

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 Anonymous Referee #2

Thanks for taking your time to review our manuscript. 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?**

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$\mu\text{g/g}$ 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 $49.5 \mu\text{g} \cdot \text{g}^{-1}$ (1.76 mM). Ultrapure water (resistivity $18.2 \text{ M}\Omega\text{m} \cdot \text{cm}$) was used to prepare the nutrient solutions and to weekly restock water taken up by the plants. Detail composition can be found in supplementary methods S1."

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.

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:

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“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 weighted without the lid and the plants, and the mass of transpired water was replenished with ultrapure water ($18.2 \text{ MOhm} \cdot \text{cm}$). The weight difference to the previous week is considered the mass of water taken up 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 have rephrased the sentence:

“For sampling, 40 mL were taken after replenishing water loss due to plant uptake and mixing of the solution.”

Line 115: What kind of extracellular Si deposits? Do you simply mean that you

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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 extra-cellular Si deposits and adhered 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 ($1\text{mg} \cdot \text{g}^{-1}$) to reduce matrix effects 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:

“Based on the concentration measured, an aliquot of each nutrient solution containing approximately $1000 \mu\text{g Si}$ was dried down in silver crucibles on a hotplate at $80\text{-}95 \text{ }^\circ\text{C}$.”

Line 131: estimates based upon what?

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

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“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) 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 SiO_2 as Si^{4+} , counterbalanced by $2 O^{2-}$. Therefore, we do not need to convert Si into a cation. The NaOH accelerates the dissolution of the oxide, and after the addition of water silicon is present as silicic acid (H_4SiO_4 and depending on the pH also in the form of $H_3SiO_4^-$ (see e.g. Stamm et al., 2019, their Fig. 1 for an aqueous Si species in equilibrium diagram).

We hesitate to include the entire Supplementary Method S3 into the main text, but if both reviewers ask, we will gladly do this.

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

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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, but if both reviewers ask, we will gladly do this.

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 guttation. 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.

We hope that these answers clarify your immediate questions and invite you to continue the review of our manuscript.

Best regards,
Daniel A. Frick

Literature:

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