Reviewer 1:

General comments

1. How are hypotheses 1 and 4 different? It makes sense to combine them: “Greater base cation uptake is dependent on base cation transporter gene family copy numbers that result from evolutionary expansions”. This would require some reorganization of the results and discussion, but would them clearer and more concise.

We have combined hypothesis 1 and 4 as suggested (see abstract and introduction, lines 20-24 and 118-123) and altered throughout the manuscript accordingly.

“We hypothesised that 1) greater base cation uptake is dependent on base cation transporter gene family copy numbers that result from evolutionary expansions, 2) mineral weathering results in base cation uptake by Suillus *growing in pure culture with mineral additions, and 3) base cation uptake by members of the genus* Suillus*, frequently reported to perform mineral weathering, will be greater when growing in pure culture with mineral additions compared to other fungal species. A correlation between base cation uptake and base cation transporter gene family copy numbers would provide a mechanistic explanation for mineral weathering.”*

2. Seventy-three supplemental figures is far too many. The authors should condense the expansion/contraction information from supplemental tree figures S3-S17 (which all appear to use the same phylogenomic tree) into one figure with a heatmap next to the tree, since they all represent the same idea. Likewise with figures S18-S56 for the partial dataset. The total number of gene family expansions/contractions shown in Fig 2 could also be included in this figure. This can be done in anvio by running anvi-import-misc-data for each tip of the tree.

Thank you for the good suggestion. We have condensed the supplementary figures S3-S17 (now S3), S18-S40 (now figure 3), and S41-S56 (now S4) into three figures, each with one summary tree and heatmaps showing the copy number change for each transporter gene family.

3. The gene family evolution results are basically presented as a list, and could be made much more concise. I suspect that condensing and synthesizing gene expansion/deletion data from supplemental figures would lead to a broader-scale presentation of results that reads more naturally. Same idea for the mycelial cation correlation results.

Overall, the manuscript is very long. Significant sections require editing or removal to be made more concise or clear–more is not always better. I’ve given some examples below.

We have made significant changes to reduce the length of the manuscript, the specific changes suggested by the reviewer were addressed and indicated below.

4. **Introduction**

a. Rewrite run-on or unclear sentences in lines 52-55, 57-60, and condense section from lines 80-91.

We have altered the text to make it clearer (see lines 56-59 – previously 52-55):

"Base cation mobilisation through mineral weathering by ECM fungi primarily takes place in the E and B horizons (van Breemen et al., 2000; Jongmans et al., 1997; Mahmood et al., 2024). While fine root density in these horizons is lower than in the O horizon, the extensive mineral soil volume hosts up to two-thirds of ECM root tips, nearly half of all ECM taxa found across a boreal forest profile, and numerous ECM taxa that do not occur in other soil layers (Rosling et al., 2003)."

(see lines 61-64 – previously 57-60):

"Mahmood et al. (2024) demonstrated that weathering in the mineral soil is driven by C sinks created through ECM-mediated N mobilisation from the O horizon. Additionally, variations in organic matter availability in the O horizon were found to influence fungal C allocation to the E and B horizons, thereby altering the abundance of different functional fungal guilds."

(see lines 89-97 – previously 80-91):

"In the presence of minerals, several Suillus *species have been observed to increase production and exudation of LMWOAs (Adeleke et al., 2012; Olsson and Wallander, 1998; Wallander and Wickman, 1999), and take up base cations such as potassium (K), Mg and iron (Fe) (Balogh-Brunstad et al., 2008a); Fahad et al., 2016). Mahmood et al. (2024) found that Mg2+ was taken up from B horizon soil solution in reconstructed podzol microcosms containing Pinus sylvestris seedlings growing in natural forest soils, where* S. bovinus *was the most abundant taxon. Other ECM fungi involved in mineral weathering include* Paxillus involutus *and* Pisolithus tinctorius *(Bonneville et al., 2009); Gazze et al., 2012; Van Scholl et al., 2006; Paris et al., 1995, 1996; Balogh-Brunstad et al., 2008b). However, previous studies primarily relied on abundance data and chemical analyses of ECM fungal biomass, plant biomass, or growth medium of experimental systems, without specifically investigating the mechanisms or regulation of base cation uptake."*

5. **Methods**

a. Line 220: I assume you meant micrometers instead of mm.

