si$, Ding et al., 2005) and -0.79 ± 0.07 (Sun et al., 2016a); banana: -0.77 ± 0.21 ‰* (Opfergelt et al., 2006) and -0.68 ‰* (Delvigne et al., 2009); and corn and wheat: -1.00 ± 0.31 ‰* (Ziegler et al., 2005). The only positive fractionations for Si isotopes reported are by Y. Sun and co-workers (Sun et al., 2016b) for rice (+0.38 and -0.32 ‰) and cucumber (+0.27 and +0.20 ‰). Previous experiments with the same rice species by L. Sun *et al.* however yielded a fractionation factor of -0.30 ‰ (Sun et al., 2008). The authors speculate that an active uptake mechanism preferentially incorporates heavy Si isotopes – a hypothesis that is not supported by our results, or that the different fractionation factors "could also be also be affected by the silicon isotopic composition fluctuations in different batches of nutrient solutions caused by the frequent replacement" (Sun et al., 2016b). For all negative bulk plant Si isotope fractionation factors the range found (-0.32 to -1.02 ‰) is larger than that found in our study (-0.33 to -0.55 ‰). These differences can arise from differences in species, chosen experimental conditions such concentration of nutrient solution or a temperature in the experiments that were hydroponic throughout.

4.4 Silicon fractionation between the roots and shoots

The presence or absence of the efflux (Lsi2-like metabolically active) transporter allows to explore its influence on isotope fractionation in the root and during further transport. (1) If Lsi2 has a similar functionality as Lsi1, a preference for the light ^{28}Si as caused by diffusion should emerge which would be indistinguishable from the passive diffusion in the absence of Lsi2. (2) Alternatively, the presence of Lsi2 could also induce equilibrium isotope fractionation during a change in the speciation of silicic acid, causing the preferential transport of either ^{28}Si or ^{30}Si. (3) The third possibility are indirect effects in the roots such

as precipitation of silicic acid in the roots which enrich the remaining silicic acid which is transported into the shoots in heavy ^{30}Si.

The three crop species show large differences in their root Si isotopic composition. Mustard and spring wheat preferentially store light ^{28}Si in their roots ($^{Mustard}\Delta^{30}Si_{roots}$ -0.77 ± 0.15 ‰, $^{Wheat}\Delta^{30}Si_{roots}$ -1.04 ± 0.34 ‰, relative to the nutrient solution) whereas tomato does not show a preference for either the lighter or heavier silicon isotopes ($^{Tomato}\Delta^{30}Si_{roots}$ -0.01 ± 0.16 ‰). The further transport of Si from the roots into the xylem seems not be driven by a diffusion process through Lsi2. Thus, hypothesis (1), that Lsi2 has a similar functionality as Lsi1 and transports Si in a diffusive process, is not likely. For mustard and wheat orthologues of Lsi2 have been shown to be involved in the Si transport (Deshmukh et al., 2016; Sonah et al., 2017). The current understanding of the molecular functionality of Lsi2 however, provides not enough evidence for an equilibrium process where a preferential transport of ^{30}Si over ^{28}Si into the xylem would be expected (hypothesis 2).

The isotopic difference between the Si in the shoots and in the roots ($^{30}\Delta_{Root\text{-}Shoot}$) for mustard and wheat amounts to -0.72 and -0.98 ‰, respectively, and can be explained by Si precipitation in the roots. Indeed, we observed mustard root phytoliths; see Fig. S2. Mineral deposition in wheat roots has also been observed by Hodson & Sangster, (1989), supporting hypothesis (3). Precipitation of biogenic silica in the root would enrich the residual mobile silicon pool in heavy ^{30}Si, which is then transported into the shoots. Köster *et al.*, 2009, showed that rice mutants with a defective Lsi2 lead to an additional (compared to non-mutants) preferential transport of heavy ^{30}Si into the straw. This could be explained by an oversaturation in the roots due to the missing efflux transporter (Lsi2), leading to additional biogenic silica precipitation in the roots. The positive $^{30}\Delta_{Root\text{-}Shoot}$ of +0.37 ‰ for tomato, where Lsi2 is absent, indicate that the pool of Si in the roots was depleted in ^{28}Si by a preferential diffusion process of the lighter isotope.

Within the shoots, Si is not homogenously distributed. Several ~~researcher~~researchers have observed an enrichment of ^{30}Si along the transpiration stream (Ding et al., 2005; Hodson et al., 2008; Sun et al., 2016b), compatible with a Rayleigh-like fractionation within the shoots. A possible explanation for this observation is the formation of phytoliths. Early in the transpiration stream, the kinetically controlled condensation of silicic acid leads to the preferential incorporation of ^{28}Si into phytoliths (e.g. Frick *et al.*, 2019), whereas the remaining silicic acid in the fluid is enriched in ^{30}Si and further transported along the transpiration stream.

5 Conclusion

The amount of Si uptake into crop plants and the distribution of Si within them is species-specific, and the uptake strategies are in operation in variable relative proportions. However, regardless of the uptake strategy (active and rejective) all three crop species studied preferentially incorporate light silicon (^{28}Si) with a fractionation factor $1000 \cdot \ln(\alpha)$ for tomato -0.33 ‰, for mustard -0.55 ‰ and for wheat -0.43 ‰ which are indistinguishable within uncertainty. This similarity indicates that the

physico-chemical processes governing Si uptake, whether active or passive, or with Lsi1-like transporters present or absent, are identical. The incorporation and fractionation of stable Si isotope ratios at the root epidermis is likely governed by the preferential diffusion of the lighter homologue of silicic acid. In contrast, at the root endodermis, for species with the Lsi2-like transporter (wheat and mustard), the further transport of silicic acid from the roots into the xylem and shoots is not controlled by the preferential diffusion of light ^{28}Si. Rather the ^{28}Si-enriched precipitation of biogenic silica in the roots governs the isotope composition of remaining Si transported into and deposited within the shoots that is thus enriched in ^{30}Si. For plant species in which no biogenic silica is precipitated in the roots the Si is transported in an isotopically unmodified form. Any further transport is governed by a diffusion in which ^{28}Si is preferentially transported into the shoots. A full description of the isotope fractionation during transport of silicic acid and precipitation of biogenic silica requires knowing the bio-molecular processes involved in the dehydration of silicic acid and its conversion into amorphous silica (He et al., 2015; Leng et al., 2009). Towards this task an additional facet of the toolbox of isotope geochemistry is well-poised: employing isotope-spiking during plant growth and -ripening. Such ~~experiment~~experiments will provide insights into the mobility and pathways of the different pools and sources of silicic acid, while the associated simultaneous stable isotope fractionation can be attributed to the biochemical processes involved.

Author Contribution

All authors designed the study, D. A. F. and R. R. have grown the plants, D. A. F. has analysed the samples and evaluated the data, prepared the figures. D. K. has prepared and imaged the phytoliths. All authors have contributed to the discussion, interpretation and writing of this manuscript.

Competing interests

The authors declare that they have no conflict of interest.

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Data availability

All data used in this study are available in the supplementary, containing the tables S1– S64.

