Response to Reviewer's Comments
All responses, corrections, and changes have been marked as BLUE color.

General Comments

Introduction is well structured. More detail should be added however on the selection of the Aspergillus strain. For example, environments found in and function, use in other rock weathering studies, use in industry (strains used for citrate production). This is important justification which grounds the theory present in the introduction to the specifics of this study and the use of Aspergillus.

We have added the details about the *Aspergillus* strain in the Introduction. **Please refer to line 65-70 page 3.**

There are some methodological issues (for example, lack of control treatments and number of replicates) that minimise the power of the analyses and conclusions.

1) The study primarily aimed to QUALITATIVELY (rather than quantitatively) evaluate fungus-Assisted bioweathering on fluoridated apatite. In this study, four experimental pairs were conducted, each comprising two treatments: (i) CPSF@Soil and LPSF@Soil, (ii) CPSF and LPSF, (iii) CFAP@CO_2 and LFAP@CO_2, and (iv) CPSF@CO_2 and LPSF@CO_2.
2) For the soil incubation experiments, fungal effects were evaluated by directly comparing apatite surface micro-morphology at hyphae-attached sites with adjacent hyphae-free sites on the same apatite thin section within the same microenvironment. This within-sample comparison effectively minimized soil heterogeneity and environmental variability, allowing the fungal influence to be isolated at the microscale. Therefore, an external abiotic soil control was not required for the primary objective of these experiments.
3) For the culture experiments, pristine apatite and single-factor treatments (fungi or elevated CO_2 alone) served as **control treatment** to assess individual and combined effects. Therefore, the absence of a fixed control treatment does not affect the analysis of the results.
4) In addition, as the primary aim of this work is qualitative and mechanistic, focusing on reproducible micro-scale dissolution and alteration features, the study does not include traditional statistical replication. Each apatite thin section was prepared and examined independently at least twice, and the same surface features and dissolution patterns were consistently observed, supporting the robustness of the qualitative conclusions.

The discussion requires significant work to bolster conclusions by referencing to relevant literature, appropriately calling out results and adjusting the structure for readability. Numerous unsupported claims are made that require support from the literature.

We have restructured the Discussion section to improve readability and logical

flow. In addition, we have added relevant references to support key statements.

Specific Comments

Lines 80 -84: two sentences discuss results presented in supplementary information in introduction. Understandable as to why, however results/context from other work should be presented here, discussion of results should be saved for discussion.

We have already adjusted the relevant information into the discussion. **Please refer to line 262-265 page 13.**

Two replicates per treatment were used – the statistical power of this should be noted in the statistics section. More replicates would be preferred or the adoption of a gradient method (e.g. a range of CO2 concentrations).

Statistical analysis was performed only for the assessment of the effects of elevated CO_2 on apatite surface roughness. For each treatment (elevated CO_2 and ambient atmospheric CO_2), atomic force microscopy (AFM) analyses were conducted on three randomly selected surface locations of the apatite samples. The roughness values obtained from these three locations were used for statistical analysis. We have clarified this in the Statistics section of the manuscript. **Please refer to line 199-205 page 10.**

Section 2.3 details the soil experiments. This is difficult to follow, and would benefit from stating the number of bottles used, consistent use of 'samples' or 'glass sheets', and explanation of why all FAP slides were not removed after 30 days (only 2 what about the others?).

To ensure consistent incubation conditions, glass slides carrying cross and longitudinal sections of fluorapatite (FAP) were incubated together within the same glass bottle and considered as two treatments.

After 30 days of incubation, all glass slides carrying FAP sections were removed from the bottles, resulting in a total of four slides (two cross and two longitudinal sections). We have revised Section 2.3 to clearly state the number of bottles, slides, and the terminology used, and to clarify the sampling procedure. **Please refer to line 144-146 page 7.**

A potentially significant issue is the lack of controls, it is not clear that incubations were performed without Aspergillus inoculation in the soil experiments. For example, in the soil incubation the role of abiotic soil factors on weathering was not accounted for (or appear not to be). The soil was acidic, which could result in weathering.

In the soil incubation experiments, treatments without fungal inoculation were not included, as the primary objective was to compare the relative weathering effects of phosphate-solubilizing fungi on different crystallographic orientations of fluorapatite rather than to quantify absolute biotic versus abiotic weathering rates.

Cross and longitudinal fluorapatite sections were incubated together in the same glass bottle, ensuring identical soil chemistry, moisture, pH, and microbial conditions.

