

## Response to Reviewer Comment RC3

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### General comments

This manuscript describes a study intended to demonstrate the effect of a microbial inoculation of soya seeds on the ability of a soil-plant system to remove atmospheric CO<sub>2</sub>. The authors found statistically significant increases in soil inorganic carbon content, but weak or no differences in compositions of leachates collected from the pots. If correct, these findings show that natural soils are able to increase the rate of pedogenic carbonate formation as a consequence of inoculation with selected bacteria. This finding is well-known from work on microbially-induced carbonate precipitation, a technique used by geotechnical engineers to stabilise soils. They achieve this by adding a calcium salt to inoculated soils.

We appreciate the reviewer's summary and feedback of our work. There is one difference that we would like to highlight between this work and the previous studies of microbially-induced carbonate precipitation: as the reviewer suggests MICP often involves addition of a calcium salt to facilitate carbonate formation. Here we show that the MP1 microbe can, in the presence of divalent cation bearing silicates, provide Ca and Mg to form carbonates on its own, and that this process represents a potential carbon dioxide removal pathway when the alkalinity generated from silicate weathering is captured with the carbonate minerals that have been formed.

### Specific comments

This study demonstrates the importance of understanding both accuracy and precision, especially when using commercial laboratories. I'll explain the weaknesses below.

When compositional differences are slight, as in this study, it is really important to know what the analytical precision is for the experimental work, as sometimes the error bars on the lab analysis are greater than what is seen statistically as variability between replicates, if the numbers are taken at face value. This is especially true when external laboratories are used. They will usually quote precision under ideal conditions; the labs have to be tested by submitting a single sample split to give 4 or 5 'unknowns', carefully each given a unique label, so the researchers can then calculate the precision that applies (for all analytes) for their specific study. That hasn't been done in this case, so it is difficult to know if the statistical analysis that has been carried out can be trusted.

This is a good recommendation but one that can be unreasonably expensive for cost intensive analyses. We are confident that the contract laboratories used in this study

adhere to the highest possible data quality standards, and as such we believe the laboratory reported precision represents reasonable working conditions. We will now include the laboratory quality assurance metrics in the XRF data table (Supplemental Table S1).

## Updated Supplemental Table S1

**Table S1. The unnormalized abundance of major oxides measured by XRF in the SBX70 soil.** Samples were analysed at the Washington State University XRF laboratory and laboratory reported data quality metrics for precision and limits of detection (2-sigma) are included for reference. Loss-on-ignition (LOI) represents the mass of volatile compounds in the sample, including organic matter and structural water in clays.

| Results                        |      | WSU XRF Metrics          |               |
|--------------------------------|------|--------------------------|---------------|
| Elemental Oxide                | wt%  | replicate R <sup>2</sup> | LOD (2-sigma) |
| SiO <sub>2</sub>               | 62.5 | 0.99975                  | 0.26          |
| TiO <sub>2</sub>               | 0.6  | 0.99996                  | 0.012         |
| Al <sub>2</sub> O <sub>3</sub> | 11.7 | 0.99995                  | 0.097         |
| FeO                            | 3.8  | 0.99977                  | 0.14          |
| MnO                            | 0.1  | 0.99983                  | 0.002         |
| MgO                            | 1.6  | 0.99999                  | 0.073         |
| CaO                            | 1.3  | 0.99995                  | 0.061         |
| Na <sub>2</sub> O              | 1.0  | 0.99992                  | 0.029         |
| K <sub>2</sub> O               | 2.0  | 0.99999                  | 0.010         |
| P <sub>2</sub> O <sub>5</sub>  | 0.1  | 0.99997                  | 0.003         |
| LOI                            | 15.0 | Not Reported             | Not Reported  |

Regarding accuracy, the analysis of the soil demonstrates some of the problems that simply taking the numbers as true can present. There is no way that a soil with 65% quartz and 28% feldspars (XRD) can have the chemical composition reported by XRF. So the XRD and XRF analyses differ in their accuracy. Taking into account the ignition loss, I recalculated the composition using the XRD results and obtained: 72.4% SiO<sub>2</sub>, 6.8% Al<sub>2</sub>O<sub>3</sub>, 0.5% CaO, 1.6% Na<sub>2</sub>O and 1.1% K<sub>2</sub>O.