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Figures

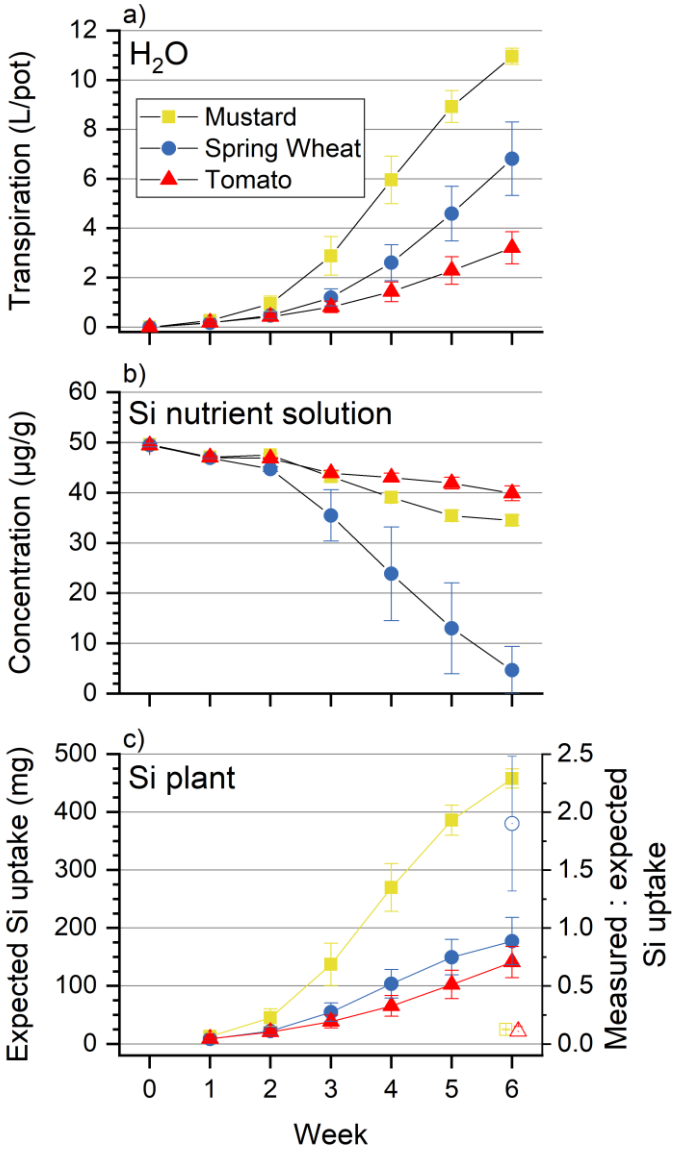

Figure 1: Cumulative transpiration (panel a), Si concentration in the nutrient solution (in µg/g, panel b) and the expected Si uptake through transpiration of tomato, mustard and spring wheat during 6 weeks (panel c). Shown is the mean ± standard deviation from 3 pots with 4 plants each. In panel c) a ratio of measured and expected Si uptake (open symbols) of greater than 1 indicates an active uptake mechanism, a ratio much smaller than 1 a rejective strategy.

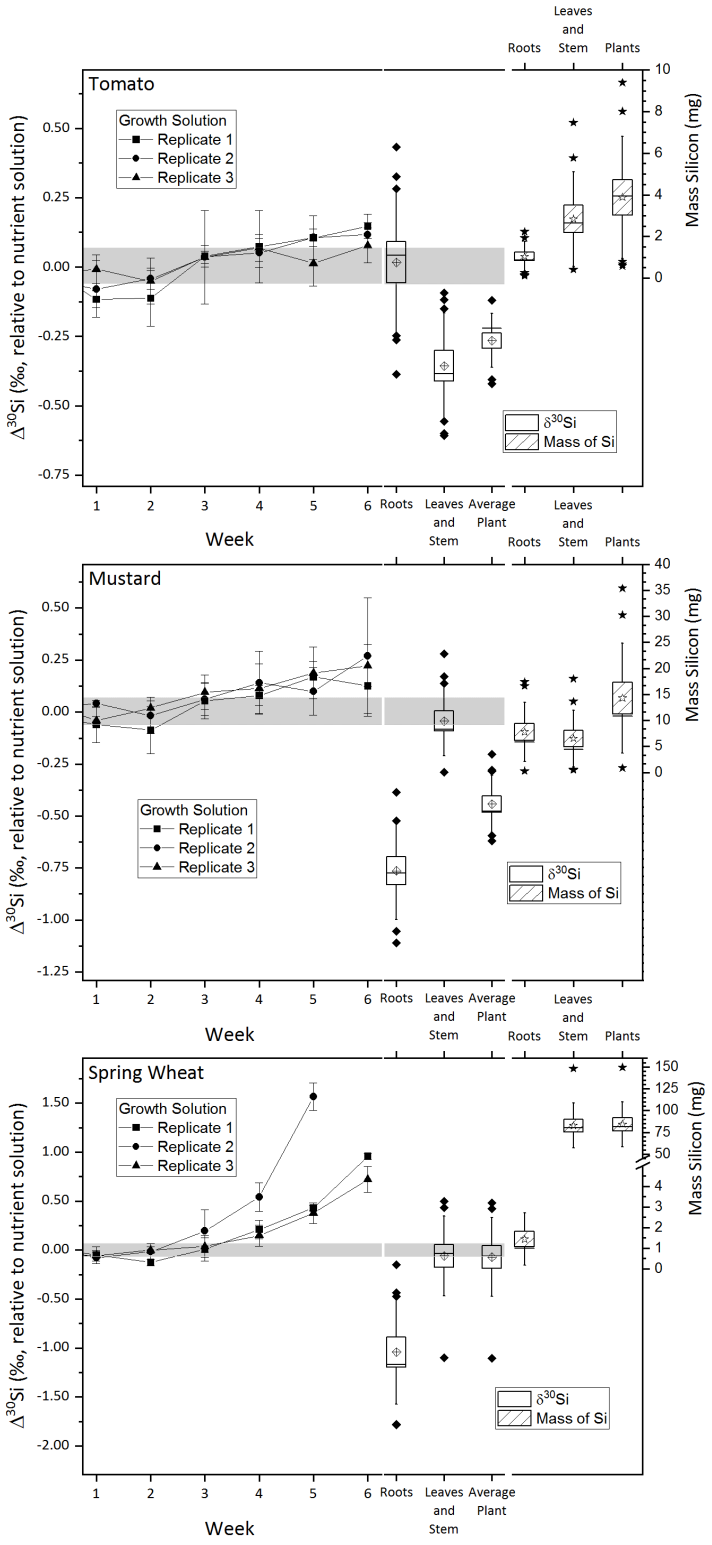

Figure 2: Silicon isotope composition (left) and mass of silicon taken up (right) during the growth of tomato, mustard, and wheat. The left y-axis shows the $\delta^{30}Si$ in ‰ relative to the nutrient solution, the right y-axis the mass of silicon incorporated by the plants in mg incorporated. The line connects $\delta^{30}Si$ from the weekly sampled nutrient solution (week 1 to 6). The box plots denote $\delta^{30}Si$ (left) and plant organ Mg mass (right), per species 12 roots and 12 leaves and stem samples were analysed, plant averages were weighted by organ mass (calculated using Eq. (2). Uncertainty bars are based on 2 standard uncertainties, grey area denotes the silicon isotopic composition of the starting solution ± two standard deviations. All box sizes denote one standard uncertainty, whisker indicate one standard deviation, horizontal line in the box shows the median, empty diamond/stars in the box indicate the mean and filed diamonds/stars show outliers, outside of one standard deviation.

