

## Reply to Reviewer 1

### Biological weathering and its consequences at different spatial levels – from nanoscale to global scale

*Reviewer 1 makes a number of helpful comments that will no doubt greatly improve our manuscript if we are permitted to submit a revised version.*

The ms is intended as a review of current progress made in understanding biological processes contributing to weathering. It is based on a very extensive reading in the literature (I counted 215 references) on a very wide range of topics, including chemistry, physics and biology, combining empirical and modelling approaches, based on a large range of experimental techniques. The authors also make a very laudable attempt to scale up, both spatially (as indicated by the title) and temporally, when they link past and current weathering processes as an evolutionary and ecological force. The ms is also very well written.

*We are pleased the reviewer considers the text well written.*

Despite all this initial praise, reading the ms did not fully satisfy me. In my view this is to a large extent due to the fact that it succeeds very well as a review, but succeeds to a lesser extent as a synthesis. Several empirical observations seem to contradict other observations, and one would like to read how much consensus has been reached on the biology of weathering. In that respect I found the final section (key questions and knowledge gaps) somewhat disappointingly short.

*We agree fully that the final section is too short and intend to expand it. (see below).*

Considering the lack of consensus on the importance or generality of several processes, a more cynical reader may easily be inclined to think that almost anything goes in biological weathering. In fact this kind of mild cynicism is almost encouraged by the authors: after having presented so many data the authors (p. 17, l. 33 – p. 18, l. 1; note that one of the authors of the paper referred to is also an author of this ms) state that “Smits & Wallander (2017) consider that there is *no clear evidence* [emphasis mine] that processes at the laboratory-scale play a significant role in soil-scale mineral dissolution rates”. If so, what is the main message of this paper?

*We do not agree that the lack of consensus reflects “cynicism”. **Indeed, one of the aims of this article was to examine differences in the degree of consensus about research conducted at different temporal and spatial scales.** (Admittedly this is not very well explained at present, but one of the take-home messages was meant to be that there is greater consensus on the large-scale, systemic effects of microorganisms on weathering than on the overall significance of micro-/nano-scale observations). Science proceeds by first identifying conflicting opinions and then, often at a later stage, resolving conflicts by collecting additional data – often in newly designed experiments or by using new techniques. It would be surprising if the **eleven** authors of this article had exactly the same opinion about every single process discussed, but we do agree that we should have worked much harder to try to resolve conflicting results to provide the “**synthesis**” the reviewer wants. At any one point in time it is often not possible to resolve conflicts of information completely but, in that case we agree that it is important to identify new approaches or key questions to ask in new experiments and to provide clear guidance about the approaches required. We will re-write the text to improve this aspect and expand the final section – as suggested above.*

Let me try to back up my dissatisfaction with a couple of general observations. Before providing more detailed comments.

1. The point of departure for the study is that weathering is the only or main supplier of base cations and phosphorus to compensate for losses through harvesting and leaching. However, on p. 17, l. 21 the reader is informed about atmospheric deposition (the only mention of this input source) where we are informed that a study found that atmospheric deposition was four times as important as weathering; and that the weathering flux was less than 0.3% of calcium uptake. This statement then raises questions about its quantitative importance over ecological time scales and evolutionary time scales, an issue treated very implicitly at best.

*We agree that alternative sources of different nutrients and base cations should be discussed and that there may be significant input under some circumstances from atmospheric deposition – examples of significant P input from atmospheric deposition to coastal Fynbos systems (eg. Brown et al. 1984) and the Florida everglades (Redfield, 2002) have been shown. These possible alternatives are now included in our discussion. However, this does not call into question the validity of all weathering studies and the clear stable isotope results from our own mesocosm experiment suggest that in boreal forest soils mobilization of Mg is probably not primarily from litter re-cycling in surface soil as shown by Dijkstra and Smits (2002) for Ca.*

2. Despite the generality of the title (biological weathering), the focus is almost exclusively on the role of mycorrhizal fungi plus associated mycorrhizosphere bacteria and the trees with which they associate (I like the focus on the plant as holobiont). Lichens, generally considering as major weathering agents in the first stages of primary succession, are mentioned only once (p. 12, l. 10-12). There the authors state that “the ubiquity and significance of lichens (..) as a model for understanding weathering (...) are well understood.” However, the reader is not informed about this understanding, nor is (s)he informed whether fungal weathering is similar or dissimilar from lichen weathering in any significant respect. There is also very limited attention for fungi other than mycorrhizal fungi, however from an evolutionary perspective this is a missed opportunity. A fungus often used in weathering studies is *Paxillus involutus*, a species derived from a clade of brown-rotting fungi characterized by oxalate production. It could be possible that the ability to produce and excrete oxalate in the environment evolved for different purposes and was even maintained in the ectomycorrhizal groups in this clade for different purposes.

