Reviewer 2:

General suggestions:

1. Please add at the beginning of the introduction some information about mineral weathering and perhaps a definition.

We have added a definition of biological mineral weathering at the beginning of the introduction (See line 39):

"In boreal forests biological mineral weathering, the disintegration and dissolution of minerals caused by biota, is driven by plant-associated ectomycorrhizal (ECM) fungi forming the dominant type of symbiosis primarily with trees of the family Pinaceae (Smith and Read, 2008)."

Additionally, we have expanded on the mechanisms ECM fungi can use to weather minerals on lines 45-48:

"To weather minerals, ECM fungi must colonise mineral rich soils and utilise plant allocated C in the application of physical force, the production and exudation of low molecular weight organic acids, free radicals, protons and siderophores, which can break down minerals leading to the mobilisation of base cations (Finlay et al., 2020; Fomina et al., 2010; Schmalenberger et al., 2015)."

2. I'm wondering if it would be helpful to have a diagram of the soil and the movement of the nutrients through weathering and fungal uptake in the introduction. Perhaps including Mg and the elements in Table 1 to bring the different parts of the paper together that feel a bit dispersive.

Thank you for the suggestion. We have added a figure to aid in the explanation of nutrient mobilisation and uptake during weathering, please see Fig. 1.

3. It is unclear overall what is the logic of the questions. For example, why did the authors look into the fungal mating-type pheromone? Justify the biology? Explain why is the expansion in pheromone receptors suggestive of adaptation to an environment?

We see the confusion of presenting these results unintroduced. We lifted this transporter gene family in our results as it was prominent in our CAFE5 analyses and is involved in sexual reproduction which can influence rates of evolution and adaptation, by increasing the occurrence of genetic recombination in a population. This may in turn have an influence on the development and continuity of mineral weathering capabilities of taxa.

In the introduction to the bioinformatics section in the materials and methods (section 2.1) we highlight that we look into transporter families which are identified as interesting in more detail (see lines 137-138):

"Additionally, transporter gene families identified as particularly relevant in our analyses were examined in greater detail."

Furthermore, we have added the following to section 3.1.3.2 (see lines 392-396):

"In addition to base cation transporter gene families, we also examined transporter gene families that were significantly expanding and/or contracting in our CAFE5 analyses and are associated with processes that may influence evolutionary rates and adaptation, such as those involved in sexual reproduction. Given that shifts in rates of evolution may, in turn, impact the mineral weathering capabilities of taxa, these gene families were considered to be of particular interest.

Our CAFE5 results indicated numerous significant expansions and contractions in the fungal mating-type pheromone receptor (MAT-PR) gene family (TCDB ID 9.B.45), which is involved in mate recognition and compatibility."

The discussion has also been expanded to properly highlight the role of sexual reproduction in environmental adaptation (see lines 525-533):

"*Significant expansions of the fungal mating-type pheromone receptor (MAT-PR) gene family were observed in several Suillus species, in both the full and partial family datasets (Fig. S3 and Fig. 3, respectively), with a notable expansion also seen in Russula emetica in the full dataset. This gene family encodes pheromone receptors located on the plasma membrane that play key roles in mate recognition, compatibility, and the reciprocal migration of nuclei during mating (Coelho et al., 2017; Di Segni et al., 2011; James, 2015; Xue et al., 2008). Sexual reproduction, which these receptors facilitate, promotes genetic recombination, enhances the selection of advantageous traits, and accelerates adaptation compared to asexual reproduction (Becks and Agrawal, 2012; McDonald et al., 2016; Nieuwenhuis and James, 2016). It is especially advantageous in unstable or nutrient-variable environments (Nieuwenhuis and James, 2016). Thus, the observed gene family expansions may reflect an increased reliance on sexual reproduction, potentially boosting adaptability in changing environments*."

4. For hypothesis 2, does the base cation uptake simulated in experimental conditions by the mineral additions?

In treatments with mineral additions, the only source of base cations is from the minerals, therefore fungi must weather the minerals to be able to take up any base cations. Then, yes, the base cation uptake is simulated by the mineral additions.

5. Are hypotheses 3 and 4 fundamentally the same? Or is there something specific about Suillus that the authors are trying to test?

Hypothesis 3 *"base cation uptake by Suillus growing in pure culture with mineral additions will be greater compared to other fungal species" is testing whether or not Suillus species are better at taking up base cations from mineral sources than other fungi"* However, the hypothesis does not say anything about the mechanism of uptake.

Hypothesis 4 *"base cation uptake will correlate with base cation transporter gene family copy numbers"* tells us if the mechanistic explanation behind increased mineral uptake is due to increased copy numbers of the transporter families involved.

However, based on a suggestion by reviewer 1, we have now combined hypothesis 1 and 4 and the hypotheses read as follows: (see lines 118-123)

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.

6. If it's just Suillus, how can we know if it's just lineage-specific? How can you exclude the evolution at the root? Or explain more clearly what the analysis is showing within Suillus.