This is an astute observation and the reviewer is correct that the reported semi-quantitative mineralogy results are, on their surface, inconsistent with the chemical composition from X-ray fluorescence. This arises from our measurement and reporting of the mineralogy, and not from the XRF-chemistry.

When performed on bulk samples, qXRD and SEM-EDS tend to underestimate clay content. This issue was further exacerbated by washing the samples prior to analysis. Importantly, our main goal for the semi-quantitative mineralogy was to constrain the composition and abundance of weatherable primary feldspar minerals, not to provide an independent estimate of chemical composition. In fact, the mineralogy data was not used in any calculations presented in the manuscript. We included those data for reference only. However, as originally presented, the mineralogy data was misleading because it implied the results were representative of the bulk soil, which is not the case. To address this concern, we will remove the full supplementary table and revise our description of those results to make it more clear that they are representative of the crystalline fraction and therefore will underestimate clay fraction mineral abundances.

In any case, at 15%, the reported LoI is really (suspiciously?) high for a sample with 2.6% organic C and a total maximum clay content of 5% phyllosilicates (the only hydrated minerals). We don't know how to account for that very high value for LoI, so there is an additional inaccuracy somewhere in the numbers.

Regarding the high LOI values from the XRF dataset, we acknowledge that this number appears questionable relative to soil organic carbon composition and the clay content from the semi-quantitative data, but as we will detail below there is no reason to believe the LOI is incorrect. For the reasons described above, the qXRD/SEM-EDS methods likely underestimate the abundance of hydrated clays. Other factors that could contribute to the higher than expected LOI include a significant abundance of SOM, carbonates, oxyhydroxides, or residual hygroscopic water (the submitted samples were air-dried prior to submission for XRF). We made separate measurements of soil organic matter content of the initial soil (mean = 5.7%, n=4). Therefore the difference mass between the two LOI approaches ( $15\% - 5.7\% = 9.3\%$ ) likely reflects some amount of initial carbonates, oxyhydroxides, and hygroscopic water.

As stated previously, we will remove the misleading table of semi-quantitative mineralogy and clarify that approach was not reflective of the bulk soil composition:

### **Proposed updates to section 2.3:**

*"Prior to the experiment, the initial homogenized soil used in the mesocosm was characterized for mineralogy of the crystalline fraction as well as bulk elemental composition. Mineralogy was estimated using a combination of the scanning electron microscope (SEM) observations on a Zeiss EVO-10 Variable Vacuum SEM and XRD analysis with the PANalytical X'Pert Pro diffractometer at the Department of Earth and Planetary Science at the University of California (Berkeley, CA, USA). The resulting semi-quantitative mineralogy was used to estimate the abundance and primary silicates available for weathering and is not an accurate estimate of bulk soil mineralogy. The total bulk*

*elemental composition of the SBX70 soil prior to the onset of the experiment was measured via X-ray fluorescence (XRF) at the GeoAnalytical Laboratory at Washington State University (Pullman, WA, USA), and soil organic matter content was determined by the loss-on-ignition method at 900 °C for a minimum of 16 hours to allow for normalization of the elemental composition on a volatile-free basis. Technical details and principles underlying these methods are described in Johnson et al. (1999) and Kelly et al. (1998). "*

Because of these observations, something has evidently gone wrong right at the start. At best, maybe two different samples have got muddled by the labs. But it is more likely one set of numbers is wrong (I would suspect the XRD). This needs to be sorted out, as associated errors affect the interpretation of the data, especially as the sequential extraction and TIC results need to obtain Ca from somewhere.

In accordance with the reviewer's assumption, and for the previously stated reasons, the semi-quantitative mineralogy data is not representative of bulk soil chemistry. It was a mistake to include those results in the original format provided because we were not systematic in our consideration of clay composition.

In contrast, we have no reason to question the results of XRF analysis. Furthermore, the XRF data are only reported as a backstop to the sequential extraction results. The XRF values are entirely consistent with the extraction data.

