Dear Prof. Bahn

In the following we summarise our responses to Dr. Delvigne, Prof. Hodson and the Anonymous Reviewer 2. We are confident that our answers provide a significant improvement of our manuscript and would like to thank the reviewers for their suggestions.

Detailed response to Camille Delvigne

The manuscript " Silicon isotope fractionation and uptake dynamics of three crop plants: laboratory studies with transient silicon concentrations" by Daniel Frick et al. brings out two important points: (a) Si isotope fractionations during plant uptake are similar no matter the Si is taken up actively or passively with water flux; (b) contrasted Si isotopes fractionations at the root-shoot interface reveal different plant Si accumulation strategies. Until now, this could only be speculated from data in the literature and for once it is clearly demonstrated. This conclusion is of great interest for the community and I'm convinced that this study will be really helpful for a large number of studies. Also, I would like to thank the authors for the high quality of their study at all steps. The experiment is well-designed and fit-for-purpose, the dataset is of high quality, the manuscript is very well written and easy to follow. It's a pleasure to read this work that is perfectly adapted to Biogeosciences. Overall, there is very little to suggest in terms of improvements but here are some minor comments.

Thank you very much for the validation of our work. It means a lot. In the following we are responding to your questions and recommended improvements in detail. We will supply the improved manuscript with track changes in the later process after the discussion has ended.

Title: I'm not sure that the term "transient" is the best one. To me, it's not appropriate but I'm not a native speaker. I would prefer something like "exhaustible" or "finite". Also, I would have loved a title less technical to attract more readers but it's a safe choice.

We agree that the title is a very technical description of the paper – after some intense discussions we have come up with a shorter title for the manuscript which still grasps the essence of our work:

Silicon stable isotope fractionation and uptake dynamics of crop species

Material and methods: I agree with Reviewer 2 that it would be useful to add details on how transpiration was measured.

We have added the information in a revised version of the manuscript. The following amendments were made in section 2.3 regarding the details how we measured the transpiration:

"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 lid, and we thus neglect evaporation."

Additionally, we have also given our definition of transpiration in *ch.* 2.6.1:

"We define the plant transpiration as the amount of water taken up by the plants followed by transpiration. The transpiration is measured weekly by weighing the pots without the lids and plants. The difference in mass to the previous week is considered 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 guttation (Joachimsmeier et al., 2012)."

L89: Have you checked the Si solubility limit at 15C? No sign of polymerization?

We have not spectroscopically searched for absence of polymerisation in the nutrient solutions. The solubility for amorphous silica at 25 °C is reported to be ~116 µg/g (Gunnarsson and Arnórsson, 2000), using their reported temperature dependence the solubility of SiO_2 at 15 °C is ~95 µg/g and for 18 °C ~101 µg/g. Our starting concentration is slightly above the solubility limit between 15-18 °C (by 2-5 µg/g Si). We did however not observe a significant change in the silicon isotope composition during the early course of the experiments. We would expect this when a significant amount of silicic acid polymerises. We have made an amendment to section 4.1 to describe this concern:

"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."

Section 2.5.1 and 2.5.3. Why don't you analyse Si isotopes of nutrient solutions directly after a cationic purification? The content of anions is too high? As salts are not detailed in section 2.1 it's not obvious what could compromise the analysis. It's worth mentioning what you feared with these samples. I guess you did not choose the easy way for a reason.

We have followed the procedure of (Steinhoefel et al., 2017) due to two concerns: the possible interference of the organic content which could be excreted by the roots and the relative high content anions. High temperature NaOH fusion is our 'go-to-method' and we have not evaluated a direct cationic purification. We have rephrased this: see below for the improved passage.

L126: It might be useful to rephrase this sentence that is a bit confusing. I had to read the sentence a few times to understand that the important thing is the amount of NaOH/g Si and not the molarity of the solution. It's worth explaining why you add a solution and not a powder directly as for solid samples. I guess it's to recover Si left on the crucible sides.

Well observed, this is exactly the reason we use a solution of NaOH instead of the pellets/powder. We have rephrased this: see below for the improved passage.

Have you tried this protocol with dissolved references like a solution of BHVO-2? There are so many different protocols for solution with a complex matrix that a quality check is useful. Alternatively, it is worth mentioning that your protocol is equivalent to the one of Steinhoefel et al 2017 (excluding the destruction of DOC) as you both use 1mmol of NaOH / 100g Si (if my calculations are correct).

Throughout the NaOH fusion and chromatographic separation we have used BHVO-2 and ERM-CD281 as a quality control. However, for the drying step we could not find an appropriate reference sample in liquid form which could act as an independent control (dissolved but unpurified BHVO-2 would contain already a large amount of Na due to the fusion). We have however taken some measures to assure that the drying is not affecting the silicon isotope composition:

- We controlled the yield based on the amounts we dried down and the concentration measured after the NaOH fusion to assure no loss or gain.
- The overall blank levels were contributing less than 1% to the total amount of Si processed.

The passage 2.5.1 reads now:

"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."

L 150: It would be useful to add some references (e.g., Savage et al., 2014 for BHVO-2 and Delvigne et al., 2019 for ERM-CD281) Camille Delvigne, Abel Guihou, Jan A. Schuessler, Paul Savage, Sebastian Fischer, Jade E. Hatton, Kate R. Hendry, Germain Bayon, Emmanuel Ponzevera, Bastian Georg, Alisson Akerman, Oleg Pokrovsky, Frank Poitrasson, Jean-Dominique Meunier, and Isabelle Basile-Doelsch (2019). An inter-comparison exercise of the Si isotope composition of soils and plant reference materials. Geophysical Research Abstracts, Vol. 21, EGU2019-18488, 2019.

I'm hesitant to cite a single selected publication for BHVO-2 since more than 27 publications (to my knowledge) have helped to characterise BHVO-2 for its silicon isotope composition, I have thus opted for the GeoReM database. Regarding ERMC-CD281, I am happy to include the tremendous effort you and your colleagues made to characterise plants and soils for their silicon isotope composition and cite your EGU abstract as a reference:

"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)."

L338: It is hard to find its way with all these data as you mix 30/28 and 29/28. It would be less confusing for the reader if you stick only to 30/28 fractionation factors and just specify when it is recalculated from 29/28. Also, it may be useful to remind here your own 30/28 fractionation factors to directly see that your data are within the literature range. It's also worth mentioning that all species in your list are Si accumulators.