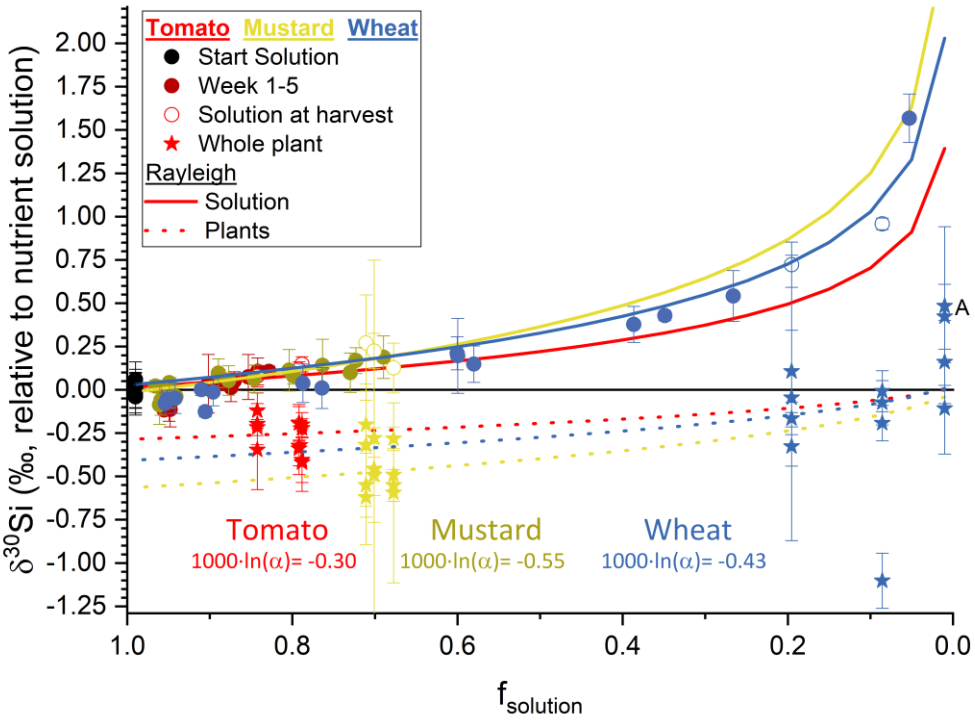

Figure 3: The silicon isotope composition (expressed in δ^{30}Si ‰ relative to nutrient solution) versus the amount of silicon taken up by the plants (expressed as dimensionless $f_{solution}$) (circles represents the nutrient solution, tomato in red, mustard in yellow and wheat in blue, starting solutions in black). Red, yellow and blue solid lines represent the best fit through a Rayleigh-like fractionation for the remaining solution, the dotted line the accumulated silicon isotope composition in the plants derived thereof. Stars are the mass-weighted average isotopic composition of the individual plants at the respective $f_{solution}$ of the container at harvest. Plant samples denoted with A have no corresponding solution value, since the concentration of silicon was below the amount required for an isotope ratio determination. Uncertainty bars are based on two standard deviations.

690 **Tables**

Parameter		Plant species		
		Mustard	**Wheat**	**Tomato**
Dry matter [g pot^{-1}]	Root	3.9 ± 1.1	2.6 ± 0.6	1.7 ± 0.2
	Shoot	25.0 ± 4.2	13.7 ± 2.0	10.3 ± 1.5
	Total plant	29.0 ± 5.2	16.3 ± 2.5	12.0 ± 1.7
Plant Si content [mg Si g^{-1} dry matter]	Root	8.6 ± 4.3	2.5 ± 2.8	3.5 ± 1.8
	Shoot	1.0 ± 0.3	24.2 ± 6.3	1.4 ± 0.7
	Total plant	2.0 ± 0.4	20.9 ± 4.0	1.3 ± 0.2
Plant Si uptake [mg Si pot^{-1}]	Root	31.1 ± 4.8	5.8 ± 3.1	4.1 ± 1.3
	Shoot	26.1 ± 3.8	331.3 ± 70.1	11.4 ± 3.6
	Total plant	57.2 ± 1.3	337.0 ± 67.9	15.5 ± 4.9
Transpiration [L pot^{-1}]	Pot	11.0 ± 0.3	6.8 ± 1.5	3.2 ± 0.6

Table 1: Dry matter, plant Si content, plant Si uptake and water transpiration of mustard, wheat and tomato after 6 weeks (hydroponic culture; mean ± standard deviation based on 3 pots with 4 plants each).

Quotient	Plant species		
	Mustard	**Wheat**	**Tomato**
Dry mass ratio [g shoot g^{-1} root]	6.5 ± 0.7	5.4 ± 0.9	5.9 ± 0.2
Si mass ratio [mg Si in shoot mg^{-1} Si in root]	0.9 ± 0.2	72.7 ± 47.8	2.7 ± 0.2
Water use efficiency [g L^{-1}]	2.6 ± 0.5	2.4 ± 0.2	3.8 ± 0.3
Si uptake efficiency [mg plant Si L^{-1}]	5.2 ± 0.3	50.3 ± 8.8	4.8 ± 0.6
Si transfer efficiency [mg shoot Si L^{-1}]	2.4 ± 0.3	49.3 ± 8.4	3.5 ± 0.4
Uptake classification (measured / expected Si uptake)	0.12±0.01	1.9±0.6	0.11±0.04

Table 2: Ecophysiological performance ratios for mustard, wheat and tomato (means ± standard deviation based on 3 pots with 4
695 **plants each). The uptake classification is based on the ratio of measured and expected Si uptake. A ratio of greater than 1 indicates an active uptake mechanism, a ratio much smaller than 1 a rejective strategy and a ratio of 1 is passive uptake.**

		Mustard			Wheat			Tomato		
	[mg]	Pot 1	Pot 4	Pot 7	Pot 2	Pot 5	Pot 8	Pot 3	Pot 6	Pot 9
Si	m_{Start}	418	421	399	425	416	411	418	415	414
	m_{End}	283	299	280	36	2	80	329	329	349
	m_{Plants}	58	56	58	299	415	297	20	15	11
	Retrieval	82%	84%	85%	79%	100%	92%	84%	83%	87%
Ca	m_{Start}	544	543	524	548	542	541	549	542	543
	m_{End}	3	0	0	382	376	423	139	182	264
	m_{Plants}	393	394	352	108	119	87	304	241	222
	Retrieval	73%	73%	67%	89%	91%	94%	81%	78%	90%
Fe	m_{Start}	39	39	38	39	40	39	39	39	39
	m_{End}	26	29	28	27	25	28	24	24	28
	m_{Plants}	4	4	3	6	4	3	5	5	2
	Retrieval	76%	85%	82%	85%	73%	80%	73%	75%	78%
K	m_{Start}	1787	1813	1742	1817	1801	1801	1803	1809	1801
	m_{End}	657	424	174	539	505	787	941	1044	1213
	m_{Plants}	1085	1218	1500	1556	1449	979	872	727	673
	Retrieval	98%	91%	96%	115%	109%	98%	101%	98%	105%
Mg	m_{Start}	121	121	116	122	120	119	122	121	120
	m_{End}	7	1	0	63	59	67	35	41	55
	m_{Plants}	82	95	73	30	26	27	52	55	33
	Retrieval	74%	79%	63%	76%	70%	80%	72%	79%	74%
P	m_{Start}	173	176	171	177	175	176	176	177	177
	m_{End}	5	2	1	0	0	11	5	20	52
	m_{Plants}	121	134	115	137	142	144	117	123	82
	Retrieval	73%	77%	68%	77%	81%	88%	69%	81%	76%
S	m_{Start}	180	183	174	182	182	182	183	182	182
	m_{End}	4	3	6	97	101	119	81	89	113
	m_{Plants}	95	88	73	61	57	33	60	55	38
	Retrieval	55%	50%	45%	87%	87%	84%	77%	79%	83%

Table 3: Major element budget for mustard, tomato and wheat. m_{Plants} is calculated based on the concentration of the element in the plant digest and the dry mass, the m_{Start} m_{End} are the element masses in mg based on the amount of nutrient solution and the element concentration at the start and the end of the experiment. Retrieval is the ratio between m_{Start} and the sum of m_{Plants} and m_{End}. The initial amount of the elements in the seeds, taken up during germination and the amount of element discharged in the wash water are not considered.

	$\delta^{30}Si$	2 s	$\delta^{30}Si$	2 s	$\delta^{30}Si$	2 s
Mustard						
	Pot 1		Pot 4		Pot 7	
Start	-0.23	0.12	-0.19	0.06	-0.15	0.06
End	-0.20	0.30	-0.04	0.38	-0.09	0.26
Wheat						
	Pot 2		Pot 5		Pot 9	
Start	-0.18	0.03	-0.18	0.13	-0.24	0.07
End	-0.39	0.30	0.05	0.23	-0.12	0.27
Tomato						
	Pot 3		Pot 6		Pot 9	
Start	-0.20	0.08	-0.25	0.10	-0.23	0.02
End	-0.09	0.19	-0.11	0.31	-0.14	0.31

Table 4: Silicon isotope budget (calculated using Eq. (45)) for mustard, wheat and tomato at the start of the experiment (based on the isotopic composition of the nutrient solution) and the end (based on the plants and nutrient solution isotopic composition).

best fit	**Mustard**	**Tomato**	**Wheat**	**All data**
1000·ln(α) [‰]	-0.55 ± 0.40	-0.33 ± 0.32	-0.43 ± 0.09	-0.43 ± 0.09

Table 5: $^{30}Si/^{28}Si$ isotope fractionation factor 1000·ln(α) numerically approximated by reducing root-mean-square-deviation ('best fit') using Eq. (56) and uncertainties (1 s) from Monte Carlo method with n=500 seeded individual data sets.

