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Interactive comment on "Biological enhancement of mineral weathering by *Pinus sylvestris* seedlings – effects of plants, ectomycorrhizal fungi, and elevated CO_2" *by* Nicholas P. Rosenstock et al.

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Referee comment: This paper has dealt with effects of plant, mycorrhiza, and future climate (elevated CO2) on mineral weathering using seedling column study. The experiment was wellde- sign and conducted to evaluate effects of mycorrhizal association and CO2 elevation on mineral weathering rates in soil. Discussion on mineral weathering is reasonable, except for one critical issue. Major issue: I am afraid of underestimation of weathering rate of Si or Si uptake by plants in this study. Plant Si uptake or Si concentration in plant materials could not be determined only by acid digestion.

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Discussion paper

The majority of Si will remain as residue in acid solution and the residue need to be included as Si uptake by plants. This problem has already been raised by VC Farmer. The Si solubility increases with increasing pH. This can explain the greater Si weathering in the planted column with the higher pH. If actual plant Si uptake is considered, Si weathering in planted column will be further greater.

Author Response: Thank you for bringing this to our attention. We were aware that Si in plant tissue will not be fully digested in aqua regia digestion, but had decided, due to space constraints, and because the underaccounting for biological silica would only make the effects we observed more conservative, not to discuss it. On further consideration, we agree with the reviewer that this warrants attention in the discussion and that were this plant uptake to be fully accounted for this would increase the observed weathering of Si in the planted treatments, increasing the observed stimulation of silica weathering by plants. We have now addressed, in section 4.3 of the discussion, both the inadequacy of aqua regia to solubilize biological silica as well as how this might affect our silica budget

Changes in manuscript: “Biological uptake of Si was likely underestimated due to the inadequacy of aqua regia as a solvent for biological Si. Considering a total seedling biomass of 4 – 5 g and a wide range of potential SI concentrations (0.01 % - 0.5%; taken from measurements of Douglas Fir, Black pine, and Norway spruce plant biomass from a mature forest; Cornelis et al., 2010), plant Si pools could vary between 14 and 700 μmol per seedling; the contents we observed after aqua regia digestion averaged 15.2 μmol per seedling. The potentially incomplete digestion of biogenic Si from seedling biomass means that we have likely underestimated biotic enhancement of Si weathering.”

Referee comment: Minor issue: The dominant low molecular weight organic acids detected in this study are formic, lactic, and acetic acids. They are relatively weak agents for mineral weathering, compared to chelating oxalic and citric acids that are well-known to contribute to mineral weathering in podzol E horizon. Is there any idea to explain the difference of root exudates between the previous studies (e.g., Ahonen-Jonnarth et al., 2000) and this study? Possible reasons may be high availability of P in added solution, low Al levels in soil solution, and precipitation of Ca oxalate. Reasoning is beneficial to generalize the result obtained. The function of formic acid, the dominant organic acid in this study, by roots needs to be further discussed.

Author Response: We agree with the referee that the monocarboxylic acids present in the highest concentrations in our rhizosphere solution are not among those considered to exert significant stimulation of mineral weathering and we have noted this in the manuscript, as well as that concentrations of oxalic and citric acid we measured in the soil solution were too low to result in significant weathering stimulation. Our findings of considerably higher concentrations of formic, acetic, and lactic acids than of the weathering promoting citric and oxalic acids seems to be more the norm than the exception in the literature (Strobel et al., 2001; van Hees et al., 2006; van Hees et al., 2002; Ray and Adholeya, 2009). Those studies that find particularly high concentrations of oxalic acid, appear to be all, or nearly all, from pure culture studies, where microbial degradation of LMWOAs (which is extremely rapid, more so even than for glucose) is excluded. In addition to Ahonen- Jonnarth et al., 2000 using pure-cultures, they only observed very high oxalic acid concentrations in response to aluminum additions, and formic acid was in some cases present in higher concentrations than oxalic acid. While EMF are often mentioned in the literature to produce significant amounts of LMWOA's, our findings are congruent with a number of studies examining the EMF role in LMWOA production which fail to find an increase in LMWOA production when comparing EMF and non-EMF seedlings (van Scholl et al., 2006; van Hees et al. 2005).

Changes in manuscript: We have added text in section 4.1 of the Discussion "Growth and organic acid concentrations" to discuss the mycorrhizal effect on organic acid exudation and composition, and how our findings compare to other studies. "While EMF are often mentioned in the literature to produce significant amounts of LMWOA's, our findings seem to fall in line with the majority of studies examining the EMF role in

LMWOA production which fail to find an increase in LMWOA production when comparing EMF and non-EMF seedlings (van Scholl et al., 2006; van Hees et al. 2005). However, many of these studies do find that EMF significantly alter the composition of LMWOA's produced, particularly increasing oxalic acid concentrations (van Scholl, 2006; van Hees et al. 2006, Ahonen-Jonnarth, 2000), which we did not. However, a number of studies in ectomycorrhizal systems have also observed higher soil solution concentrations of formic, acetic, and lactic acids than of the weathering promoting citric and oxalic acids (Strobel et al., 2001; van Hees et al., 2006; van Hees et al., 2002; Ray and Adholeya, 2009). Studies that find particularly high concentrations of oxalic acid, are typically from pure culture studies with high nitrogen availability (Rosling 2009), where rapid microbial degradation of LMWOAs is excluded. Despite the fact that our rhizosphere lysimeters were placed in the area of highest root density it is possible that the organic acid profile we observed is primarily the product of microbial activity, following partial decomposition of plant exudates and other SOM. It is, however, interesting to note that the planted treatments all had far higher LMWOA concentrations, and that LMWOA concentrations were correlated with seedling biomass. Elevated CO2 was associated with significantly higher total LMWOA concentrations, potentially indicating greater carbon allocation to root exudation by seedlings under elevated CO2, in agreement with previous studies (Fransson and Johansson, 2010). However this stimulation by elevated CO2 appeared to be primarily a function of larger seedlings as the elevated CO2 treatment was not associated with higher LMWOA concentrations per unit seedling biomass. Similar to Fransson and Johansson (2009), we did not find that elevated CO2 increased LMWOA production beyond its effect on seedling biomass. "

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van Hees, P.A.W., Rosling, A., Finlay, R.D.: The impact of trees, ectomycorrhiza and potassium availability on simple organic compounds and dissolved organic carbon in soil. Soil Biology & Biochemistry, 38, 1912–1923, 2006.

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Referee comment: Most data are presented on a basis of column. I wonder whether the results could be transformed and presented in a generalized manner to compare with the previous studies.

Author Response: There was one seedling in each column, and the column was the experimental unit, and this is why we have presented data on a per column basis. We have also presented both chitin and elemental contents in seedling on a per unit seedling mass basis. We have also presented organic acid concentrations as concentrations in soil solution (μmol/L). It is not immediately clear to us which results that we have presented per column would be better presented in other units. As our experiment was conducted in columns filled with an artificially constructed mineral mixture, and planted with seedlings, converting results to soil surface area or volume in order to compare to field experiments is problematic.

Please also note the supplement to this comment:
https://www.biogeosciences-discuss.net/bg-2019-46/bg-2019-46-AC1-supplement.pdf

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