A problem also affects the determination of organic C by difference. Here there is a propagation of analytical error, as the combustion-based determination of total carbon has an analytical precision that needs to be added to the precision of the inorganic carbon determination to estimate the precision of the calculated value for organic carbon. It is a pity that the organic C content was not determined directly by combustion – something that is easily done using thermal analysis or an instrument such as the LECO RC612. Given that a single soil is used for all the columns, it would be worth thorough analysis of its carbon content through direct determination using these techniques.

We understand the reviewers' concern regarding our reported measurement of total organic carbon. However, we'd like to point out that it is standard practice in the soil community to calculate TOC by the difference between TC and TIC.

Given our interest in TIC accumulation and the low concentrations, we opted to measure that parameter directly using our in-house GC-based method (Yip et al., 2025). Calculating TOC as the difference from TC ensured carbon mass balance in our accounting and avoided issues with TIC removal prior to direct TOC measurement. Although it was not apparent in the original manuscript, we've gone through great lengths to confirm these results. Specifically, we measured SOM via low-temperature

LOI and we contracted an additional set of bulk soil carbon measurements (including TC, TOC, and TIC) from a second external laboratory. Both sets of results are consistent with the originally reported values. We chose to report the combination of our in-house TIC measurements and TC from our primary contract laboratory because we have evidenced that our in-house quantification of TIC is more precise at low concentrations.

*Yip, C.; Weyman, P. D.; Wemmer, K. A.; Yang, Y.-Y.; Chowdhury, A.; Traag, B. A.; Timmermann, T.; Fuenzalida-Meriz, G. Quantification of Soil Inorganic Carbon Using Sulfamic Acid and Gas Chromatography. PLoS One 2025, 20 (5), e0320778. <https://doi.org/10.1371/journal.pone.0320778>*

The description of the method is given in detail, and so other workers would be able to reproduce the experiment. However, it is stated that the seeds were inoculated with 1 ml of water. That is greatly in excess of the size of the seed, and so I wonder if they were inoculated with 1 microlitre of water – which would then enable the statement in line 99 to make sense.

We apologize for the confusion regarding the inoculation of seeds during planting. To be clear, we did not inject the seed with 1 ml of water. Rather, we applied 1 ml of water, with or without MP1 spores, to the surface of the seed when the seed was placed in the soil at the time of sowing. We will modify the text to clarify this important point.

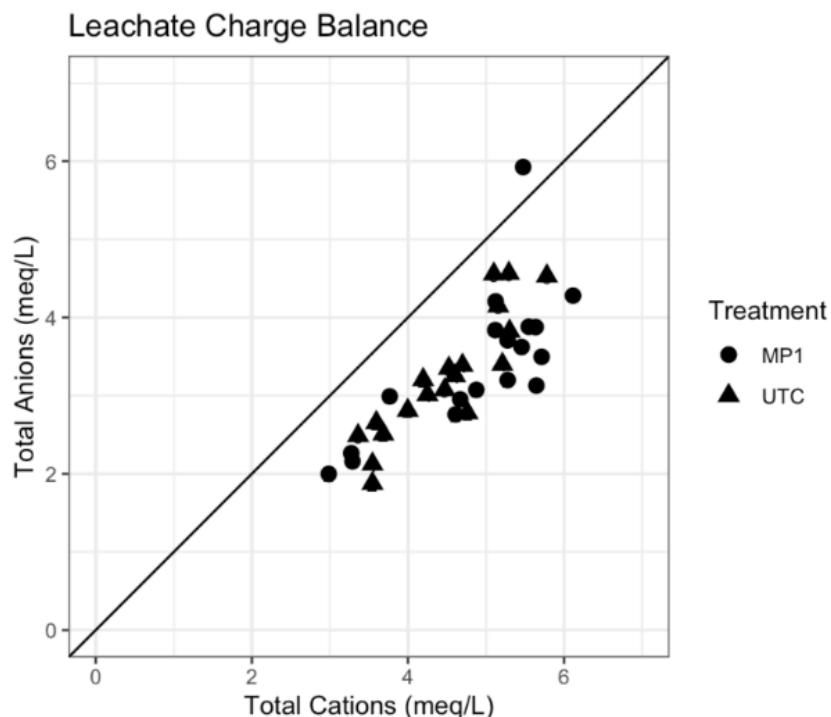
Charge balance has been assessed for the leachate analyses. The calculated charge balance should be made known to the reader. It is acceptable to assume that a positive surplus might be counterbalanced by bicarbonate, but how does that calculated value compare with the measured values? In my experience, charge imbalance often arises from error in the determination of anions.