Supplement to: Silicon ~~stable~~uptake and isotope fractionation ~~and uptake~~ dynamics ~~of~~by crop species

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Figures

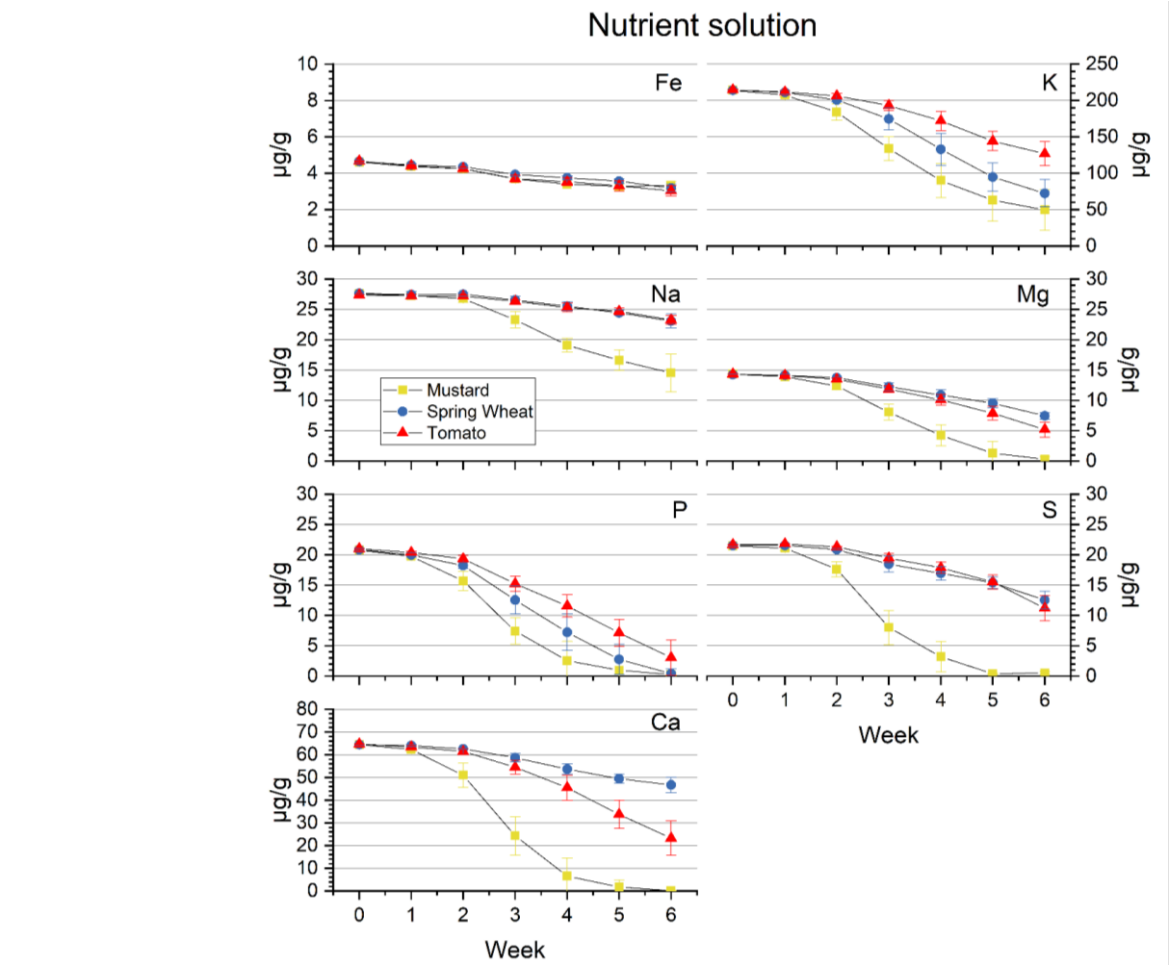

Figure S 1: Temporal evolution of nutrient concentrations during the hydroponic growth of the different plant species. Concentration (Fe, K, Mg, Ca, P, S, Na in µg·g^{-1}) are based on the mean of the three replicated containers, uncertainty shown is 1 standard deviation of those replicated containers.

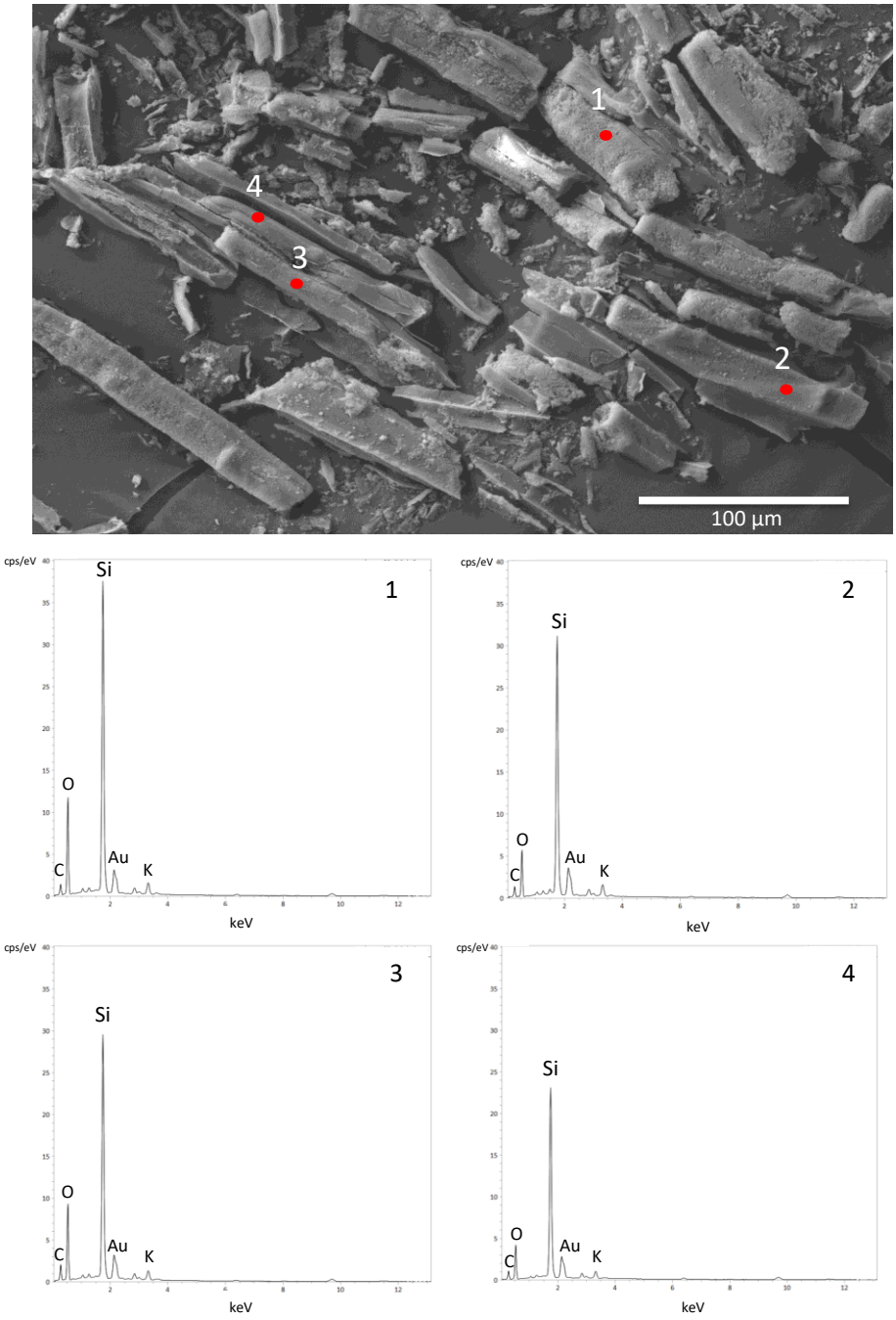

Figure S 2: Representative SEM-EDX micrograph of Si precipitates (phytoliths) in mustard roots extracted from dried root samples. See SEM-EDX analysis of mustard root phytoliths for detailed extraction and measurement methods.

