

Interactive comment on “Silicon isotope fractionation and uptake dynamics of three crop plants: laboratory studies with transient silicon concentrations” by Daniel A. Frick et al.

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Dear Prof. Hodson

Thanks for taking your time to review our manuscript. 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.

This paper represents an interesting investigation into Si isotope fractionation in three contrasting crop plants. I am not aware that anyone has taken this ap-

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proach 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. We will supply the improved manuscript with track changes in the later stage.

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 adaptations and accounted for this throughout the manuscript. The major changes are:

Line 12: The incorporation of silicic acid from the soil solution into the plants forms a broad spectrum, from varieties which reject to species which actively incorporate silicic acid, these classifications are however to subject to an intense debate.

Line 40: Higher plant species form a continuous spectrum to what extent Si is incorporated, depending on the relative amounts of Si taken up they are grouped into three categories: active, passive and rejective (Marschner and Marschner, 2012).

Line 185: Plants form a continuous spectrum of different uptake characteristics, from almost no silicic acid incorporation, to actively accumulating silicic acid. The uptake characteristics were can be classified based on the ratio of measured and theoretical

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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 Si uptake was calculated based on the amount of transpired water taken up and the nutrient solution Si concentration.

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 preceding precipitation of silicic acid 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 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 however, 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 include her in the author list.

The following changes are made to the manuscript:

Line 327: Phytolith formation, which was observed in mustard roots (see Fig. S2) could explain the lower Si transfer efficiency of mustard.

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Line 370: The isotopic difference between the Si in the shoots and in the roots ($^{30}\Delta$ Root-Shoot) for mustard and wheat, amounts to 0.72 and 0.98 ‰ respectively, and could be explained by precipitation reactions in the roots (see Fig S2 for the observed mustard root phytoliths, for wheat mineral depositions in the roots have also been observed see Hodson and Sangster, 1989, supporting hypothesis 3).

Added the following items to the supplement:

“Figure S2: SEM-EDX micrograph of Si precipitates (phytoliths) in mustard roots extracted from dried root samples. See Method S4 Phytolith separation and SEM-EDX analysis for detailed extraction and measurement methods.”

Method S4 Phytolith separation and SEM-EDX analysis

Phytolith separation: One gram of plant material (roots, shoots) was taken for analysis. Removal of organic matter was conducted by burning the samples in a muffle furnace at 500 °C for 5h. Next, the residue material was subject to additional oxidation using 30% H₂O₂ for 0.5h, the carbonates were dissolved by 80 °C in HCl (10Vol.%) for 10 min. The plants residue was washed with water, bulked, and dried at 105 °C. SEM-EDX analysis: 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.

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*

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the family of Brassicacea (Sonah et al., 2017), but not in tomato (Sun et al., 2020)."

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 d_{30Si} 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 ^{28}Si isotope being more reactive, and thus more likely to be deposited in phytoliths. Thus, in wheat, proportionately more ^{28}Si isotope would be deposited in the culm phytoliths, and a greater proportion of ^{30}Si and ^{29}Si 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.

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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 and 4.5 to highlight this confirmation and included the Hodson et al. 2008 reference:

Line 390: "*Several researcher have observed an enrichment of ^{30}Si along the transpiration stream (Ding et al., 2005; Hodson et al., 2008; Sun et al., 2016), which represents a second Rayleigh-like fractionation process internally 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, whereas the remaining silicic acid in the fluid is enriched in ^{30}Si and further transported along the transpiration stream. This process could be analysed spatially resolved using an in situ technique to target individual phytoliths (e.g. Frick et al., 2019).*"

Line 404: "*Our results demonstrate that the fractionation between roots and shoots is variable in direction and is controlled by internal plant processes, which are also present within subparts of the roots and shoots (Ding et al., 2005; Hodson et al., 2008).*"

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.

We hope that our answers clarify your questions and remarks. Thank you for the

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suggested improvements and clarification for our manuscript.

Best regards on behalf of all my co-authors,
Daniel A. Frick

Literature:

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