We agree that it is a very crowed section, we have taken your advice and only report 30/28 and indicate where we re-calculate the fractionation factor from a reported 29/28 ratio. Thank you also for pointing this out that we have likely measured the first Si fractionation factors for non-accumulating Si species, we have added this information:

"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)."*

L384-392: The link with the previous section is a bit poor. It's too bad to end the discussion with a weak paragraph:

In retrospective we agree and have decided to remove the paragraph.

L 394: It would be more careful with the "species-specific" term as your study demonstrates that your fractionation factors are rather similar despite your 3 plants have very different Si strategies. This might be confusing and sounds contradictory.

This is true and was not the intended meaning of species-specific. We have rephrased the sentence and hope this is now clearer:

"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·ln(α) for tomato -0.33 ‰, for mustard -0.55 ‰ and for wheat -0.43 ‰ which are indistinguishable within uncertainty."

Detailed response to Martin Hodson

This paper represents an interesting investigation into Si isotope fractionation in three contrasting crop plants. I am not aware that anyone has taken this approach before. I have seen that another referee has concentrated on the methodology, and I will not go over these points again. Rather I will look mostly at the interpretation of the results, and give some suggestions for improvements in the discussion.

We have responded to the comments from *Anonymous Reviewer 2* and Dr. Delvigne and have clarified our Materials and Method section.

Major Points

Line 12 and elsewhere. I am not sure that I would use "a variety of strategies (rejective, passive and active)." As we have come to understand Si uptake by plants it has become obvious that the different species form a spectrum. You mentioned Hodson et al. (2005) and the spectrum is very evident there. I would just say that you took species that take up Si to different extents.

We understand that the silicic acid uptake classification (active, passive or rejective) is not a strict metric and still source of an intense debate (see also Anonymous Reviewer 2 comment RC1 and RC4 regarding this topic). We have made adaptions and accounted for this throughout the manuscript. The major changes are:

Abstract: "*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."*

Ch. 1: *"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)."*

Ch. 2.6.1: *"The plant Si uptake characteristics can be classified based on the ratio between the measured 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."*

Line 22. Not always at the endodermis (rice)- some species have much more dispersed transporters in the root.

We have accounted for this and rephrased the sentence:

"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.

Line 24 and elsewhere. The finding of significant biogenic silica deposition in the roots of mustard is novel. As far as I am aware it is the first time in a non-woody dicot. The only dicot mentioned in the recent review of silicification of roots by Lux et al. (2020) is beech. I don't think you can really just

say "unpublished observations". We need to know more about this- is it endodermal deposition? A picture would help.

We have currently gathered only little data regarding the mustard root phytoliths and have decided not to include these results. One of the reasons is, that we do not have analysed '*fresh'* mustard roots and can thus not provide in depth review where those phytoliths are deposited. Based on your recommendation we have added our observations (SEM-EDX measurements of phytoliths extracted from dried mustard roots). Our colleague Danuta Kaczorek has obtained these results and we will thus include her in the author list.

The following changes are made to the manuscript:

Ch. 2.7 Method description for the phytolith extraction and SEM-EDX measurement.

Ch. 3.5 Results of the SEM-EDX measurements.

Ch. 4.2: *"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)."*

Ch. 4.4: *"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)."*

Added the following items to the supplement:

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."

Line 95 onwards: Sun et al. (2019) found that while there are two Si transporter homologues present in tomato (SILsi1, a homologue of the rice LSi1 influx transporter; and SILsi2, a homologue of the rice LSi2 efflux transporter), only SILsi1 is active. They suggest that the absence of active SILsi2 explains the low levels of Si accumulation in this species.

Thanks for bringing this study to our attention: we have included it:

Line 102: *"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."*

Line 321 onwards. As already stated phytoliths in the mustard root is a novel finding, and "data not shown" is not really good enough.

See comment on Line 24. We have added SEM-EDX images of the root phytoliths of mustard.

Line 340 and elsewhere: I really don't like reviewers that try to increase their citations by recommending their own papers! However, there are some cases where this is justified. I am very surprised that you did not mention the work of Hodson et al. (2008) on Si isotopes in wheat. Our plants were grown in soil to maturity, and so it was a different setup. But one thing is very clear: there is significant fractionation within the wheat shoot. This does not invalidate your results, but it should be noted (our culm d30Si is negative, but leaf sheaths and blades are positive leading to a

positive value overall). The second point is that we also found that the lighter isotopes were deposited first. We could not measure Si in the roots because of soil contamination, but we said "It is apparent that there are two main routes for Si transport within the wheat plant, and that heavier isotopes increase towards the end of both routes: (1) culm » leaf sheath » leaf blade; and (2) culm » rachis » inflorescence bracts. A similar pattern was reported by Ding et al. (2005) working on rice. They considered that the process of Rayleigh fractionation explained the accumulation of heavy isotopes in the upper parts of the plant. Essentially, this would involve the lighter 28Si isotope being more reactive, and thus more likely to be deposited in phytoliths. Thus, in wheat, proportionately more 28Si isotope would be deposited in the culm phytoliths, and a greater proportion of 30Si and 29Si would continue in the transpiration stream to the leaf sheath. In the sheath the same fractionation occurs, leading to an even greater concentration of heavier isotopes in the leaf blade." This is exactly the same process that you postulate is happening in the wheat roots before Si flows on to the shoots. So our work confirms your ideas in section 4.4.

In the paragraph starting on line 338ff we discuss the literature for which we were able to report fractionation factors. The Hodson *et al.* 2008 manuscript does unfortunately not provide the soil water or soil silicon isotope composition to calculate the fractionation factor. We acknowledge that there is significant internal fractionation observed (e.g. Ding et al., 2005; Hodson et al., 2008) which is one of the reasons we have decided to investigate the silicon isotope fractionation on bulk shoots and roots and not in greater detail. We made changes in section 4.4 to highlight this confirmation and included the Hodson et al. 2008 reference:

Line 390: *"Within the shoots, Si is not homogenously distributed. Several researcher 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."*

Minor correction Line 43 Yan

We have used the official notation used by *Journal of Integrative Agriculture* (https://www.sciencedirect.com/science/article/pii/S2095311918620374) for Gua-chao YANs last name.

