

Response to Reviewer Comment RC4

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Lawrence et al. present results of a 63 day mesocosm experiment testing whether inoculation with a microbial strain, *B subtilis*, increases silicate weathering, and resulting carbon dioxide removal, in an agricultural soil. The authors use sequential selective extractions to trace how potential weathering products are distributed amongst different soil, plant, and leachate pools. I found the study interesting and relevant for SOIL readership. However, I share some of the concerns of the other reviewers regarding the characterization of the soil used in the experiment, the lack of baseline sequential extraction measurements, the statistical treatment of the data, and the strength of some of the conclusions drawn from the study. I believe that major revisions are necessary before the study could be published in SOIL.

We appreciate the reviewers' time and perspective on our manuscript. Many of the points flagged here and by earlier reviewers are easily addressable. We've updated the statistical tests used to compare treatments and added more details about the experimental soil. Please see our responses to comments RC1 and RC3 for additional details.

Major Comments:

1. Soil characterization

I agree with Reviewer 2 that the mineralogical and elemental characterization of the soil needs to be clarified. The results do not seem to match particularly well, given the very high proportion of quartz and feldspar reported. Because the interpretation of the experiment depends on silicate minerals being the source of the additional base cations, I think this discrepancy should be explained more clearly. I was also surprised by the 15% LOI. The measurement at 360 C is useful for an operational estimate but not equivalent to a high temp LOI. Given the reported ~2.6% SOC and only 5% phyllosilicates, 15% mass loss seems unexpectedly high. As some of the reported treatment effects are relatively small, it would be helpful to see some more information on the analytical uncertainty, particularly detection limits and measurement precision.

Thank you for this comment. The perceived discrepancy in our results was driven by our inclusion of semi-quantitative mineralogy data that was not adequately described. Most importantly, those data were presented in a way that suggested they were representative of bulk soil mineralogy, which was incorrect for a couple reasons.

First, our intent for the mineralogy measurements was to quantify the abundance of weatherable primary silicate minerals in the study soil. The samples were washed prior to processing for measurement, which removes clays, and as a result the reported

values are not reflective of bulk soil mineralogy and almost certainly underestimate the abundance of hydrated clays. We'd like to further emphasize that these data were included in the original manuscript for reference only, they were not used in any calculations or referenced in our interpretation of the results.

Second, there was a mistake in our reporting of the LOI temperature used prior to the XRF analyses. We stated it was 360°C, when in fact the temperature used was 900°C (for no less than 16 hours). The discrepancy resulted from the fact that we separately measured SOM content using an LOI approach, which was performed at the lower temperature. The results of those analyses (initial soil SOM = 5.7%) are consistent with our estimates of TOC and provide further support for the presence of significant hydrated clay content (on the order of 10% by mass).

We will correct these mistakes and remove the misleading mineralogy data from the supplemental materials. Please also see our detailed responses to reviewer 2's comments.

2. Experimental baseline and control treatment

I agree with Reviewer 1 that the lack of baseline sequential extraction measurements complicates the interpretation of the results a bit since there is no direct measurement of how the cation pools changed relative to initial conditions. What was the rationale behind no baseline extraction? Perhaps it is also worth discussing the lack of a control with no soybean plant? While the experiment can isolate the additional effect of MP1 in the presence of soybean, it cannot separate background/abiotic weathering from plant/rhizosphere driven weathering. I think these limitations should be acknowledged more clearly, particularly when discussing the sources of cations and the responsible mechanisms for the observed treatment effects.

Lack of inclusion of the initial soil in the sequential extraction experiment was an unfortunate and unintended oversight on our part. However, it does not undermine our ability to assess treatment effects. Furthermore, we can use bulk soil data to help confirm that available cations in both treatments either stay the same or increase relative to the initial soil. For more detail, please see our detailed response to reviewer #1's original comment.