20 **Tables**

	Concentration measurements	**Si isotope ratio measurements**
Instrument	Varian 720ES ICP-OES	ThermoFisher Neptune Plus
Spraychamber	cyclonic, glass	APEX
Nebuliser	concentric, glass	concentric, PFA
Sample uptake rate	ca. 2 ml/min (pumped:15 rpm)	160 µL/min
Cones	standard cone	N-sampler / H-skimmer
Plasma RF power	1.0 kW	1200 W
Ar cool gas	15 L/min	15 L/min
Ar aux gas	1.5 L/min	0.8 L/min
Ar nebuliser pressure /flow rate[a]	280 -320 kPa	1.0 L/min
Analysis integration time	10 s	4 s
Integration replicates per analysis	3	30
Rinse time between samples	60 s (pumped at 50 rpm), 0.3 M HNO_3	160 s, 0.1 M HCl
Analytes (wavelengths in nm for ICP-OES or isotopes for MC-ICP-MS)	Ca 422.673, Fe 238.204, K 769.897, Mg 280.270, Na 588.995, Si 288.158, S 181.972, P 213.618	^{24}Mg, ^{25}Mg, ^{26}Mg ^{28}Si, ^{29}Si, ^{30}Si medium mass resolution mode: Δm/m (5%/95% intensity limits): >5000
[a]Optimised during each analytical session		

Table S1: Instrument settings for concentration and silicon isotope ratio measurements.

ERM-CD281			
$\delta^{29}Si/^{28}Si$	2 s	$\delta^{30}Si/^{28}Si$	2 s
-0.12	0.04	-0.25	0.05
-0.18	0.05	-0.33	0.06
-0.16	0.04	-0.26	0.05
-0.19	0.05	-0.24	0.07
-0.15	0.05	-0.27	0.05
-0.19	0.05	-0.28	0.07
-0.15	0.06	-0.18	0.07
-0.25	0.04	-0.45	0.05
-0.26	0.04	-0.47	0.05
-0.28	0.04	-0.44	0.07
-0.27	0.05	-0.46	0.07
-0.31	0.04	-0.42	0.07
-0.25	0.04	-0.38	0.07

BHVO-2			
$\delta^{29}Si/^{28}Si$	2 s	$\delta^{30}Si/^{28}Si$	2 s
-0.14	0.06	-0.26	0.07
-0.13	0.04	-0.24	0.07
-0.18	0.04	-0.30	0.06
-0.18	0.04	-0.32	0.06
-0.22	0.05	-0.35	0.07
-0.15	0.05	-0.29	0.06
-0.27	0.14	-0.40	0.15
-0.07	0.08	-0.25	0.09
-0.17	0.05	-0.24	0.07
-0.11	0.05	-0.27	0.07
-0.16	0.05	-0.26	0.07
-0.11	0.08	-0.23	0.09
-0.14	0.06	-0.27	0.10
-0.14	0.04	-0.30	0.06
-0.17	0.04	-0.29	0.06
-0.17	0.04	-0.29	0.06
-0.18	0.05	-0.32	0.07
-0.14	0.05	-0.22	0.07
-0.19	0.04	-0.35	0.06
-0.18	0.05	-0.31	0.07
-0.15	0.05	-0.29	0.06
-0.13	0.05	-0.29	0.06
-0.16	0.06	-0.26	0.07
-0.22	0.05	-0.32	0.07
-0.21	0.05	-0.21	0.07
-0.16	0.04	-0.29	0.05
-0.16	0.04	-0.33	0.05
-0.20	0.04	-0.31	0.06
-0.13	0.06	-0.25	0.09
-0.16	0.05	-0.35	0.06
-0.17	0.04	-0.24	0.07
-0.18	0.05	-0.32	0.07
-0.18	0.05	-0.30	0.07
-0.17	0.05	-0.36	0.07
-0.13	0.04	-0.25	0.05
-0.12	0.04	-0.29	0.04
-0.15	0.06	-0.29	0.06
-0.09	0.04	-0.26	0.07
-0.16	0.04	-0.27	0.05
-0.15	0.04	-0.23	0.06

Table S2: Individually repeated analysis of BHVO-2 and ERM-CD281 for their silicon isotope composition.

		Ca	Fe	K	Mg	P	S	Si	$\delta^{30}Si$	2 SD
		µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	‰	
Mustard	**Pot 1**	64.2	4.6	210.8	14.3	20.4	21.2	49.3	-0.23	0.12
	Pot 4	64.3	4.6	214.8	14.3	20.9	21.6	49.8	-0.19	0.06
	Pot 7	65.0	4.7	216.1	14.4	21.2	21.6	49.5	-0.15	0.06
Spring Wheat	**Pot 2**	64.3	4.6	213.2	14.3	20.7	21.3	49.9	-0.18	0.03
	Pot 5	64.5	4.7	214.0	14.3	20.8	21.6	49.4	-0.18	0.13
	Pot 8	64.8	4.6	215.5	14.2	21.0	21.7	49.2	-0.24	0.07
Tomato	**Pot 3**	64.9	4.7	213.3	14.4	20.9	21.7	49.4	-0.20	0.08
	Pot 6	64.5	4.7	215.4	14.4	21.1	21.6	49.5	-0.25	0.10
	Pot 9	64.7	4.7	214.7	14.2	21.1	21.7	49.4	-0.23	0.02
Average									-0.21	0.07

Table S 3: Starting composition (major element concentration (in µg g^{-1}) and silicon isotopic composition) of the nutrient solutions for the individual pots.

The Table S4 is on the following pages.

Table S4: Dry weight, major element concentration (in mg·g^{-1}) and Si isotope composition (in ‰) of the plants separated into shoot and root.

The Table S5 is in the following pages.

Table S5: Composition (major element concentration (in µg g^{-1}) and silicon isotopic composition) of the weekly sampled nutrient solutions for the individual pots. See Table S3 for the starting composition (week 0).

Difference between		Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
		Week 0	Week 1	Week 2	Week 3	Week 4	Week 5
		[g]	[g]	[g]	[g]	[g]	[g]
Mustard	**Pot 1**	407.5	671.2	1842.2	2707.5	2863.3	2277.8
	Pot 4	239	929.9	2469.8	3397.6	2627.5	1673.4
	Pot 7	137.8	449.4	1500.7	3119.5	3413.4	2152.7
Spring Wheat	**Pot 2**	231.1	374.7	791.1	1416	2083.7	2471.3
	Pot 5	147.6	331.3	914.9	1816.3	2306.7	2422.1
	Pot 8	133.5	189	474.7	996.6	1576.9	1756.8
Tomato	**Pot 3**	206.7	316.2	526.6	772.6	998.9	951.6
	Pot 6	224.1	233.2	352.6	625.2	913	1005.8
	Pot 9	127.1	164.6	292.4	444.6	672.3	800.2

Table S 6: Weekly transpiration (in g), determined by weighing the pots without the plants. The transpired water was replenished weekly with ultrapure water.