We have expanded our explanation about the CAFE5 analysis and the use of the Agaricomycotina tree. The CAFE5 analysis in the Agaricomycotina tree allows us to compare Suillus with other lineages within Agaricomycotina, which in turn allows to understand whether the changes in copy number are occurring only or mainly in Suillus or not (see lines 176-179):

"The same data was used to estimate gene family evolution of transporters across the Agaricomycotina phylogeny using CAFE5 v5.1 (Mendes et al., 2020). This approach facilitated the comparison of changes in transporter gene family copy number between multiple lineages, enabling evaluation of whether these changes were isolated to the Suillus lineage or more broadly distributed."

7. Add the goal/context for the bioinformatics section (2.1) to understand the questions those methods will help address.

We have added a paragraph introducing the bioinformatics used and how they aid in answering our questions in section 2.1 (see lines 132-138):

"Bioinformatic analyses were performed to investigate whether evolutionary expansions have occurred in base cation transporter gene families in mineral-weathering species, such as those in the genus Suillus, and to assess the relationship between gene copy number and mineral weathering capacity. To achieve this, a phylogeny of Agaricomycotina taxa with diverse lifestyles was constructed. Evolutionary expansions and contractions of all transporter gene families, including those involved in base cation transport, were then analysed to enable a comparative assessment of the copy number variations across the phylogeny. Furthermore, transporter gene families identified as particularly relevant in our analyses were examined in greater detail."

8. Provide more context for looking into Mg transporter family.

The importance of Mg and studies reporting it's uptake by ECM fungi have been raised in the introduction (see lines 67-69 and 89-93):

"Mahmood et al., (2024) also showed that mobilisation and uptake of magnesium (Mg), which acts as an activator for many enzymes and plays a key role in photosynthesis, occurred primarily in the deeper mineral horizon and was driven by carbon allocation to ECM mycelium (Black et al., 2007; Bose et al., 2011)."

"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."

We have also included more context about why Mg and the Mg transporter gene family is of interest in sections 2.1.4 and 3.1.4.

2.1.4 (see lines 208-209):

"Our CAFE5 results and subsequent correlations between base cations and base cation transporter gene family copy numbers led us to investigate the Mg transporting MgtE transporter gene family further."

3.1.4 (see lines 411-415):

"The Mg transporting MgtE transporter gene family was highlighted as particularly relevant by our CAFE5 results, as one of the most frequently significantly expanding and contracting base cation transporter gene families in both the full and the partial datasets. It was also found to be frequently significantly expanding and contracting in the clade containing Suillus species in the partial dataset. Additionally, copy numbers of the MgtE transporter gene family were found to have significant correlations with mycelial Mg concentration in isolates grown in pure culture in axenic systems. Furthermore, Mg plays an important role in plants and fungi, and has been found to be taken up by ECM fungi from minerals and mineral soils (Fahad et al., 2016; Mahmood et al., 2024)."

9. Why is there no figure with the whole Agaricomycotina tree?

We present a tree of the whole Agaricomycotina in Figure S1. We think that it is not possible to add this tree as a main figure in a way that it fits an A4/letter size paper and that it can be easily read and interpreted. Therefore, we have decided to keep it as a supplementary information so that the reader can have access to it and can zoom in to relevant parts.

10. I have a few specific suggestions:

a. Figure 1 - what is the input matrix to calculate the PCAs? counts of genes? Perhaps a multipanel figure with also the phylogeny might help explain the analysis?

We have added in the figure legend (see Fig. 3)(lines 385-390) and the text (lines 268-172) that copy number counts per transporter family were used as input, also we have included a table in the supplement with the copy number count data per transporter gene family per species (Tab. S3).

"Relationships between transporter gene family copy numbers and fungal taxa were analysed and visualised by performing a principal component analysis (PCA) on count data of transporter gene family copy numbers (Tab. S3) using CANOCO 5.10 (Microcomputer Power, Ithaca, NY, USA; Braak and Smilauer, 2012)."

b. Figure 2 - add branch lengths. The reason for this tree is to show the taxa that are tested in the lab? Since the bioinformatics section talks about a Agaricomycotina tree, I am puzzled by the selection of these taxa and what the purpose of this tree is.

We have presented a tree of the whole Agaricomycotina (the full dataset) with true branch lengths in figure S1. The reason for not including it as a main figure is because some of the branch lengths (particularly in the Suillus clade) are very short and it is difficult to see the branches properly.

We have clarified that both a full dataset with all 108 Agaricomycotina taxa and scaled copy number values, and a partial dataset with 28 taxa and true count values of copy numbers were used and their usefulness. We have also expanded on our explanation for the selection of the 28 taxa used in the partial dataset (see lines 184-193):

"Although the values are scaled, it remains valuable for observing expansions and contractions across the entire Agaricomycotina phylogeny.