*Apart from ectomycorrhizal fungi in forests we do also mention 1. proteoid roots of in highly weathered soils, 2. calcicole plants in calcareous soils, 3. non-mycorrhizal fungi such as different *Aspergillus* species and 4. different bacterial species. However, we agree that a slightly better description of the potential role of bacteria, lichens and non-mycorrhizal fungi as weathering agents should be included and will include more information in the revised version of the manuscript. This will include evolutionary aspects (discussed by Fahad et al., 2016) and also the desired information about *Paxillus involutus* – recently discussed by Nicolás et al 2019. (ISME J. 13: 977-988).*

3. There are many parts in the ms where the possible difference in weathering ability between arbuscular mycorrhizal fungi+plants and ectomycorrhizal fungi+plants are mentioned. Some of these are quite explicit in suggesting

that the ectomycorrhizal symbiosis allows higher weathering rates than the arbuscular mycorrhizal symbiosis. However, we also learn that weathering evolved in the arbuscular mycorrhizal symbiosis (p. 11, l. 26) and that some studies did not find differences in weathering rates under ectomycorrhizal and arbuscular mycorrhizal vegetation (p. 21, l. 26-27). The reader of his paper will therefore remain in doubt what the current consensus view is (if there is consensus), what likely hypotheses exist to explain such different data and what kinds of research approaches exist to resolve that issue. (One option would be a common-garden experiment with sister clades of plants with the different mycorrhizal symbioses, in analogy of the approach by Koele et al. (New Phyt. 196: 845-852. 2012) when they tested for stoichiometric differences (leaf N:P ratio) between both guilds. I am sure there must be other ways to make progress as well.) Another group of mycorrhizal fungi + plants, which form the ericoid mycorrhizal symbiosis, is mentioned once (p. 7, l. 21) even though they have been suggested to be strong weathering agents as they can produce copious amounts of low-molecular-weight organic acids (Martino et al., Soil Biol. Biochem. 35: 133-141. 2003).

*We agree that some of these ideas are not currently included and will mention them in the revised manuscript. One of the arguments about possible differences between arbuscular mycorrhizal and ectomycorrhizal fungi concerns evolutionary differences of the C-fixation properties of their plant hosts (work of KJ Field and D Cameron) and these ideas will be discussed in the revised manuscript.*

4. Addressing (and putatively answering) the question of the role of different mycorrhizal symbioses in weathering is, in my view, particularly relevant when it comes to understanding mechanisms. If weathering is driven by the production of LMWOA and siderophores, then it should be clear that the ectomycorrhizal symbiosis is much more important for weathering than the arbuscular mycorrhizal symbiosis (as AMF have not been reported to produce LMWOA, the AMF symbiosis has been reported to downregulate LMWOA production by plants (Ryan et al., Plant Cell Environ. 35: 2170-2180. 2012), and AMF do not produce siderophores as far as I know). If other mechanisms are more important (e.g., acidification driven by excess uptake of cations over anions and proton exudation to maintain charge balance; or dissolved CO<sub>2</sub> as a consequence of respiratory activity), the contribution by both guilds could be more important – with differences still related to the amount of extraradical hyphal biomass and / or respiratory activity.

*This is an interesting subject area and there are probably questions that cannot be fully resolved with currently available information but we will add some comments. Generalisations should always be made with care but one aspect that could be relevant is differences in decomposition rates. Since ectomycorrhizal (and ericoid) fungi typically dominate systems characterized by recalcitrant organic substrates and slow decomposition rates, whereas AM fungi dominate systems with higher decomposition rates in which the input from turnover of nutrients from organic residues may be higher. Possible future approaches include common-garden experiments, as mentioned above, as well as the use of mutant plants with altered regulation of proton-pumping and some of these ideas will now be mentioned in the revised manuscript.*

5. The issue about the relative importance of weathering mechanisms has been debated since mycorrhizal researchers entered that field in the early 2000s.