Detailed response to the Anonymous Referee #2

We have considered your suggestion and have further clarified the materials and methods section. In detail we provide our answer to your questions and suggestions:

Line 89: What is this? A somewhat unconventional unit. Do you mean & Line 90: Do you mean 49.5 mg/L? Is this the concentration of Si or the salt?

µg/g is a SI unit for concentration. Our measurements are based on weighing the solutions, thus we report the concentration as 'per g' and not as 'per mL'. For the convenience of the reader we expanded the sentence and provide the concentration in mM and specified that the concentration refers to Si:

"Silicon was added in the form of $NaSiO_4$ 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."

Line 94: What does this mean? How can they 'reject' silicic acid? & Line 94: Active uptake of silicic acid? Where is the evidence that this occurs?

Active, passive and rejective Si uptake is a concept which has been proposed by several groups: see e.g. (Hodson et al., 2005; Takahashi et al., 1990) and also the review by M. Hodson for this manuscript: https://www.biogeosciences-discuss.net/bg-2020-66/bg-2020-66-RC2.pdf). The classification is based on the amount of silicon is taken up in relation to the water uptake and is also explained in Line 171ff. As also M. Hodson remarked in his review, the uptake of Si is not a strict classification, but a spectrum which allows to qualitatively describe the Si uptake. We have accounted for this and clarified it:

Abstract: "*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."*

Ch. 1: *"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)."*

Ch. 2.6.1: *"The plant Si uptake characteristics can be classified based on the ratio between the measured 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."*

Line 99: Really, so silicic acid does not follow water into either mustard or tomato? Do you have evidence to support this?

This is not what has been stated in the text. We justify the selection of the plant species and provide information which additional transporter channels / proteins are present in the investigated plants.

Line 102: Added Si, but how much Si was present in these solutions?

The amount of Si introduced by the other nutrient salts and the water was not resolvable using the ICP-OES, thus we considered these negligible. We have changed the sentence to:

"Plant seeds were germinated in Petri dishes with half-strength nutrient solution used for the later growth experiment that contained no added $NaSiO_4$."

Line 104: What about the significant increase in sodium content, did you have a control for this?

We did not counterbalance or remove the Na which has been introduced by the addition of $NaSiO_4$.

Line 105: How did you measure the volume of transpired water?

The pots were weighted weekly without the lid and plants, using a balance. The weight difference to the previous week is reported as volume taken up by the plants, assuming a density of 1 g/mL. We replenished the pots by filling up with ultra-pure water to the weight from the previous week. The pots were closed with a lid, and we thus neglect evaporation. The term transpiration is thus referred to the water taken up, which is either lost by transpiration and guttation or stored in the biomass. Based on previous reports (e.g. Joachimsmeier et al., 2012) the amount of fluid lost through guttation, was considered negligible during the course of the experiment. We have added this information:

"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 lid, and we thus neglect evaporation."

Line 112: What about other forms of water loss such as guttation?

See question before. We considered water loss through guttation negligible. We specified how we defined plant transpiration in Ch. 2.6.1:

"We define the plant transpiration as the amount of water taken up by the plants followed by transpiration. The transpiration is measured weekly by weighing the pots without the lids and plants. The difference in mass to the previous week is considered 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 guttation (Joachimsmeier et al., 2012)"

Line 115: What kind of extracellular Si deposits? Do you simply mean that you washed off the nutrient solution?

Thanks for bringing this to our attention, we have clarified the sentence:

"The roots were immersed multiple times in ultrapure water to remove potential extracellular Si deposits and attached nutrients."

Line 118: how?

We have added a link to chapter 2.5.2 where the digestion procedure is explained.

Line 123: Why are all essential details of methods in Supplementary files, they need to be here in M&M.

We have expanded the section and explained how we have performed the concentration measurements by ICP-OES:

"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."

Line 125: What do you mean? How do you know that the aliquot contains this amount of Si? Where are the methods?

The concentration is known from the measurement by ICP-OES, we have clarified this part:

"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."

Line 131: estimates based upon what?

The concentration was estimated by analysing an exploratory experiment, we have clarified this:

"50-800 mg of plant material, depending on the Si concentration determined in an exploratory study, 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 133: what does this mean?

We removed this information since the results were not presented in this study.

Line 134: what is the Si content of this salt?

We have specified what the Si content of NaOH was:

"After cooling 400 mg NaOH (TraceSELECT, Sigma-Aldrich, checked for low Si blank levels) was added."

Line 137: Does plant silica dissolve under these conditions?

The high temperature fusion of silicates, silicon, and bio silica (e.g. diatoms, phytoliths) using NaOH has been proven to be quantitative. The silicate is transformed in this fusion into its silicic form which can be dissolved in water.

Line 138: How? You convert Si to a cation? You need to fully explain these methods.

The dissolution procedure of silicates, silicon and bio silica is state of the art in geosciences. Si is present in SiO2 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).

We hesitate to include the entire Supplementary Method S3 into the main text, since this is a routine method applied in chemistry and geochemistry to dissolve silicates quantitatively.

Line 140: Again, the methods should be here and not in Supplementary files.

We have clarified that in the supplementary files a step-by-step procedure can be found. We hesitate to include the entire Supplementary Method S3 into the main text, since this is a routine method applied in chemistry and geochemistry to dissolve silicates quantitatively.

Line 161: It would seem that all measurements rely upon accurate measurements of water intake. Where have you written about how you measured the amount of transpired water? Why do you assume that all water uptake is reflected by this transpired volume? Again, what about processes like gutation. Even if your measurements of transpiration are accurate, they do not represent water uptake into the plant.

See response to your question on Line 105.

I think that what is actually demonstrated is that silicon as silicic acid follows water and that this is only a passive process. See attached.

We do not agree with your observation.

The expected Si uptake was calculated based on the amount of transpired water and the nutrient solution Si concentration. This expected Si uptake equals the amount of the passive process, where silicic acid follows the water. Our results, comparing the expected and the actual amount that plants taken up during growth (Fig. 1c), show a clear evidence that active, metabolism-driven processes or mechanisms must have been involved for wheat. There is no other explanation for the 2-fold excess of the theoretically taken up amount of Si which we observe for wheat. Of course, this does not mean that the sub-processes you have indicated did not also occur passively.

Additional changes

We have made the following additional changes to clarify the manuscript.