"CAFE5 was also run on a smaller dataset with true count values of copy numbers to account for any error introduced by the scaling and allow for observation of expansions and contractions calculated with true copy number values in key species of interest. Twenty-eight taxa were selected for this smaller dataset including all Suillus species, as they have been frequently reported to perform mineral weathering and have been found to inhabit the mineral B horizon in boreal forest soil profiles. Piloderma species were selected as they are also ECM fungi and have been found to inhabit both the organic and mineral horizons in boreal forests. Finally, two saprotrophic fungi, Coniophora puteana and Serpula lacrymans, were selected to allow comparison between the ECM and saprotrophic lifestyle."

Additionally, we have elaborated on the reason for the selection of these species in section 2.2 (see lines 218-223):

"The ECM genus Suillus has been reported to possess mineral weathering capabilities and inhabit the mineral horizon in boreal forest soils, and was therefore studied in greater depth. Isolates of Suillus, the ECM genus Piloderma, which inhabits both the organic and mineral

soil horizons (Mahmood et al., 2024; Marupakula et al., 2021), and two saprotrophic fungi, Coniophora puteana and Serpula lacrymans, were included to compare between ECM and saprotrophic lifestyles. All isolates were grown in pure culture axenic systems with and without mineral additions to determine base cation uptake during mineral weathering."

c. Figure 3 - highlight panels with significant slopes and mention statistical test in caption.

We have added the statistical test used and highlighted significant panels in the figure legend (Now Figure 4; lines 434-436):

"Pearson's correlations between mycelial magnesium (Mg), calcium (Ca) and iron (Fe) concentrations (mg/kg) and transporter gene copy numbers. Element concentration is given as the estimated mean values in eight different fungal species grown in pure culture in gabbro, granite, limited and rich treatments, and the gene copy numbers of the Mg2+ transporter-E family (TCDB ID: 1.A.26) in the corresponding fungal genomes. Significant correlations with $p < 0.05$ are found in panels a, b, c and g."

Additionally, we realised that we miswrote in the materials and methods section where we said that a Spearman's correlation was used, but it was in fact a Pearson's (see lines 304-305):

"Correlations between base cation transporter gene family copy numbers and means of mycelial elemental concentration for all isolates in each mineral treatment and the control treatments were tested using Pearson's correlation."

d. Figure 4 - highlight different species on the figure with boxes around the names or labels. Report significance and statistical tests used in the caption.

Figure 4 (now figure 6) has been adjusted to make it clearer which species different isolates belong to and with the addition of a horizontal line to highlight isolates which are significantly different from each other.

The materials and methods have been adjusted to describe more explicitly how the ratios were estimated and reported (see lines 300-303):

"Ratios were calculated from the means of all replicates per isolate per treatment, yielding one ratio per comparison. Ratio means are reported with 95% confidence intervals, based on model uncertainty and pooled variance across isolates. Ratios with non-overlapping confidence intervals were considered significantly different."

The figure legend has also been adapted (see lines 460-468):

"Figure 5 – Ratios of mycelial calcium (Ca) concentration (mg/kg) between gabbro and limited treatments for each fungal isolate. Isolates include Suillus bovinus (Sb1–4), S. luteus (Sl1–3), S. variegatus (Sv1–3), S. granulatus (Sg1–3), S. grevillei (Sg4), Piloderma byssinum (Pb), P. aff. fallax (Pf), P. olivaceum (Po), P. sphaerosporum (Ps), Piloderma sp. 1, Piloderma sp. 2, Coniophora puteana (Cp), and Serpula lacrymans (Sl). Data points represent the mean

ratio of Ca concentration between gabbro and limited treatments for each isolate, with error bars indicating the 95% confidence interval. Isolates with non-overlapping confidence intervals are considered significantly different from one another. The red dashed line represents a ratio of 1.78, distinguishing isolates with significantly higher Ca concentrations above the line (S. luteus, S. variegatus, and all S. bovinus isolates except Sb16) from those below it, which include all Piloderma species except P. sphaerosporum."

e. L18 list species of saprotrophic fungi

We have now added "*Coniophora puteana*" and "*Serpula lacrymans*" to line 18.

f. L397-401 sentence is incredibly long

We have re-written the long sentence (see lines 423-425):

"Of the 25 base cation transporter gene families that were found to be significantly expanding or contracting, 11 showed significant correlations between gene family copy number and base cation uptake. From these, six are specifically involved in the transport of Ca, Fe, K, Mg, Na and P and five are base cation transporters not linked to a specific base cation. "

L417 onwards, is there a way of summarising the information on significance so that it's not so difficult to read through? perhaps a table?

We have included a table (Tab. 2) summarising the R values with an asterisk indicating a significance of $p \leq 0.05$ to make it easier to read (see lines 440-445)

"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 in bold with p values where $p \leq 0.05$ indicated 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."

g. L447 italicise luteus

We have now italicised *luteus*.