After incubation, etch pits were observed exclusively on the transverse sections, whereas no such features were detected on the longitudinal sections. Although the acidic soil may have induced some abiotic apatite weathering, this effect would have acted equally on both crystal orientations under the shared conditions and therefore cannot explain the contrasting surface features.

We note that the acidic soil environment may partly explain why no distinct etch pits were observed on apatite surfaces in subsequent culture-medium experiments following fungal inoculation. However, this does not contradict our main conclusion that phosphate-solubilizing fungus exerts intense weathering effect on the cross-section of fluorapatite. We have added clarification of this experimental rationale and limitation in the revised manuscript.

How were cell concentrations determined? Not presently stated.

1) Spore concentrations were determined using a hemocytometer. More specifically, fungal spores were harvested and suspended in sterile water and thoroughly mixed.
2) Spore concentrations were determined using a hemocytometer under a light microscope. An aliquot of the spore suspension was loaded into the counting chamber, and spores were counted in five representative squares according to standard counting rules.
3) The average number of spores was used to calculate spore concentration, taking into account the chamber volume and dilution factor. All measurements were performed in duplicate, and the mean value was used for subsequent experiments.

The method for measuring spore concentration has been added. **Please refer to line 125-126 page 6.**

Soil sterilisation requires detail. Sterilisation at 121 C in a dry sterilisation mode? Using what instrument (autoclave, oven…)? Heat treatment can alter organics structure; did it affect organic P mobilisation from the soil? Gamma sterilisation would be preferable, why was it not used?

1) We have clarified the sterilization procedure in the revised manuscript. Soil samples were sterilized using a steam autoclave at 121 ºC for 1 h. The soils were placed in glass bottles, and the bottle openings were sealed with sterile breathable sealing film, which allows gas exchange while preventing contamination, thereby ensuring effective sterilization.
2) Heat treatment may alter the structure of soil organic matter. However, the red soil used in this study is characterized by low organic matter and P (total P is 0.19 g/kg). Inorganic P (Fe-P and Al-P) dominating the total P pool. Under these conditions, any heat-induced alteration of organic matter is expected to have a negligible effect on organic P mobilization and does not affect the interpretation of the results. Moreover, all treatments were subjected to the same sterilization procedure, ensuring internal consistency and comparability among treatments.

3) In this study, Gamma sterilization was not used as the sterilization at 121 ºC is well accepted for fungal experiments.

The addition of a table of treatments in the methods would be a useful look up tool when reading the discussion.

For clarification, all the treatments were listed in Table 1 below.

Table 1. All the treatments in the four experiments

Experiments	Treatment name
Bioweathering of FAP in Soil	CPSF@Soil and LPSF@Soil
Weathering of FAP in Medium (i: Weathering of FAP by PSF)	CPSF and LPSF
Weathering of FAP in Medium (ii: Weathering of FAP by elevated CO_2: Weathering of FAP by PSF and elevated CO_2)	C_{FAP}@CO_2 and L_{FAP}@CO_2
Weathering of FAP in Medium (iii: Weathering of FAP by PSF and elevated CO_2)	CPSF@CO_2 and LPSF@CO_2

Line 176: How was roughness gauged. Not enough to say they were compared. How were they compared? Visual assessment is not sufficient, it is a qualitative method. Number and size of pits/etches/deposits is an example of data that could be used. Covered later, to some extent in section 3.3, but more explicit description on how roughness was quantified in methods is required.

We have clarified the method used to quantify surface roughness in the revised manuscript. Surface roughness of the fluorapatite (FAP) slices was evaluated using atomic force microscopy (AFM), which provides quantitative topographic parameters rather than qualitative or visual assessment. Multiple surface parameters, including step height, surface roughness, and adhesion force can be measured via AFM, as commonly reported in previous studies (De Oliveira et al., 2012; Li et al., 2016; Li et al., 2021).

In this study, Roughness values represent the means of measurements obtained from three randomly selected locations on each FAP slice. The detailed procedure has been added to the Methods section. **Please refer to line 164-166 page 8.**

De Oliveira, R.R.L., Albuquerque, D.A.C., Cruz, T.G.S., Yamaji, F.M. and Leite, F.L. (2012) Measurement of the nanoscale roughness by atomic force microscopy: basic principles and Applications, in: Bellitto, V. (Ed.), Atomic Force Microscopy-Imaging, Measuring and Manipulating Surfaces at the Atomic Scale. InTech, pp. 147-174.