Yes, thank you for pointing that out, it has now been corrected (see line 242)

b. Line 246: How was biomass harvested? A brief description or citation would be sufficient.

We have added a brief description (see lines 250-251):

"A sterile cellophane sheet, rinsed in deionised water and autoclaved, was placed on each

plate to prevent mycelial growth into the agar and to aid harvesting, whilst allowing the transfer of soluble compounds."

(see lines 258.259):

"Both control treatments were adjusted to pH 5.5 and covered with a sterile cellophane sheet."

(see line 266-267):

"Biomass was harvested by gently removing mycelia from the cellophane and the agar plug…"

c. Commas are needed to provide clarity on lines 160, 192, 279, and elsewhere in the manuscript. A period is missing in line 265.

Thank you for pointing this out. Commas have now been added to improve readability and the full stop has been added.

d. Table 1: Why not simply specify the absolute concentrations of each mineral in each medium, rather than as a relative increase from the minimum medium?

The concentration of these elements is zero in the limited treatment, and we now added the limited treatment to the table to make this clear.

Results/Figures/Supplementals

a. Line 300: Replace "There are" with "In the clade *Agaricomycotina*, we identified…"

We have changed "There are" to "In the clade Agaricomycotina, we identified". See line 320

b. Line 305: Assume you meant MFS instead

Thank you for pointing this out, we have now corrected "MSF" to "MFS".

c. Figure 1a: Curious to know if you observe a separation between the non-Suillaceae, non-Russulaceae ECM and the non-ECM if you plotted on one of the other primary axes. Do *all* ECM fungi have different transporter gene composition from non-ECM fungi?

No, they do not have completely different transporter gene compositions from non-ECM fungi, but good question!

Similar comment for the correlations (Figures S58-S68), but do something like plot the R2 values to condense these into a single figure.

We tried condensing the plots by having one plot per transporter gene family per element with all four treatments in one, but found it to be visually too noisy. We instead opted to condense the supplementary figures into a table with only the R values as suggested and an asterisk indicating a significance of $p \leq 0.05$, please see Table 2 on line 440

"Table 2 – Summary of R values for each of the correlations between estimated mean mycelial elemental concentration and base cation transporter gene family copy numbers of eight different fungal species grown in pure culture in gabbro, granite, limited and rich treatments. Element, Treatment and TCDB IDs of base cation transporter gene families are indicated. R values with significant p values ($p \leq 0.05$) are indicated in bold and with an asterisk. Correlations were not performed for cases where a base cation transporter gene family is not associated with the transport of the respective element, as indicated by empty cells."

c. Fig. 2: Include in the legend that + refers to an expansion and – a contraction (I assume)

We have modified the figure and figure legend (now figure 3, and now with the heatmaps as well) (see lines 380-390):

"Figure 3 - Summary of copy number changes in significantly expanding and contracting transporter families (TFs) across the partial dataset. The number of TFs expanding and contracting at each node of the phylogenomic tree (not to scale) are indicated with a positive (+) number for expanding families or a negative (-) number for contracting families. The heatmaps show changes in gene copy number of significantly expanding and contracting TFs, with contractions in blue and expansions in red at each node (left heatmap) and tip (right heatmap). Significant expansions and contractions are indicated with an asterisk (*). The heatmap for internal nodes (left) has gene copy number changes ranging from -22 to +49, and dashed red lines indicating which node corresponds to with row of the heatmap. The heatmap for tips (right) has gene copy number changes ranging from -48 to +101. For both heatmaps, base cation TFs are positioned to the left and separated from other TFs which are positioned to the right."

d. Fig. 3: Why show (or even mention, line 408) all the non-significant correlations? It distracts from the correlations you did find.

We have now removed the mention of the non-significant correlations in the gabbro and granite treatments, referred to the above-mentioned table of R values and highlighted Mg uptake and the MgtE gene family (see lines 428-429):

"Significant correlations are indicated in Table 2. In particular, mycelial Mg concentration was significantly positively correlated with genome copy numbers of the MgtE gene family in the gabbro, granite and limited treatments, and there was a positive correlation in the rich treatment (Fig. 4a-d, respectively)."

e. Line 444: Specify statistical test and results. Was this an ANOVA?