New-Coauthor: Danuta Kaczorek from the Leibniz Centre for Agricultural Landscape Research (ZALF) has extracted the phytoliths and obtained the SEM images.

Abstract and introduction: we have revised the language in those two chapters.

Fig 1c: Changed the axis label to “Expected Si uptake (mg)” and “Measured : expected Si uptake” and clarified this in section 2.6.1.

Fig 2, caption: We have rephrased the caption of Figure 2.

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Silicon stable isotope fractionation and uptake dynamics of crop species~~Silicon isotope fractionation and uptake dynamics of three crop plants: laboratory studies with transient silicon concentrations~~

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Abstract. That ~~Silicon~~ silicon ~~has been recognized~~ is an important element in global biogeochemical cycles ~~for a long time~~is widely recognized. Recently, its relevance for global crop production ~~gains~~ gained increasing attention too. Silicon is beneficial for plant growth and is taken up in considerable amounts by crops~~,~~ like~~wise~~ rice or wheat. However, plants differ in the way ~~The~~ they take up ~~incorporation of~~ silicic acid from ~~the~~ soil solution. Correspondingly ~~into the plants~~species ~~is~~encompass ~~forms~~ a broad spectrum, from varieties ~~which~~that reject silicic acid to species ~~which~~that actively incorporate ~~silicic acid,~~ it. Yet these classifications are ~~accomplished by a variety of strategies (rejective, passive and active) that are however to~~ subject to ~~an~~ intense debate. To forge a new perspective on the ~~underlying~~ processes involved, we investigated ~~how~~ the dependence of silicon stable isotope fractionation ~~during plant growth depends~~ on silicon uptake strategy, transpiration, water use, and Si transfer efficiency. Crop plants with ~~a~~ 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~~,~~. ~~they are~~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 preceding precipitation of~~the amount of ~~silicic~~ silicon ~~acid~~ previously precipitated in the roots and the presence of active transporters ~~at~~ in the root~~s endodermis~~. 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 ~~governed~~ 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). Developing and validating geochemical tools to trace plant Si uptake~~,~~ will improve our ability to ~~answer open~~address questions ~~about~~ on weathering, ecosystem nutrition strategies, and geo-pedo-~~sphere~~-biosphere interactions.

Despite having a disputed biochemical role, Si is considered beneficial for plant growth, including crops: Si increases abiotic stress mediation (heavy metal sequestration, 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~~.~~ ~~can be grouped into three categories depending on the relative~~According to the amount~~s~~ 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. (Coskun et al., 2019a; Exley, 2015; Exley et al., 2020). Genome sequencing has ~~uncovered~~ disclosed the transporter and mechanism that regulate 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). ~~The influence of~~How these different Si transporter and passive Si pathways and their ~~ir respective~~ resulting ~~relative~~ magnitude of Si uptake ~~on~~ affect the mobility of silicic acid within plants remains however unknown.

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 and Si (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)., and tThere 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. 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, with ~~a finite~~ nutrients being ~~supply~~ 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 ~~M~~methods

2.1 Nutrient s~~S~~olution

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 Na_2SiO_3 to an initial Si starting concentration of 49.5 µg·g^{-1} (1.76 mM). Detail compositions~~s~~ 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-~~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). ~~There is some~~An on-going 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$_3$. After cotyledons formed, seedlings were transferred into a foam block 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$_3$ 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 lid, and we thus neglect evaporation. 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 %. 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 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~~,~~. ~~b~~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. ~~For details of the analytical method and an extended verification see 'S1 Description of analytical methods' in Schuessler *et al.*, 2016.~~

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, ~~After~~ based on the concentration measured~~ments~~, 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 ~~C~~crucibles were then filled with ~~a solution containing~~ 400 mg NaOH (~~prepared from~~ Merck pellets, p.a. grade, previously checked for low Si blank levels) ~~in~~ 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 ~~together with~~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 ~~estimated~~ Si concentration determined in an exploratory study, 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 ~~the loss of ignition was determined and~~ 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 ~~high temperature~~ 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 ~~more~~ a detailed account of the procedureal steps~~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 measured weekly by weighing the pots without the lids and plants. The difference in mass to the previous week is considered 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 guttation (Joachimsmeier et al., 2012). ~~In order to~~To compare the plant species with respect to their ~~water uptake~~ transpiration, ~~as well as~~ Si uptake, and Si transfer the following performance ratios were calculated at the end of the experiments:

1. Water use efficiency: total phytomass ~~kg~~ 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. The plant Si uptake characteristics ~~The uptake characteristics were~~ can be classified based on the ratio ~~of~~ between the measured and the ~~theoretical~~ 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. ~~The theoretical expected Si uptake was calculated based on the amount of transpired water and the nutrient solution Si concentration.~~

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):

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

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):

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

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 revile[d] ~~re~~markably high Si concentration and amounts in comparison to the other two crop specie[s] 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. [T]he 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 ~~very~~ 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. Mustard showed the highest, wheat

intermediate and tomatoes the lowest cumulative transpiration. The water use efficiency 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 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).

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 solution's 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) (Mariotti et al., 1981):

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

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 ($1000 \cdot \ln(\alpha)$ = -0.33 ‰), for mustard an $\alpha_{Plant-solution}$ of 0.99945 ($1000 \cdot \ln(\alpha)$ = -0.55 ‰), and for

wheat an $\alpha_{Plant\text{-}solution}$ of 0.99957 (1000·ln(α) = -0.43 ‰), respectively (Figure 3). We use a Monte Carlo approach to estimate uncertainty on $\alpha_{Plant\text{-}solution}$, by calculating $\alpha_{Plant\text{-}solution}$ on 500 permutations of the dataset in which values for δ^{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 µg·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) 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).

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

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 ~~(in~~with 1000·ln(α) ~~space)~~of -0.33 to -0.55‰. ~~than the ideal diffusion coefficient ratio (-0.41 ‰ versus -1.61 ‰). The overestimation of t~~The theoretical diffusion coefficient ~~to~~ exceeding the measured coefficient has been observed in other systems ~~before~~ (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.