Li, M., Wang, L.J., Zhang, W.J., Putnis, C.V. and Putnis, A. (2016) Direct Observation of Spiral Growth, Particle Attachment, and Morphology Evolution of Hydroxyapatite. Cryst Growth Des 16, 4509-4518.

Li, Z.B., Liu, L.W., Lu, X.C., Zhao, L., Ji, J.F. and Chen, J. (2021) Mineral foraging and etching by the fungus to obtain structurally bound iron. Chem Geol 586.

The discussion begins with reference to supplementary figures - given importance to the discussion they should be included in the main body of the manuscript.

Figure S1 has now been moved from the Supplementary Material to the main body of the manuscript.

Lines 234-242: cause of reductions in structural stability of mineral crystals, with regards to 'screw dislocation', not discussed with reference to LFAP and CFAP.

We supplemented the relevant information in the updated discussion. **Please refer to line 262-277 page 13.**

Lines 238-239 require referencing of the relationship of screw dislocation to reduced stability. Further discussion on how screw dislocations and how they result in reduced stability also needed.

The relationship between screw dislocations and the reduction in structural stability of fluorapatite crystals had been added in the discussion, with appropriate literature references. **Please refer to line 262-277 page 13.**

Paragraph 2 discussion should be split into two at "fungal hyphae can accelerate..." because it deals with different weathering concepts/causes than start of paragraph.

The first part of this paragraph provides evidence that *Aspergillus niger* preferentially weathering the surface of CFAP, while the second part elaborates on the mechanism of apatite weathering by *Aspergillus niger*. This paragraph described a continuous and integrated process of *A. niger*-induced bioweathering of FAP, linking surface colonization, nutrient acquisition, and subsequent chemical and physical weathering mechanisms. Therefore, we consider it appropriate to retain them within a single paragraph to preserve the logical continuity of the discussion.

Line 257: Aspergillus niger's ability to accelerate bioweathering through fungal growth requires referencing, specifically with reference to Aspergillus niger.

1) We have added references to substantiate biomechanical contribution of *Aspergillus niger* to mineral weathering in the revised manuscript.
2) Hoffland et al. (2004) emphasized that filamentous fungi can generate turgor pressure and mechanical forces during hyphal growth, inducing micro-fractures when penetrating mineral fissures or grain boundaries.
3) As a typical filamentous fungus, *A. niger* likely exerts similar localized physical stress on mineral surfaces. In addition, Gadd (2007) noted that *A. niger* hyphae can penetrate mineral cracks and exert minor mechanical pressure, although biochemical dissolution via organic acid secretion remains the dominant process. These references have been added to support our discussion of *A. niger*-induced bioweathering mechanisms.

Please refer to line 293-296 page 14.

Gadd, G.M. (2007) Geomycology: biogeochemical transformations of rocks,

minerals, metals and radionuclides by fungi, bioweathering and bioremediation. Mycol Res 111, 3-49.

Line 260: the link between screw dislocations and increased susceptibility to bio weathering is inferred not explicitly tested. It should be stated that it 'may' have resulted in increased susceptibility.

Updated. **Please refer to line 297 page 14.**

Line 262: Potential for fluorine toxicity introduced, but not relevant to the rest of paragraph and not fully discussed (how was it determined, why is it relevant…)

The mention of potential fluorine toxicity was indeed not sufficiently discussed and was not directly relevant to the focus of this paragraph. To improve clarity and maintain the focus of the discussion on CO_2- and fungus-induced weathering of fluorapatite, we have removed the statements related to potential fluorine toxicity.

Line 264: reference to literature. How does carbonic acid increase weathering?

Apatite minerals weather congruently (that is, the mineral weathers completely to dissolved products in one step) with dissolved carbon dioxide:

$Ca_5(PO_4)_3OH + 4H_2CO_3 \leftrightarrow 5Ca^{2+} + 3HPO_4^{2-} + 4HCO_3^- + H_2O$ (Filippelli, 2008).

Carbonic acid provides protons which first adsorb onto surface phosphate groups, transforming PO_4^{3-} into HPO_4^{2-} and weakening adjacent Ca–P bonds. The protonation on phosphate surface induces local lattice relaxation and bond cleavage, facilitating the release of Ca^{2+} and HPO_4^{2-} into solution (Dorozhkin, 2012).