			dry mass	Ca	Fe	K	Mg	P	S	Si	δ^{30}Si			δ^{30}Si		
Mustard		Plant ID	[g]	mg/g	mg/g	mg/g	mg/g	mg/g	mg/g	mg/g	‰ NBS28	2 s / *95 % CI		‰ nutrient solution	2 s / *95 % CI	
Pot 1	Roots	19-5-1R-S1	0.96	3.9	1.0	23.3	2.6	5.9	4.0	13.2	-0.83	0.17		-0.62	0.17	
		19-5-1R-S2	1.48	2.2	0.5	29.6	1.9	4.6	3.3	11.2	-1.15	0.05		-0.94	0.05	
		19-5-1R-S3	0.58	2.3	0.5	27.7	1.2	4.5	3.3	11.3	-0.92	0.05		-0.72	0.05	
		19-5-1R-S4	0.05	4.2	0.9	15.2	0.9	3.2	2.2	5.0	-0.73	0.12		-0.53	0.12	
	Shoot	19-5-1S-S1	4.12	13.5	0.10	51.4	3.7	4.3	4.6	0.90	-0.27	0.08		-0.06	0.08	
		19-5-1S-S2	12.12	18.7	0.07	46.0	3.7	5.1	3.7	1.12	-0.38	0.04		-0.18	0.04	
		19-5-1S-S3	4.43	20.5	0.06	44.9	3.0	5.0	3.6	0.90	-0.50	0.08		-0.29	0.08	
		19-5-1S-S4	0.63	18.4	0.15	53.1	3.0	8.1	7.4	0.89	-0.38	0.08		-0.17	0.08	
Pot 4	Roots	19-5-4R-S1	0.71	3.3	0.67	15.2	1.8	3.2	3.7	7.23	-0.59	0.04		-0.39	0.04	
		19-5-4R-S2	0.62	2.7	0.37	15.5	1.6	4.4	2.8	4.75	-1.26	0.08		-1.06	0.08	
		19-5-4R-S3	0.61	2.6	0.40	17.8	1.8	4.1	1.6	9.00	-1.32	0.07		-1.11	0.07	
		19-5-4R-S4	1.58	2.1	0.38	18.1	1.6	4.1	2.1	8.19	-0.82	0.04		-0.62	0.04	
	Shoot	19-5-4S-S1	5.84	34.1	0.10	50.9	5.1	4.0	4.4	1.68	-0.32	0.11		-0.11	0.11	
		19-5-4S-S2	3.08	12.4	0.12	47.1	3.7	5.7	4.2	0.95	-0.25	0.18		-0.05	0.18	
		19-5-4S-S3	4.83	11.0	0.08	44.4	3.3	4.9	2.2	1.11	-0.33	0.05		-0.12	0.05	
		19-5-4S-S4	10.54	9.0	0.08	47.5	3.0	5.3	2.8	1.04	-0.18	0.08		0.02	0.08	
Pot 7	Roots	19-5-7R-S1	1.98	1.6	0.21	18.5	1.6	3.7	2.3	3.08	-1.19	0.07		-0.99	0.07	
		19-5-7R-S2	0.22	2.7	0.89	24.1	1.2	3.1	3.5	17.95	-0.74	0.07		-0.54	0.07	
		19-5-7R-S3	0.80	2.0	0.16	21.2	0.9	3.1	3.4	3.85	-1.05	0.10		-0.84	0.10	
		19-5-7R-S4	2.15	1.4	0.19	24.8	1.1	3.2	2.8	8.07	-1.07	0.09		-0.86	0.09	
	Shoot	19-5-7S-S1	8.90	6.9	0.08	37.7	2.0	3.1	1.6	0.53	-0.07	0.09		0.13	0.09	
		19-5-7S-S2	0.93	4.6	0.06	44.2	1.9	2.5	1.7	0.52	-0.04	0.10		0.17	0.10	
		19-5-7S-S3	5.30	17.8	0.06	48.4	2.5	4.7	3.2	0.77	-0.38	0.09		-0.18	0.09	
		19-5-7S-S4	14.40	12.7	0.06	52.4	2.3	3.0	1.8	1.25	0.07	0.06		0.28	0.06	
Average	Roots		0.98	2.59	0.51	20.90	1.52	3.92	2.92	8.57	-0.97	0.15	*	-0.77	0.15	*
	Shoot		6.26	14.96	0.08	47.34	3.10	4.63	3.44	0.97	-0.25	0.11	*	-0.05	0.11	*
Sum	Plants		86.88													
* Uncertantiy based on 95 % CI																

Table S4: Dry weight, major element concentration (in mg·g^{-1}) and Si isotope composition (in ‰) of the plants separated into shoot and root.

			dry mass	Ca	Fe	K	Mg	P	S	Si	δ30Si			δ30Si		
Spring Wheat		Plant ID	[g]	mg/g	mg/g	mg/g	mg/g	mg/g	mg/g	mg/g	‰ NBS28	2 s / *95 % CI		‰ nutrient solution	2 s / *95 % CI	
Pot 2	Roots	19-5-2R-W1	0.8	4.6	0.46	62.9	1.1	5.2	1.9	1.31	-0.68	0.14		-0.48	0.14	
		19-5-2R-W2	0.9	3.8	0.37	52.3	1.2	4.2	1.9	1.30	-0.64	0.05		-0.44	0.05	
		19-5-2R-W3	1.0	3.5	0.38	60.2	0.9	5.5	1.9	1.80	-1.42	0.08		-1.21	0.08	
		19-5-2R-W4	0.7	3.9	0.32	56.8	0.9	4.9	1.8	1.29	-0.89	0.12		-0.68	0.12	
	Shoot	19-5-2S-W1	3.7	6.7	0.31	95.1	1.7	7.6	3.6	22.18	-0.21	0.09		-0.01	0.09	
		19-5-2S-W2	3.5	7.4	0.23	56.9	2.0	7.6	2.6	18.59	-0.28	0.10		-0.07	0.10	
		19-5-2S-W3	4.2	4.7	0.30	105.2	1.8	8.0	4.0	15.36	-1.31	0.16		-1.10	0.16	
		19-5-2S-W4	3.5	7.0	0.46	105.6	1.6	9.1	4.3	23.42	-0.40	0.05		-0.19	0.05	
Pot 5	Roots	19-5-5R-W1	0.51	4.9	1.11	54.4	1.9	5.4	1.4	1.10	-1.45	0.13		-1.25	0.13	
		19-5-5R-W2	0.63	5.1	0.71	61.3	1.4	4.6	1.7	1.59	-1.99	0.12		-1.79	0.12	
		19-5-5R-W3	0.47	5.6	0.72	62.6	1.4	4.4	1.6	1.58	-1.64	0.03		-1.44	0.03	
		19-5-5R-W4	0.76	4.0	0.73	62.1	1.3	4.2	1.9	1.31	-1.60	0.06		-1.39	0.06	
	Shoot	19-5-5S-W1	3.31	7.0	0.13	66.2	2.0	8.9	3.0	23.69	0.22	0.03		0.43	0.03	
		19-5-5S-W2	3.58	9.0	0.11	107.2	0.3	9.8	4.4	41.44	0.29	0.09		0.50	0.09	
		19-5-5S-W3	3.14	7.0	0.18	81.8	2.1	8.3	3.2	26.64	-0.04	0.05		0.17	0.05	
		19-5-5S-W4	4.83	6.3	0.13	92.5	1.7	8.5	3.5	21.05	-0.31	0.08		-0.10	0.08	
Pot 8	Roots	19-5-8R-W1	0.62	3.9	0.47	63.1	1.6	8.5	1.5	2.10	-1.00	0.09		-0.79	0.09	
		19-5-8R-W2	0.28	4.3	0.68	57.6	1.7	7.7	1.2	1.30	-1.34	0.13		-1.14	0.13	
		19-5-8R-W3	0.46	2.7	0.50	56.3	1.9	7.7	1.7	11.18	-0.36	0.02		-0.15	0.02	
		19-5-8R-W4	0.74	2.2	0.29	55.5	1.5	8.3	2.0	3.29	-1.99	0.06		-1.78	0.06	
	Shoot	19-5-8S-W1	3.42	7.3	0.15	68.0	1.6	10.1	2.8	23.25	-0.09	0.06		0.12	0.06	
		19-5-8S-W2	1.60	8.1	0.20	79.8	2.8	11.6	2.7	26.05	-0.53	0.10		-0.32	0.10	
		19-5-8S-W3	2.87	6.4	0.13	78.5	2.2	11.2	2.2	24.93	-0.38	0.10		-0.17	0.10	
		19-5-8S-W4	3.47	7.0	0.14	78.2	2.3	11.9	2.8	27.33	-0.21	0.04		-0.01	0.04	
Average	Roots		0.65	3.99	0.57	58.39	1.43	5.94	1.71	2.53	-1.25	0.34	*	-1.04	0.34	*
	Shoot		3.43	7.02	0.20	83.62	1.85	9.54	3.22	24.70	-0.27	0.26	*	-0.06	0.26	*
Sum	Plants		48.92													
* Uncertantiy based on 95 % CI																