We have rephrased the text in the materials and methods, and in the results for clarity (see lines 284.286 – previously 264-265):

"Linear models were used to perform ANOVA for each element, biomass, and C:N ratio to assess the effects of isolate (or species in the case of C:N ratio), treatment, and their interactive effect on individual response variables."

(see lines 447-448 – previously 443-444):

"For mycelial concentration of each base cation measured, ANOVA showed that there were significant (p < 0.001) overall effects of isolate, treatment (gabbro, granite, limited or rich) and the interaction between isolate and treatment."

6. **Discussion**

   a. Line 535: This point would be made more strongly if presented after synthesizing the base cation uptake results.

We have now added the point of base cation uptake levels in the beginning of the discussion, to support hypothesis 1. In addition, we moved up the section about discussing correlation directly after "Significantly evolving base cation transporters and their functions". (see lines 516-518 and 586-603)

"…Additionally, we identified 28 significant correlations between different elements and the copy numbers of expanding or contracting base cation transporter gene families associated with their uptake. ***These findings support our first hypothesis that greater base cation uptake is driven by evolutionary expansions of base cation transporter gene families."***

   b. Line 540: How exactly might the choice or number of taxa influence the numbers of gene families found to have significant expansions/contractions, and what consequence might this have for interpreting these results? Please cite an example if possible.

In a larger dataset many more families might be included since they can be present at the root but not in a more exclusive dataset, for example family IA6 and 3D3 are present in the Agaricomycotina dataset, but not in the 28-taxa dataset. CAFÉ5 uses a birth-death process to model evolutionary expansions and contractions, when a family is present in some of the taxa but inferred to be absent from the root then it is excluded from the analyses as a family cannot "die" if it has not been "born", therefore when having a more inclusive dataset more families can be included. With this reasoning in mind, we decided to analyse the different datasets to identify some contractions/expansions that might be missing due to the size and membership of the different datasets.

Line 580: Add a reference to a figure here.

Figure S6 has now been referenced here.

   c. Awkward/unclear phrasing: lines 582-584

We have altered the text to make it clearer (see lines 560-561 – previously 580-584):

“This supports previous findings of Mg^{2+} uptake from B horizon soil solution, where S. bovinus was the most abundant species (Mahmood et al., 2024).”

d. Line 601: Mention that this is hypothesis 2 (here, instead of in the next sentence that only references previous studies).

We changed the text accordingly (See lines 1175-1183)

“In addition to significant expansions in the MgtE gene family, S. variegatus also possesses significant expansions in the nucleobase:cation symporter-1 gene family (TCDB ID 2.A.39) which is associated with Na uptake. In pure culture, S. variegatus exhibited significantly higher mycelial Ca and Fe concentrations in the gabbro compared to the limited treatment, further supporting our second hypothesis about mineral weathering resulting in base cation uptake when fungi are growing with mineral additions. Previous studies have shown that S. variegatus can take up K^+ from granite particles (Fahad et al., 2016), produce high levels of organic acids in the presence of minerals (Olsson and Wallander, 1998; Wallander and Wickman, 1999), and exhibit increased weathering activity when in symbiosis with Pinus sylvestris compared to non-mycorrhizal plants (Wallander and Wickman, 1999). “

e. Line 612: Mention that this is hypothesis 3, since hypotheses were numbered in the abstract.

Done (see line 607)

f. Lines 628, 632: Add references to figures here.

Done (see line 589)

g. Line 624: Mention that this is hypothesis 4

Hypothesis 4 is removed and added in together with hypothesis 1 as suggested, please see comment above.

h. Lines 625-634 belong in the results, not the discussion.

This has been moved to the results. See lines 432-435

i. Line 634: typo?

We have deleted the extra “t”

j. Lines 649-652: run-on sentence

We have updated the text to (See lines 627-630):

“Contrary to our hypotheses which were confined to Suillus, we also observed significant expansions and mycelial uptake of elements in the genus Piloderma. Therefore, future controlled pure culture experiments should investigate mineral weathering capabilities across a wider range of species. Additionally, future work on the transcriptional regulation of transporter proteins in Suillus and other ECM species could provide deeper insights into the mechanisms underlying mineral weathering and base cation uptake.”