4.2 Si uptake strategies

The ratio between measured Si uptake and the ~~theoretical~~ 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 c~~C~~losely related cereals have such transporters, ~~therefore~~ 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 ~~a~~ 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·L^{-1}). The transfer efficiency for tomato is significantly higher than mustard (3.5 ± 0.4, and 2.4 ± 0.3 mg shoot Si·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 ~~the P~~phytolith formation~~, which was observed in mustard roots (data not shown~~see Fig. S2). ~~could explain the lower Si transfer efficiency of mustard.~~ 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 ~~show that there are~~disclose two distinctly different Si uptake mechanisms ~~present~~: 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·ln(α) of -0.41 ± 0.09 ‰ (1 s). We can only speculate on the reasons for the plants~~'~~ ~~preference~~ preferring ~~for~~ ^{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)) 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 ~~including~~ include rice~~,~~: -0.30 ‰ (Sun et al., 2008), -1.02 ± 0.33 ‰* ~~-0.53 ± 0.17 ‰~~ (* indicates results ~~for~~ ~~$^{29/28}$Si,~~ recalculated from $^{29/28}$Si to $^{30/28}$Si~~,~~: ~~-1.02 ± 0.33 ‰,~~ Ding et al., 2005) and -0.79 ± 0.07 ~~(Sun et al., 2016a),~~ (Sun et al., 2016a); banana~~,~~: -0.77 ± 0.21 ‰* ~~0.40 ± 0.11 ‰~~ (~~for $^{29/28}$Si, recalculated to $^{30/28}$Si: 0.77 ± 0.21 ‰,~~ Opfergelt et al., 2006) and -0.68 ‰* ~~0.35 ‰~~ (~~for $^{29/28}$Si, recalculated to $^{30/28}$Si: 0.68 ‰, Delvigne et al., 2009),~~ Delvigne et al., 2009); and corn and wheat~~,~~: -1.00 ± 0.31 ‰* ~~0.52± 0.16 ‰~~ (~~for $^{29/28}$Si, recalculated to $^{30/28}$Si: 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). ~~These~~ 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 ~~applicable~~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 ~~could~~ can be explained by Si precipitation ~~reactions~~ in the roots. I~~(~~ndeed, we ~~see Fig. S2 for the~~ observed mustard root phytoliths; see Fig. S2~~,~~. ~~for wheat m~~Mineral deposition~~s~~ in ~~the~~ wheat roots ~~have~~ has also been observed ~~see~~ by Hodson & Sangster, ~~(~~1989~~)~~, supporting hypothesis ~~(~~3). Precipitation of biogenic silica in the root would enrich the residual mobile silicon pool ~~in the root~~ 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 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.

~~**4.5 Implications for terrestrial Si isotope cycling**~~

~~Several field~~ field ~~based studies have investigated the isotope fractionation induced by plants~~ . ~~In contrast to field based studies, the stable silicon isotope fractionation determined on bulk plants show a very narrow range from -1.02 to -0.30 ‰ with the exception of the positive fractionation factors by Sun et al., 2016b. The determined Si isotope fractionation factors~~ determined ~~in laboratory experiments~~ under a broad range of conditions ~~indicate that the physico-chemical processes governing Si uptake in a wide range of different plant species, are identical under a broad range of laboratory (environmental) conditions. The broader and larger magnitude of isotope fractionation observed in natural settings could be an observational bias when analysing and extrapolating from individual plant parts to whole plant fractionation and the challenges associated with~~ isotopic ~~characterisation of the plant~~ plant ~~available silicon pool. Our results demonstrate that the fractionation between roots and shoots is variable in direction and is controlled by internal plant processes, which are likely also being present within subparts of the roots and shoots~~ .

~~This implies that Si which~~ that ~~is liberated through weathering reactions, may be recycled multiple times through plants, re-dissolved into soil solution and precipitated into secondary minerals before being exported from the ecosystem. Based on plant and phytolith data aggregated by Frings et al., 2016, biogenic silica is unlikely one of the main export flux of Si from the ecosystems. Plants are thus an important factor for the internal ecosystem element cycling (Uhlig and von Blanckenburg, 2019), but not for the particulate Si export. The plant- internal processes whichthat distribute, and deposit Si have however, influence on the amounts and chemical form of Si which is cycled through the ecosystem, and these processes can be traced using stable isotopes to identify the mechanism.~~ ~~as biogenic silica, secondary clays, or as dissolved Si. The relative magnitude between these fluxes depends however on the environmental conditions (Frings et al., 2016; Sommer et al., 2006, 2013). The isotope composition of the dissolved Si in river water shows almost exclusively a heavier silicon isotope signature than the bedrock they drain (Frings et al., 2016; Opfergelt and Delmelle, 2012). To close the Si isotopic mass balance therefore requires an isotopically light solid counterpart (Bouchez et al., 2013). The plant and phytolith data aggregated by Frings et al., 2016~~

~~suggest that biogenic silica is unlikely one of the main export flux of Si from catchments. Plants are an important factor for the internal ecosystem element cycling (Uhlig and von Blanckenburg, 2019), but not for the particulate Si export.~~

5 Conclusion

~~We have confirmed that t~~The amount of Si uptake into crop plants and the distribution of Si within them is species-specific ~~and complex~~, ~~involving~~ and the ~~rejective, passive and active~~uptake ~~mechanisms~~strategies ~~processes in~~are in operation in ~~varying~~ variable relative proportions. However, ~~R~~regardless of the uptake strategy (active and rejective) all three crop species studied preferentially incorporate light silicon (^{28}Si) with a fractionation factor 1000·ln(α) for tomato -0.33 ‰, for mustard -0.55 ‰ and for wheat -0.43 ~~‰.~~ ‰ which are indistinguishable ~~Within~~ within uncertainty~~, the fractionation factors between these species are indistinguishable~~. 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~~, that is enriched in ^{28}Si over ^{30}Si.~~ governs the isotope composition of ~~the mobile Si pool. A likely change in the chemical environment in the roots results in~~ is the precipitation of biogenic silica, ~~which~~that ~~is enriched in ^{28}Si over ^{30}Si. The~~ remaining ~~silicic acid~~Si ~~which is~~ transported into and deposited within the shoots that is thus enriched in ^{30}Si. For plant species ~~where~~ in which ~~the precipitation of~~no biogenic silica ~~precipitation~~ is ~~absent,~~precipitated in the roots the Si ~~pool~~ is transported in an isotopically unmodified form. ~~unchanged, and the~~Any further ~~the~~ transport is governed by a diffusion ~~process and hence~~in which ~~preferentially light silicon~~ ^{28}Si is preferentially transported into the shoots. A full description of the isotope ~~and elemental~~ fractionation during transport of silicic acid and precipitation of biogenic silica requires ~~a better understanding of the~~knowing the ~~biological~~ bio-molecular processes ~~and molecules~~ involved in the dehydration of silicic acid and its conversion into amorphous silica (He et al., 2015; Leng et al., 2009)~~ and ideally model system where these processes can be followed~~. ~~Here~~Towards this task~~,~~ an additional facet of the toolbox of isotope geochemistry is well-poised~~, :~~ e.g.~~such as by~~ employing ~~temporal~~ isotope-spiking ~~experiments~~ during ~~a short period of the~~ plant growth and ~~-~~ripening. Such experiment ~~can~~ will ~~deliver~~ provide insights into the mobility and pathways of ~~newly and formerly acquired~~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– S4.