Regarding the lack of no-plant control: In a mesocosm experiment, where the starting conditions are the same for control and treatment (soil, seeds, growth conditions), and the single differential factor is the addition of the MP1 microbe, the results observed must be attributed to the influence of that factor at some level. It is also well established that *Bacillus* spp. have the ability to increase silicate weathering (e.g., Uroz, Picard, and Turpault, 2022. Trends in Microbiology). Very importantly, this rhizosphere

microbe requires plants to survive and thrive in the soil (i.e., root exudates are the main C and N sources), and, on the other hand, root respiration concentrates and redirects atmospheric CO₂ belowground, which is necessary to support weathering via MAW. Given that and the logistical limits to the number of experimental units, we did not feel that treatments with either no plants and no microbe or no plant with the microbial amendment would have added useful information. We've previously demonstrated that this same microbe can increase available base cations (Timmermann et al., 2025). The goal here was to confirm that these increases come from silicate weathering.

Timmermann, T.; Yip, C.; Yang, Y.; Wemmer, K. A.; Chowdhury, A.; Dore, D.; Takayama, T.; Nademanee, S.; Traag, B. A.; Zamanian, K.; González, B.; Breecker, D. O.; Fierer, N.; Slessarev, E. W.; Fuenzalida-Meriz, G. A. Harnessing Microbes to Weather Native Silicates in Agricultural Soils for Scalable Carbon Dioxide Removal. Global Change Biology 2025, 31 (5), e70216. <https://doi.org/10.1111/gcb.70216>

3. Statistical analysis

I agree with Reviewer 1 that the statistical analysis could better reflect the experimental design. At the moment, many of the depth-specific results are tested separately, even though the three depth intervals come from the same mesocosm and are therefore not independent. A treatment x depth analysis or mixed effects model with mesocosm included as a random effect seems more appropriate. Additionally, the methods state that either a student's t-test or Mann-Whitney U/Wilcoxon rank-sum tests were used, while the captions for Figures 1 and 3 report Wilcoxon signed-rank tests. Were the UTC and MP1 columns paired in some way? If so, please describe the pairing in the experimental design; if not, please clarify which statistical test was used. There are also a large number of separate tests across depths, elements, and extraction fractions, so it would be helpful to clarify whether multiple testing was considered.

We've heeded this advice and updated our statistical methods to now use a linear mixed effect model, with experimental columns as the random effect for all comparisons of treatment with depth. For all other contrasts, we now use only the Mann-Whitney U/Wilcoxon rank-sum tests. Please see our response to reviewer 1 for more details.

Minor comments:

L26-L65: The introduction is generally well referenced, but a few of the broad claims could use citations, particularly those regarding agricultural co-benefits, preference of EW for acidic soils, and CDR consequences of carbonate precipitation.

Citations will be added as requested.

L65-70: "definitively link" seems too strong given limitations of study. I would also consider changing "all potential sinks" to "major measured sinks"

We will update as requested.

L75: "microbial amended" -> "microbially amended"

We will correct this mistake in the revised manuscript.

L98-100: Please check the inoculation-rate calculation. The manuscript states that 3.9×10^6 spores were added per mesocosm and that this corresponds to 3.9×10^3 spores/g soil but this does not seem consistent with either the wet or dry soil mass.

Thank you for pointing this out. The correct value is 1.8×10^3 spores/g dry soil. We will update the manuscript accordingly.

L215–225: I would state more clearly here that the SIC/TIC treatment difference was not statistically significant. The text currently emphasizes the ~37% increase, whereas the following section notes that TIC was near the detection limit and not significant.

We will revise these lines to make clear the lack of significance in the bulk soil TIC.

L350–360: The discussion of depth-dependent rhizosphere activity and pCO_2 is interesting, but neither root abundance nor pCO_2 was measured. I would make it clearer that this is a proposed explanation.

As requested, we will revise to make it clear this is our proposed explanation and not something that has been proven.