This is an important point. The charge balance calculation for leachate measurements reflected a surplus of positive charge (mean charge balance error = 17%), which is commonly interpreted to indicate errors in the quantification of anions. We chose to correct the measured concentrations of carbonate alkalinity based on the excess positive charge because we were most concerned with the bicarbonate measurements. The magnitude of this correction was on average 55% of the originally measured bicarbonate values. Given this correction, our reported results represent the maximum potential carbonate alkalinity because other possible sources of negative anions, including phosphate and organic anions (e.g. Bijma et al., 2026), were not measured. We will update the description of the charge balance correction and the associated results to better reflect these details.

We would like to point out that there were no statistically significant differences in bicarbonate fluxes from the columns regardless of whether the raw measurements or charge corrected values are used. Moreover, the charge-corrected fluxes represented a

very small fraction of the treatment-driven change in total carbonate alkalinity, with the vast majority occurring in the solid-phase carbonate fraction. Using the raw measured bicarbonate values would only strengthen this conclusion.

### New Supplemental Figure



**Figure S2: Charge balance of leachate chemistry.** The sum of measured anions (meq/L), including  $\text{NO}_3^-$ ,  $\text{SO}_4^{2-}$ ,  $\text{Cl}^-$ , and  $\text{HCO}_3^-$  and  $\text{CO}_3^{2-}$ , are plotted against the equivalent sum of cations including  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{K}^+$ , and  $\text{Na}^+$ . The mean charge balance error across both treatments was 17%, reflecting an excess in measured positively charged base cations. We assume this imbalance to be the result of errors in our measurement of the bicarbonate/carbonate anions due to atmospheric equilibration of  $\text{CO}_2$  in samples, and we corrected bicarbonate/carbonate concentrations based on the charge offset. As a result, our estimates represent the maximum potential concentrations of carbonate alkalinity in column leachate.

### Proposed updates to section 3.1.3

"At the end of the 63-day experiment, soil carbonate alkalinity ( $\text{CO}_3^{2-} + \text{HCO}_3^-$ ), measured from soil titrations, was significantly higher ( $p < 0.05$ ,  $N = 6$ ) in MP1 compared with UTC columns (Figure 2A). Alkalinity in leachate is reported as the total cumulative flux, corrected for charge imbalance, and normalized by the mass of soil in each column (mEq/kg soil) so that it can be directly compared to soil measurements. The magnitude of the charge correction was on average 55% of the value measured from the titrations. The corrected values likely represent a maximum-estimate of carbonate alkalinity since some of the excess positive charge may be attributed to organic alkalinity (e.g., Bijma et al., 2026), which was not measured directly. Regardless of whether the measured or charge-corrected values are used,

*leachate alkalinity was similar between treatments, thus, differences between treatments in the total alkalinity of the system (soil + leachate), were mainly driven by the solid phase, resulting in a higher total alkalinity for MP1-treated soils in comparison to UTC soils (Figure 2A). Inorganic carbon in leachate and soil showed similar trends, with higher mean values of SIC and TIC in the MP1-treated columns ( $\approx 37\%$  increase over UTC) (Figure 2B)."*

So, overall, I think there are issues regarding the experimental data that need to be resolved before we can judge the validity of the statistical interpretation of the results. The interpretation of the base cation data for the carbonate step of the extraction should also be reconsidered, as there is no reason why K or Na should be associated with dissolution of a carbonate mineral – K and Na carbonates are unlikely to be present in a natural soil.