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690 **Figures**

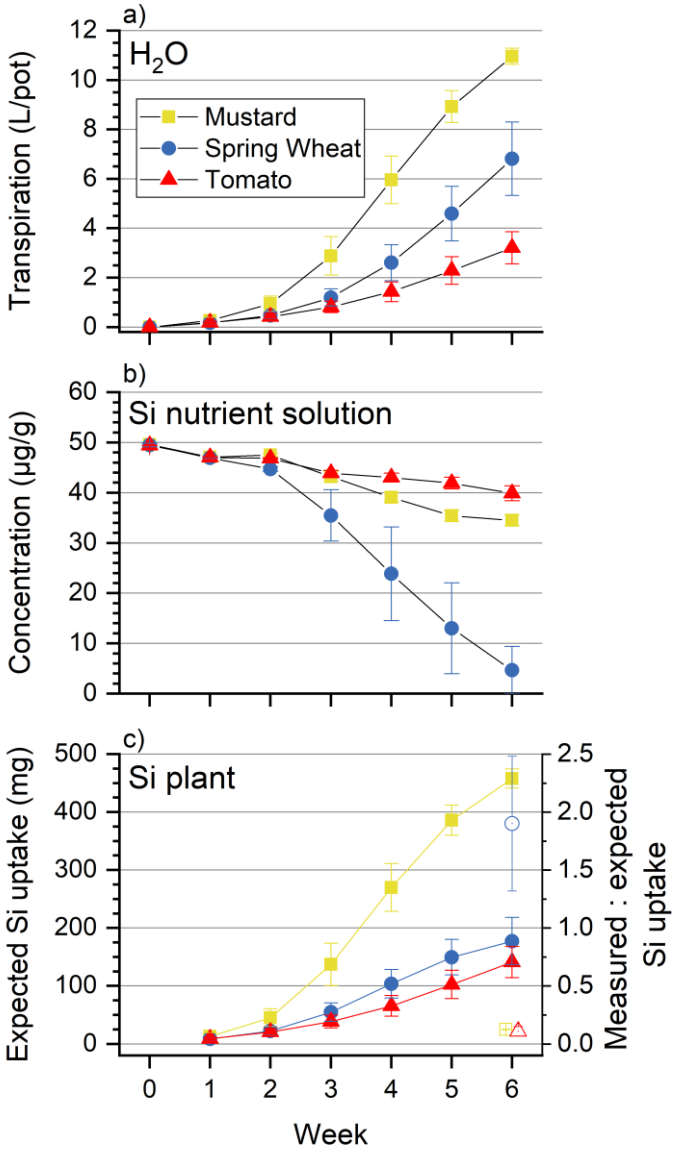

Figure 1: Cumulative transpiration (panel a), Si concentration in the nutrient solution (in µg/g, panel b) and the ~~theoretical~~ expected Si uptake through transpiration of tomato, mustard and spring wheat during 6 weeks (panel c). Shown is the m~~M~~ean ± standard deviation from 3 pots with 4 plants each. In panel c) ~~A~~ a ratio of measured and ~~theoretical~~ expected Si uptake (open symbols) of 695 **greater than 1 indicates an active uptake mechanism, a ratio much smaller than 1 a rejective strategy.**