We have updated this paragraph and added references. **Please refer to line 298-304 page 14-15.**

Dorozhkin, S.V. (2012) Dissolution mechanism of calcium apatites in acids: A review of literature. World Journal of Methodology 2, 1-17

Filippelli, G.M. (2008) The global phosphorus cycle: Past, present, and future. Elements 4, 89-95.

Lines 269-271: conclusion that "PSF would survive and perform its solubilizing ability under elevated CO2" does not follow from results. It can or may survive. Further, that "that elevated CO_2 not only accelerates chemical dissolution but also sustains fungal colonization and activity on mineral surfaces" also doesn't follow from the results. The results do not show fungal colonisation was sustained by elevated CO2 rather were not inhibited. Increases in weathering markers between CO2 and fungi+CO2 treatments necessary to support these conclusions. If they are present, explicitly detail them.

1) *Aspergillus niger* dissolves FAP through oxalic acid secretion, leading to the formation of calcium oxalate as a direct bioweathering product. Although the

amount of oxalic acid released by fungi on the FAP surface could not be quantitatively determined, the presence of calcium oxalate provides clear mineralogical evidence of fungal-mediated dissolution for FAP.

2) Calcium oxalate was observed only on FAP surfaces colonized by *A. niger* (see Figs. 6a, b, d, e), whereas CO_2 treatment alone did not produce this feature.
3) Under elevated CO_2, the FAP surfaces with fungal hyphae exhibited both CO_2-induced morphological alterations (hexagonal pyramids and trench-like structures; see Figs. 6a, d) and fungal weathering signatures (calcium oxalate formation) (see Figs. 6b, e). These demonstrates that elevated CO_2 not only accelerates chemical dissolution but also sustains fungal colonization and activity on mineral surfaces.
4) Moreover, there were more calcium oxalate formation in the fungi+CO_2 treatments than fungi only treatments (see Figs. 3c, d, e and f) which demonstrates that elevated CO_2 further promote the weathering of FAP by fungi.

We have made adjustments in the updated manuscript. In this part, we will discuss the impact of CO_2 on the surface of FAP. The synergistic effects of CO_2 and fungi will be discussed in detail in the next paragraph. **Please refer to line 311-317 page 15.**

Line 272: statement requires referencing.

The relevant literature has already been cited. (Gorbushina, 2007; Warscheid and Braams, 2000). **Please refer to line 315-317 page 15.**

Gorbushina, A.A. (2007) Life on the rocks. Environ Microbiol 9, 1613-1631.

Warscheid, T. and Braams, J. (2000) Biodeterioration of stone: a review. Int Biodeter Biodegr 46, 343-368.

Line 273: statement requires referencing.

We have updated this statement. **Please refer to line 315-317 page 15.**

Line 276-278: statement requires referencing.

The relevant literature has already been cited (Gadd, 2007; Landeweert et al., 2001). **Please refer to line 323 page 16.**

Gadd, G.M. (2007) Geomycology: biogeochemical transformations of rocks, minerals, metals and radionuclides by fungi, bioweathering and bioremediation. Mycol Res 111, 3-49.

Landeweert, R., Hoffland, E., Finlay, R.D., Kuyper, T.W. and van Breemen, N. (2001) Linking plants to rocks: ectomycorrhizal fungi mobilize nutrients from minerals. Trends Ecol Evol 16, 248-254.

Line 280-281: statement requires referencing.

The relevant literature has already been cited (Fierer et al., 2003; Kuzyakov and Blagodatskaya, 2015). **Please refer to line 330-332 page 16.**

Fierer, N., Allen, A.S., Schimel, J.P. and Holden, P.A. (2003) Controls on microbial CO2 production: a comparison of surface and subsurface soil horizons. Global Change Biol 9, 1322-1332.

Kuzyakov, Y. and Blagodatskaya, E. (2015) Microbial hotspots and hot moments in soil: Concept & review. Soil Biol Biochem 83, 184-199.

Line 283-285: traditional view statement requires referencing.

The relevant literature has already been cited (De Sena et al., 2023). **Please refer to line 325 page 16.**

De Sena, A., Mosdossy, K., Whalen, J.K. and Madramootoo, C.A. (2023) Root exudates and microorganisms. Encyclopedia of Soils in the Environment, 343-356.

Technical Corrections

Lines 69-71: sentence requires reference(s).

We have deleted this statement. **Please refer to line 79-83 page 4.**

Line 185: forming should be formed.

Updated. **Please refer to line 208 page 10.**

Citation: https://doi.org/10.5194/egusphere-2025-5242-RC1