It is important to emphasize that the sequential extractions are not expected to be perfectly selective (e.g., Hass and Fine, 2010). It's therefore not surprising that some small amounts of Na and K appear in the carbonate extraction pool. The overall masses of these elements in the carbonate extracts were roughly two orders of magnitude less than the Ca and Mg (see Supplemental Table S2 for details).

*Bijma, J.; Hagens, M.; Hammes, J. S.; Planavsky, N.; Pogge Von Strandmann, P. A. E.; Reershemius, T.; Reinhard, C. T.; Renforth, P.; Suhrhoff, T. J.; Vicca, S.; Vienne, A.; Wolf-Gladrow, D. Reviews and Syntheses: Carbon vs. Cation Based MRV of Enhanced Rock Weathering and the Issue of Soil Organic Carbon. Biogeosciences 2026, 23 (1), 53–75. <https://doi.org/10.5194/bg-23-53-2026>*

*Hass, A.; Fine, P. Sequential Selective Extraction Procedures for the Study of Heavy Metals in Soils, Sediments, and Waste Materials—a Critical Review. Critical Reviews in Environmental Science and Technology 2010, 40 (5), 365–399. <https://doi.org/10.1080/10643380802377992>*

I recommend a second round of review after the analytical data have been properly audited, verified and interrogated prior to statistical analysis.

The issues identified by the reviewer were the result of errors in our reporting, not a fundamental problem with the experimental design. Specifically, our inclusion of semi-quantitative mineralogy and a typo in the temperature of LOI used to remove volatiles prior to XRF analyses raised questions about the discrepancies in the chemical composition of the bulk soil. We will address those issues by removing the misleading data and clarifying our methodologies.

### **Technical corrections/typos**

Line 124: the x in X-ray should be upper case.

Corrected.

Line 126: soil organic matter determination by combustion at 360°C is a very rough estimate – some hydrated minerals report, and refractory forms of soil carbon, very much part of SOM, are stable up to higher temperatures and so don't report.

The 360°C combustion temperature for LOI associated with the XRF analysis was a typo. The actual temperature used was 900°C for a minimum of 16 hours, which is sufficient for removal of both volatile organic matter and structural water in clays. The originally quoted lower value was the temperature used for LOI based estimates of organic matter content. The measured SOM ranged from 5.2 to 6.3% in the columns at the end of the experiment, which is consistent with the SOC content that ranged from 2.4 to 2.8%.

Line 151 and elsewhere: use proper IUPAC formulae (or write the chemical name in full) rather than using the informal 'Ac' to denote acetate – it could mean something quite different.

We will update as suggested.

Line 164: which yttrium salt was used? It might well not be metallic yttrium.

For the ICP internal standard, we have used nitrate salt of yttrium ( $\text{Y}(\text{NO}_3)_3$  - 1000 mg/L Y in 2-3%  $\text{HNO}_3$ ). The specific product can be found at the following URL:

[https://www.sigmaaldrich.com/BE/en/product/mm/170368?srsId=AfmBOop1Pn8z1xcj3Vy6XZOBUD0yb\\_3S-BIM74EHqshK6Z62WfqmwEOY](https://www.sigmaaldrich.com/BE/en/product/mm/170368?srsId=AfmBOop1Pn8z1xcj3Vy6XZOBUD0yb_3S-BIM74EHqshK6Z62WfqmwEOY)

Line 172: 'equal masses' would be better.

We will change as suggested.

Line 183: say which section the description is in – it isn't in the 'previous section'.

We will clarify that the method is detailed in the soil mineralogy and chemistry section.

Line 202: explain what you mean by 'volume weighted pH'. pH is an intensive parameter, and so is independent of sample volume.

Our reported values for leachate pH represent the mean values of leachate collected from the three simulated rainfall events, weighted by the volume of leachate that was collected. We will clarify this in the methods section.

Line 257: please provide more information concerning the K (and Na) data, as the values are too small to read from the graphs. The same goes for Al in Figure 5.

All values from these graphs are shown in Supplemental Table S3. We've retained the shared y-axis for the plots to emphasize the magnitude of differences between elements (see also our response to your question about Na and K abundances in the carbonate data above).

Line 440: Would 'In contrast' be better than 'Whereas'?

We appreciate the suggestion and will change the text accordingly.