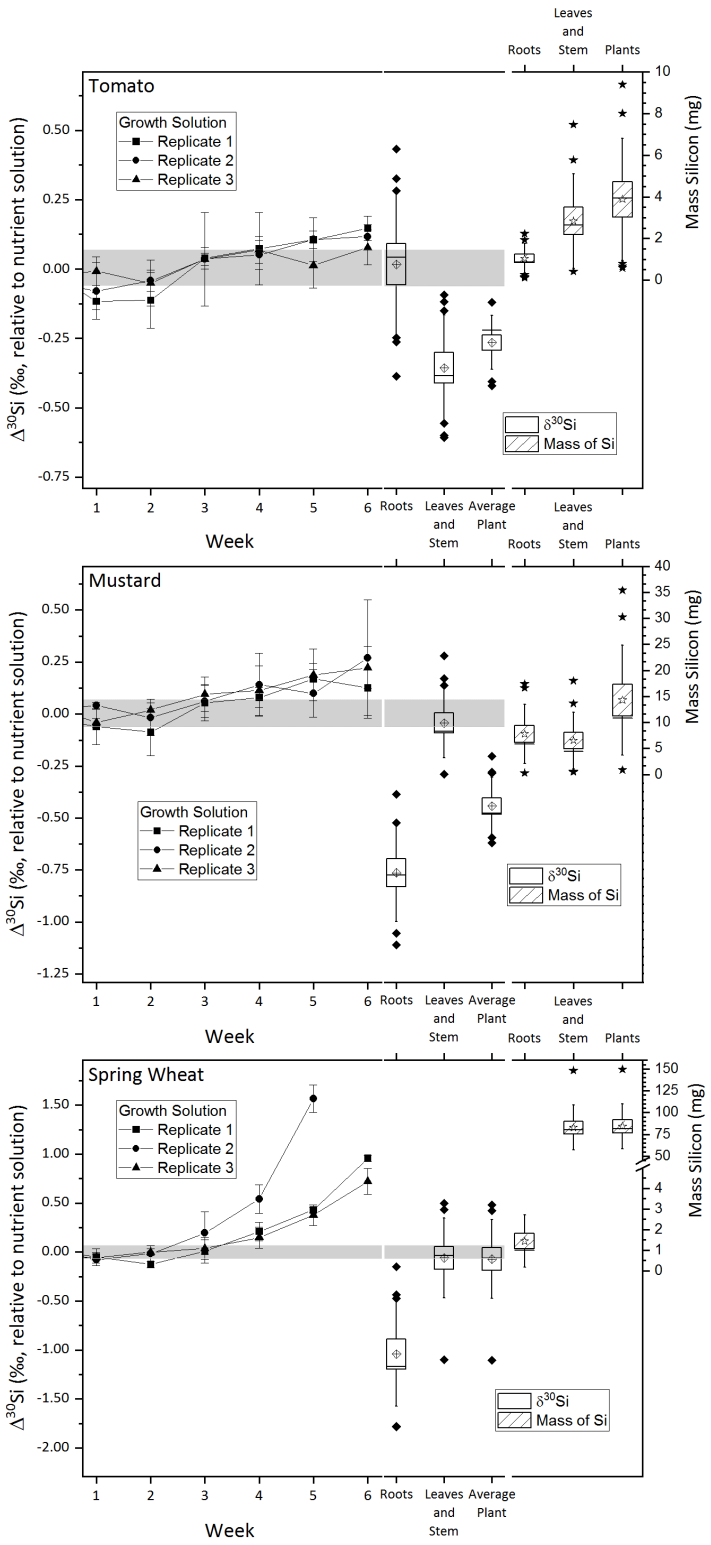

**Figure 2: Silicon isotope composition (left) and mass of silicon taken up (right) during the growth of tomato ~~(top panel)~~, mustard, ~~(mid panel)~~ and wheat ~~(bottom panel)~~. ~~On t~~The left y-axis shows the $\delta^{30}Si$ ~~composition~~ in ‰ relative to the nutrient solution, ~~is reported,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

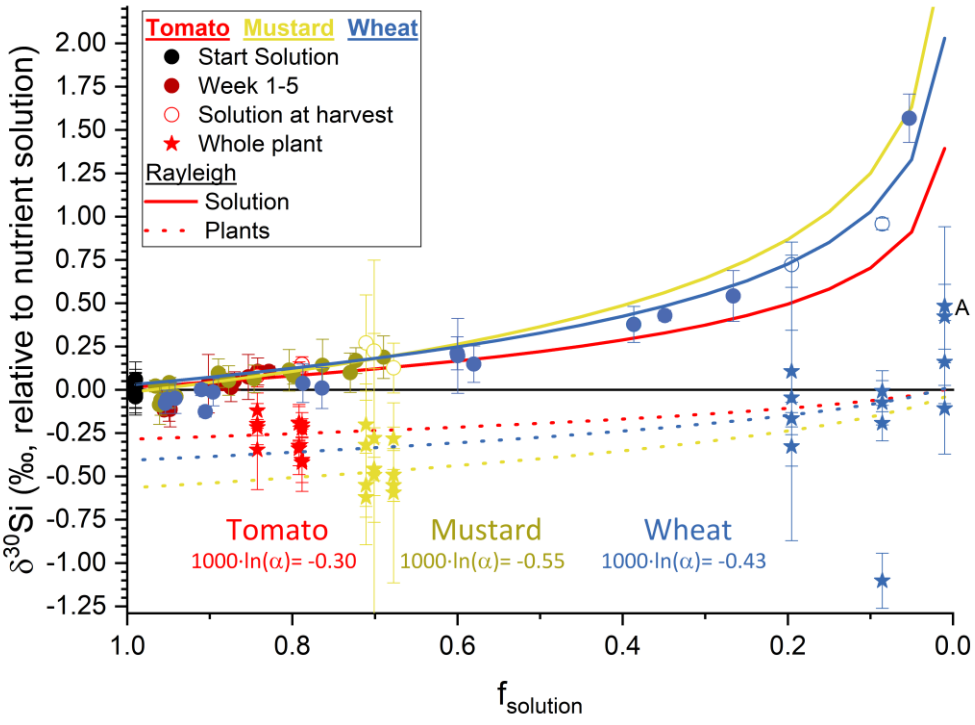

Figure 3: The silicon isotope composition (expressed in δ^{30}Si ‰ relative to nutrient solution) versus the amount of silicon taken up by the plants (expressed as dimensionless $f_{solution}$) (~~dots~~ circles represents the nutrient solution, tomato in red, mustard in yellow and wheat in blue, starting 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.

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).

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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 / ~~theoretical~~ 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 plants each). The uptake classification is based on the ratio of measured and ~~theoretical~~ 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. (4)) 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**
$1000 \cdot \ln(\alpha)$ [‰]	-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 $1000 \cdot \ln(\alpha)$ numerically approximated by reducing root-mean-square-deviation ('best fit') using Eq. (5) and uncertainties (1 s) from Monte Carlo method with n=500 seeded individual data sets.

Supplement to: Silicon stable isotope fractionation and uptake dynamics of crop species~~Silicon isotope fractionation and uptake dynamics of three crop plants: laboratory studies with transient silicon concentrations~~