Table S4: Dry weight, major element concentration (in $mg \cdot g^{-1}$) and Si isotope composition (in ‰) of the plants separated into shoot and root.

			dry mass	Ca	Fe	K	Mg	P	S	Si	δ30Si			δ30Si		
Tomato		Plant ID	[g]	mg/g	mg/g	mg/g	mg/g	mg/g	mg/g	mg/g	‰ NBS28	2 s / *95 % CI		‰ nutrient solution	2 s / *95 % CI	
Pot 3	Roots	19-5-3R-T1	0.03	8.7	1.53	65.4	4.6	6.1	5.7	7.41	-0.60	0.10		-0.39	0.10	
		19-5-3R-T2	1.08	7.3	0.99	81.1	3.8	7.5	2.7	1.77	-0.03	0.04		0.17	0.04	
		19-5-3R-T3	0.40	6.8	1.18	58.5	4.6	7.6	2.6	4.32	-0.46	0.03		-0.25	0.03	
		19-5-3R-T4	0.44	6.8	2.48	72.5	3.4	9.1	2.8	3.80	-0.19	0.01		0.02	0.01	
	Shoot	19-5-3S-T1	0.17	19.5	0.11	79.8	3.2	11.5	2.9	2.45	-0.30	0.09		-0.10	0.09	
		19-5-3S-T2	6.18	24.9	0.13	60.2	3.1	7.9	4.6	1.21	-0.77	0.01		-0.56	0.01	
		19-5-3S-T3	2.13	28.9	0.28	50.7	5.5	10.9	4.8	1.38	-0.42	0.09		-0.21	0.09	
		19-5-3S-T4	3.33	21.5	0.15	70.1	3.9	8.3	4.6	1.23	-0.81	0.06		-0.60	0.06	
Pot 6	Roots	19-5-6R-T1	0.45	6.0	1.53	58.7	4.4	10.0	3.1	1.90	0.07	0.10		0.28	0.10	
		19-5-6R-T2	0.21	8.6	3.13	62.7	5.6	13.1	3.8	4.12	-0.47	0.10		-0.27	0.10	
		19-5-6R-T3	0.45	6.4	2.63	68.7	3.9	12.2	2.3	1.79	0.22	0.10		0.43	0.10	
		19-5-6R-T4	0.69	6.7	1.22	76.4	4.7	10.6	2.8	1.92	-0.15	0.07		0.06	0.07	
	Shoot	19-5-6S-T1	2.62	14.8	0.14	53.9	4.6	8.0	4.6	0.89	-0.57	0.11		-0.37	0.11	
		19-5-6S-T2	1.40	25.9	0.23	61.6	5.1	11.7	6.2	0.98	-0.36	0.14		-0.15	0.14	
		19-5-6S-T3	2.52	24.3	0.16	62.6	4.8	11.7	5.1	1.63	-0.70	0.12		-0.49	0.12	
		19-5-6S-T4	3.64	25.6	0.15	60.1	4.3	10.0	4.5	1.01	-0.67	0.11		-0.47	0.11	
Pot 9	Roots	19-5-9R-T1	0.02	9.4	1.75	99.0	3.1	9.5	9.2	4.65	-0.35	0.07		-0.14	0.07	
		19-5-9R-T2	0.05	6.8	1.99	78.2	5.5	15.2	3.9	3.10	-0.34	0.15		-0.14	0.15	
		19-5-9R-T3	0.10	8.1	2.82	76.8	5.3	13.0	5.0	5.10	-0.14	0.04		0.07	0.04	
		19-5-9R-T4	1.30	6.6	0.70	81.4	3.2	9.8	2.8	1.71	0.12	0.12		0.32	0.12	
	Shoot	19-5-9S-T1	0.21	27.6	0.18	80.1	3.8	15.3	4.7	3.16	-0.33	0.12		-0.12	0.12	
		19-5-9S-T2	0.44	20.2	0.27	63.9	4.8	9.9	5.0	0.95	-0.43	0.15		-0.23	0.15	
		19-5-9S-T3	0.80	21.7	0.16	70.9	4.1	8.9	4.3	0.99	-0.61	0.05		-0.41	0.05	
		19-5-9S-T4	7.41	24.3	0.11	60.9	2.9	7.1	3.6	0.78	-0.82	0.08		-0.61	0.08	
Average	Roots		0.44	7.35	1.83	73.27	4.34	10.29	3.88	3.47	-0.19	0.16	*	0.01	0.16	*
	Shoot		2.57	23.25	0.17	64.56	4.17	10.10	4.59	1.39	-0.57	0.12	*	-0.36	0.12	*
Sum	Plants		36.09													
* Uncertantiy based on 95 % CI																

Table S4: Dry weight, major element concentration (in $mg \cdot g^{-1}$) and Si isotope composition (in ‰) of the plants separated into shoot and root.