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Figures

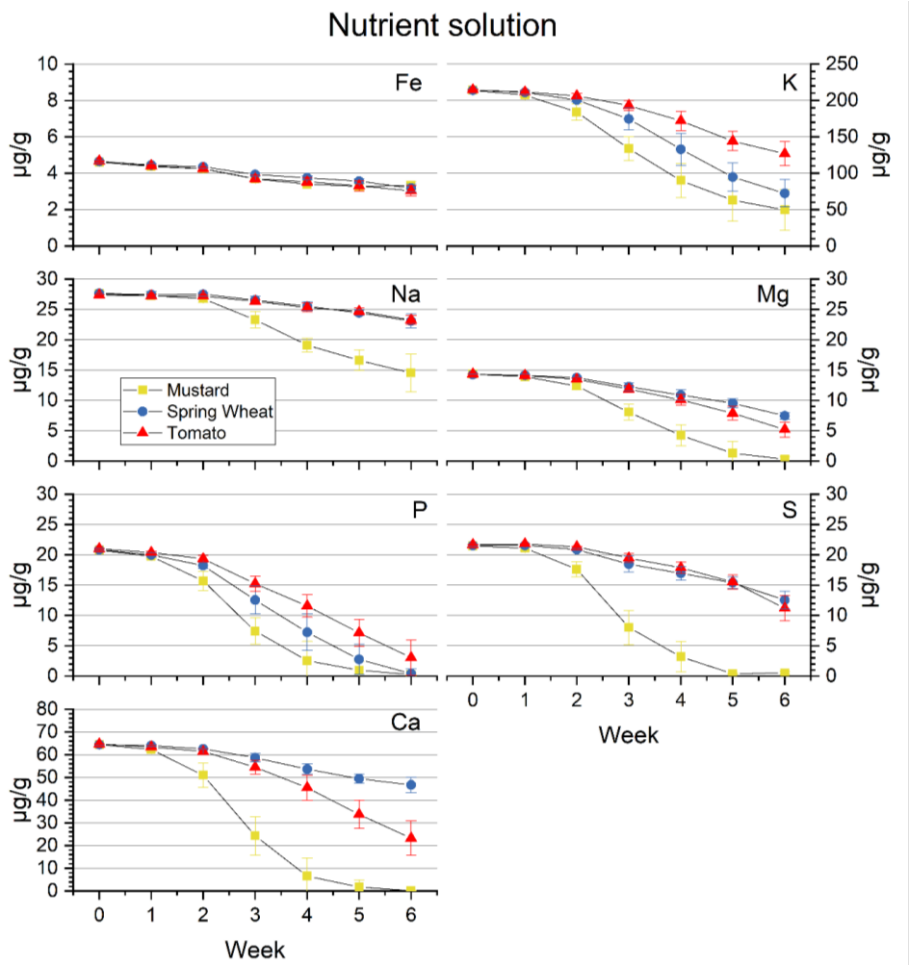

Figure S 1: Temporal evolution of nutrient concentrations during the hydroponic growth of the different plant species. Concentration (Fe, K, Mg, Ca, P, S, Na in µg·g^{-1}) are based on the mean of the three replicated containers, uncertainty shown is 1 standard deviation of those replicated containers.

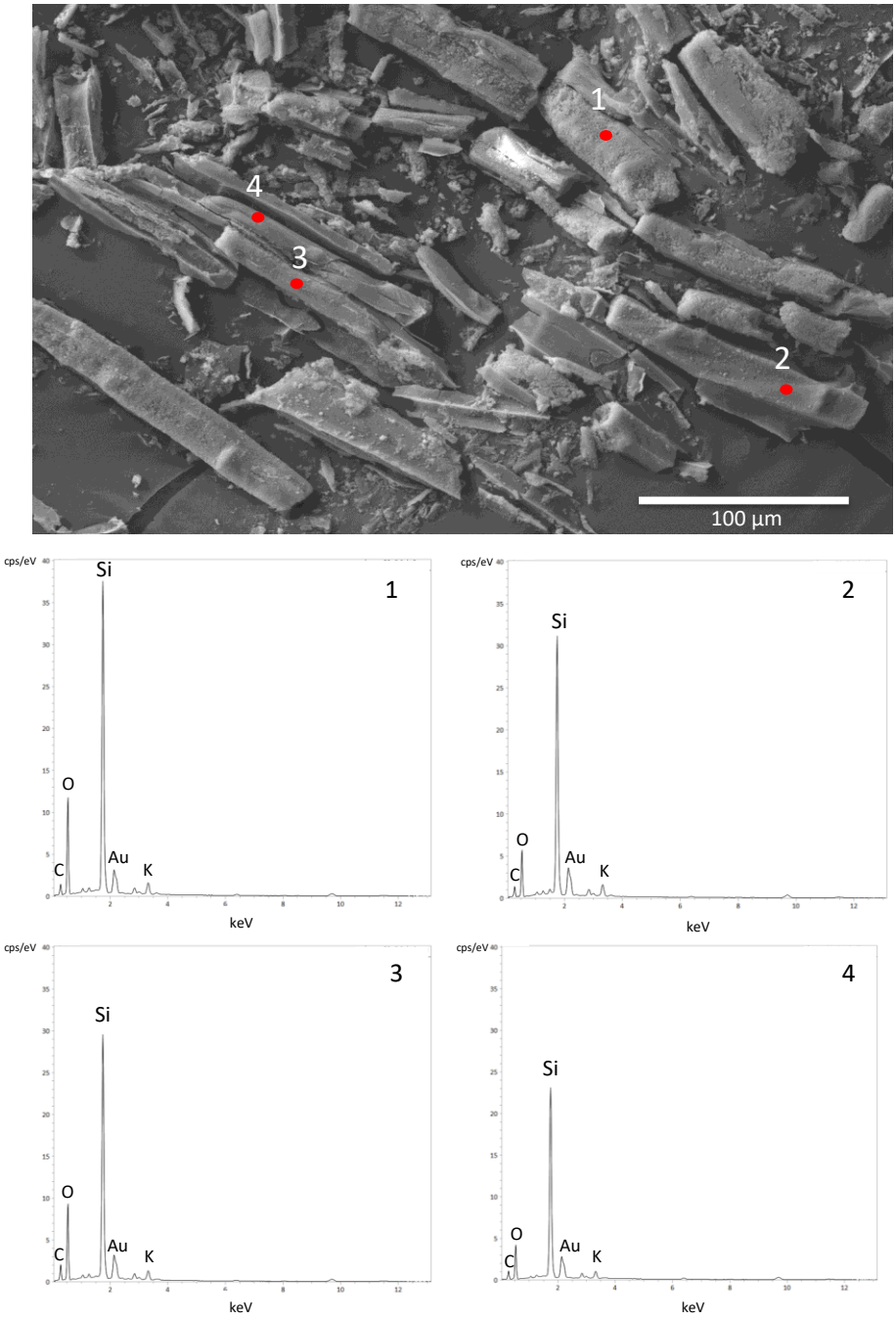

Figure S 2: Representative SEM-EDX micrograph of Si precipitates (phytoliths) in mustard roots extracted from dried root samples. See SEM-EDX analysis of mustard root phytoliths for detailed extraction and measurement methods.

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	δ^{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 S3: Starting composition (major element concentration (in µg g-1) and silicon isotopic composition) of the nutrient solutions for the individual pots.

30 The Table S4 is in 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.

35

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 $NaSiO_4$. 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 (3 cm high with a diameter of 2.5 cm) and each seedling (foam block) transferred to a PP vial (50 mL centrifuge tube) filled with half-concentrated nutrient solution without $NaSiO_4$. Two weeks later, the foam blocks 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). 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. ~~In order to~~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.