			Ca	Fe	K	Mg	P	S	Si	δ30Si	
			µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	‰ NBS28	2 s
Week 1	Mustard	Pot 1	63.3	4.4	210.5	14.0	20.2	21.4	47.3	-0.27	0.09
		Pot 4	62.0	4.4	206.2	13.9	19.7	21.3	47.3	-0.17	0.02
		Pot 7	61.7	4.4	205.2	13.8	19.4	20.6	46.6	-0.25	0.02
	Spring Wheat	Pot 2	63.7	4.4	210.3	14.0	20.0	21.3	47.0	-0.26	0.09
		Pot 5	63.9	4.5	209.1	14.2	19.6	21.5	47.1	-0.29	0.06
		Pot 8	64.6	4.5	214.1	14.3	20.4	21.9	46.8	-0.27	0.05
	Tomato	Pot 3	63.8	4.4	211.5	14.2	20.2	21.8	47.2	-0.33	0.03
		Pot 6	62.8	4.4	209.5	14.1	20.4	21.8	47.0	-0.29	0.10
		Pot 9	63.8	4.5	213.6	14.0	20.6	21.8	47.0	-0.22	0.05
Week 2	Mustard	Pot 1	54.2	4.2	188.8	12.8	16.6	18.3	47.4	-0.30	0.11
		Pot 4	44.8	4.2	171.5	11.6	13.9	16.2	47.3	-0.23	0.09
		Pot 7	54.1	4.3	192.0	12.8	16.6	18.4	47.9	-0.19	0.03
	Spring Wheat	Pot 2	62.8	4.3	200.6	13.8	18.3	20.7	45.2	-0.34	0.03
		Pot 5	62.8	4.4	198.2	13.6	17.7	20.9	44.3	-0.22	0.08
		Pot 8	62.3	4.4	203.3	13.8	18.7	21.0	44.8	-0.21	0.03
	Tomato	Pot 3	60.2	4.2	202.7	13.3	18.8	21.3	46.8	-0.32	0.10
		Pot 6	62.1	4.3	208.6	13.5	19.5	21.5	47.0	-0.25	0.04
		Pot 9	62.1	4.3	208.0	13.7	19.8	21.3	46.8	-0.26	0.08
Week 3	Mustard	Pot 1	32.7	3.7	151.5	9.3	9.9	11.1	43.3	-0.16	0.09
		Pot 4	15.8	3.7	119.4	6.8	5.8	5.4	42.2	-0.15	0.08
		Pot 7	24.5	3.7	130.4	8.1	6.5	7.6	44.1	-0.11	0.08
	Spring Wheat	Pot 2	59.0	3.9	178.1	12.4	13.2	18.5	38.1	-0.20	0.12
		Pot 5	56.8	3.8	158.3	11.5	10.0	17.2	29.6	-0.01	0.22
		Pot 8	60.4	4.0	187.5	12.8	14.5	19.8	38.7	-0.17	0.11
	Tomato	Pot 3	51.4	3.6	185.8	11.2	13.9	18.7	43.5	-0.17	0.04
		Pot 6	55.0	3.6	194.1	12.0	15.5	19.6	43.8	-0.17	0.02
		Pot 9	57.2	3.9	199.1	12.4	16.3	20.1	44.5	-0.17	0.17
Week 4	Mustard	Pot 1	15.7	3.4	116.0	6.2	6.2	5.2	39.4	-0.13	0.05
		Pot 4	1.7	3.4	83.9	3.1	1.1	4.0	38.1	-0.07	0.15
		Pot 7	2.3	3.4	70.3	3.4	0.4	0.4	39.8	-0.10	0.12
	Spring Wheat	Pot 2	54.2	3.8	138.6	11.2	8.3	17.1	30.0	0.00	0.10
		Pot 5	51.2	3.6	108.6	9.9	3.8	15.8	13.1	0.33	0.15
		Pot 8	55.7	3.9	151.9	11.5	9.6	18.0	28.5	-0.06	0.11
	Tomato	Pot 3	39.7	3.4	157.3	9.1	9.6	16.9	42.2	-0.14	0.13
		Pot 6	46.3	3.4	175.5	10.3	12.0	18.1	43.0	-0.16	0.05
		Pot 9	50.6	3.8	183.2	10.8	13.2	18.7	43.9	-0.14	0.05
Week 5	Mustard	Pot 1	5.4	3.3	92.2	3.6	2.7	0.3	35.7	-0.04	0.07
		Pot 4	0.1	3.5	61.3	0.3	0.2	0.3	36.4	-0.11	0.11
		Pot 7	0.0	3.1	35.0	0.1	0.0	0.5	34.2	-0.02	0.12
	Spring Wheat	Pot 2	49.3	3.6	93.2	9.6	3.0	15.3	17.4	0.22	0.03
		Pot 5	47.5	3.4	76.1	8.8	0.2	14.5	2.6	1.36	0.14
		Pot 8	51.7	3.7	115.1	10.3	5.2	16.4	19.0	0.17	0.10
	Tomato	Pot 3	27.5	3.1	130.2	6.8	4.9	14.3	40.9	-0.10	0.03
		Pot 6	34.1	3.2	147.1	7.9	7.4	15.7	41.7	-0.10	0.08
		Pot 9	39.8	3.6	155.6	9.0	9.3	16.6	43.2	-0.20	0.08
Week 6	Mustard	Pot 1	0.4	3.1	77.6	0.8	0.5	0.4	33.4	-0.08	0.14
		Pot 4	0.0	3.4	50.3	0.1	0.2	0.4	35.4	0.06	0.28
		Pot 7	0.0	3.5	21.6	0.0	0.1	0.8	34.7	0.01	0.10
	Spring Wheat	Pot 2	44.9	3.2	63.2	7.4	0.0	11.4	4.3	0.75	0.03
		Pot 5	44.7	3.0	60.0	7.0	0.0	12.1	0.2	0.00	0.00
		Pot 8	50.6	3.4	94.2	8.1	1.3	14.2	9.6	0.51	0.13
	Tomato	Pot 3	16.5	2.9	111.3	4.1	0.6	9.5	38.9	-0.06	0.04
		Pot 6	21.7	2.9	124.3	4.9	2.4	10.6	39.2	-0.09	0.04
		Pot 9	31.5	3.4	144.6	6.6	6.2	13.5	41.6	-0.13	0.06

Table S5: Composition (major element concentration (in µg g^{-1}) and silicon isotopic composition) of the weekly sampled nutrient solutions for the individual pots. See Table S3 for the starting composition (week 0).

Methods

Method S1 Preparation of the nutrient solution

The nutrient solution was prepared from technical graded salts and dissolved in 10 L of ultrapure water. Macro nutrients 1.23 g $MgSO_4 \cdot 7H_2O$, 3.54 g $Ca(NO_3)_2 \cdot 4H_2O$, 0.33 g Ferric sodium EDTA, 3.6 g KNO_3, 1.1 g KCl and 0.82 g KH_2PO_4. Micro nutrients: 0.55 mg $Al_2(SO_4)_3$, 0.28 mg KJ, 0.28 mg KBr, 0.55 mg TiO_2, 0.28 mg $SnCl_2$ $2H_2O$, 0.28 mg LiCl, 0.39 mg $MnCl_2$ $4H_2O$, 6.1 mg H_3BO_3, 0.55 mg $ZnSO_4$, 0.55 mg $CuSO_4$ $5H_2O$, 0.55 mg $NiSO_4$ $6H_2O$, 0.55 mg $Co(NO_3)_2$ $6H_2O$, 0.05 mg As_2O_3, 0.28 mg $BaCl_2$, 0.05 mg $Bi(NO_3)_3$, 0.05 mg Rb_2SO_4, 0.28 mg K_2CrO_4, 0.05 mg KF, 0.05 mg $PbCl_2$, 0.05 mg $HgCl_2$, 0.28 mg MoO_3, 0.05 mg H_2SeO_4, 0.28 mg $SrSO_4$, 0.05 mg H_2WO_4, 0.05 mg VCl_2). Silicon: 2.03 g ~~$NaSiO_4$.~~$Na_2O_7Si_3 \cdot 3H_2O$. pH was adjusted to 6.0 using HNO_3 (PA grade).

Method S2 Plant germination and growth conditions

Plant seeds were germinated in in Petri dishes containing a nutrient solution of half the concentration than the solution used for growth experiments (Methods S1) and in the absence of $NaSiO_4$. After cotyledons germinated, seeds and roots were clamped in a foam ~~block~~disk (3 cm high with a diameter of 2.5 cm) and each seedling (foam ~~block~~disk) transferred to a PP vial (50 mL centrifuge tube) filled with half-concentrated nutrient solution without $NaSiO_4$. Two weeks later, the foam ~~blocks~~disks including young plants were transferred to the experimental containers, four plants per container, 3 replicated container per species. These containers were opaque plastic containers 25.5 cm high, 20.5 cm deep and 20.5 cm wide (with a wall thickness of 0.5 cm). In order to reduce evaporation and to prevent algae growth in the nutrient solution, the containers were closed with opaque lids which had holes for the plants (foam ~~blocks~~disks). Germination and plant cultivation were performed in a growth chamber under controlled conditions. The temperature in the growth chamber during the day and night was maintained at 18 °C for 14 h and at 15 °C for 10 h, respectively, and the daylight intensity at the top of the container was adjusted to 350 µE m^{-2} s^{-1}) at the start of the experiment. The relative humidity was maintained at approximately 65 %. For comparability, the cultivation conditions for the three species were the same, knowingly they are not equally suited for all species. The relatively low temperatures may have inhibited the growth of the more thermophilic tomato, while the conditions for mustard and summer wheat were close to their optimum. To supply the roots with oxygen, perforated PVC tubes were used to inject (approx. 6 L) room air into the nutrient solution twice a day for two hours each. The transpired water was replenished weekly with ultrapure water.

Method S3 Dried plant and nutrient residue digestion and chromatographic purification of Si

The crucibles containing the sample (dried down nutrient solution or charred plant material with approximately 400 mg NaOH) were placed in a high temperature furnace at 750 °C for 15 min. After cooling down the crucibles were cleaned externally with ultrapure water and placed in precleaned 50mL PP centrifuge tubes and covered with ultrapure water for 24 h. Thereafter, the crucibles were placed in an ultra-sonic bath for 30 min to facilitate the dissolution of the fusion cake. This solution #1 was decanted and collected in precleaned PP flask. The silver crucibles were then stored for ~3 h in a 0.03 M HCl solution and this solution #2 was combined with solution #1 in the PP flask. Using concentrated HCl the pH was adjusted to 1.5. If the concentration was expected to be above 60 µg g^{-1} additional 0.03 M HCl solution was added. 1:10-fold dilution was analysed by ICP-OES to determining the Si content. Approximately 60 µg Si from are loaded onto precleaned and preconditioned columns using a cation exchange resin (1.5 mL, DOWEX 50WX8, Sigma-Aldrich) and eluted using 5 mL ultrapure water. The cation exchange resin is then regenerated using HCl and HNO_3. The Si yield of the fusion procedure and the column chemistry was determined in a 1:10-fold dilution by ICP-OES.