When enthusiastic claims were made for a major role of mycorrhizal fungi (and I admit having been such an enthusiast as well), these ideas were criticised by Sverdrup, who essentially claimed that weathering was driven by CO<sub>2</sub> flux and that the contribution by ectomycorrhizal fungi was around 2%. While his claim has been challenged (Van Schöll et al., *Plant Soil* 303: 35-47, 2008), I think this review would have been a good place to synthesise current understanding. Sverdrup (cited in the ms – pls note that the journal has Volume 23, Issue 4; not Volume 4) has maintained his suggestion about the major importance of respiration / CO<sub>2</sub> production, rather than the production of organic acids) as the driver for weathering, stating: “the growth of trees represents quantitatively largest single biological process that can affect weathering, followed closely by decomposition of organic matter.” It is evident that the authors of this ms disagree with Sverdrup, however, without fully discussing this alternative view. I think this is a missed opportunity. The same applies to the origin of pores, with Sverdrup claiming that they are of abiotic origin (as cited in p. 3, l. 30). How would the authors of this ms evaluate our current knowledge and understanding? (Note that because of the extent of tunnelling the contribution to weathering might be limited, irrespective of the question on their origin.)

*We have discussed the fact that tunnelling may be both biotic and abiotic and that there are ways of distinguishing the two types of tunnels. ALL tunnelling is not abiotic. However we also discuss the important studies of Smits et al. (2005) that showed that tunnelling is not quantitatively significant as the sole indicator of weathering. We now mention the review by Van Schöll et al. (2008), as well as many other more recent studies. In fragmented mineral substrates weathering of surfaces may take place without formation of tunnels. We do fully understand that the growth of trees affects weathering of minerals and have explained that removal of weathering products from these sites (by hyphae) is an important process.*

6. While I agree about the importance of upscaling, both spatially and temporally, I think that progress depends on the extent to which we can quantify rates. Unfortunately, the paper is quite frugal in giving numbers. This may give the impression that despite such many studies there has been little progress in quantifying processes. That conclusion seems also implied in p. 1, l. 31-32 (“opinion appears to be divided with respect to the quantitative significance [of interactions between microbes and minerals]”). If opinions are divided, please give equal hearing to arguments from both sides. But if a clearer picture has emerged in the view of the authors, please provide more quantitative detail. In order to have feedback mechanisms to work over both ecological and evolutionary times, we need such data.

*We agree that more quantitative estimates (rates) are necessary and will attempt to cite more quantitative estimates in the revised manuscript..*

7. The authors refer (p. 2, l. 28) to twelve testable hypotheses on the geobiology of weathering. It would help the reader to list those (rather than to invite them to look up the paper themselves) and to indicate to what extent their review helps addressing these hypotheses. For instance hypothesis 8 (elevated CO<sub>2</sub> will enhance weathering) seems to assume that weathering fluxes and its ultimate consequences of drawdown of CO<sub>2</sub> occur at very different time scales, which could put constraints on feedback

mechanisms postulated in the ms. With respect to hypothesis 2, the importance of stoichiometry, I think that the studies done of mycorrhizal weathering provide much of the needed data. In none of their hypotheses they draw attention to different kinds of mycorrhizal symbiosis (but this could be a refinement of hypothesis 1), although it may not be coincidental that their figure 1 depicts an ectomycorrhizal conifer...

*A full discussion of all 12 hypotheses discussed by Brantley et al is not possible within this article is not possible for reasons of space but some of the ideas cited in that article are now discussed in more detail in as much as they relate to biological weathering.*

Some of these comments will make the manuscript longer, so I think it may help if I indicate cases where shortening of the ms is possible. I noted several digressions (also in the Abstract) that result in a less focused paper. Examples are: reference to acquisition of N and P by mechanisms other than weathering (p. 7, l. 18-34), hydraulic lift / redistribution (p. 9, l. 6-8), oxygenic and anoxygenic photosynthesis (p. 11, 14- 24), autotrophic and heterotrophic respiration in forests (p. 13, l. 30 – p. 14, l. 6; unless the authors think that Sverdrup is, essentially, right...), differential carbon storage in ectomycorrhizal and arbuscular mycorrhizal forests (p. 14, l. 4-28; note that of the two biomes where both guilds occur larger C storage per unit N was shown for the temperate biome, not for the (sub-)tropical biome – so we should not take Averill's claim too seriously), nitrogen in the rhizosphere (p. 24, l. 1-2).

*We will make some of the suggested cuts to reduce the overall length of the article.*

### **Page-by-page comments**

#### **p. 3, l. 9**

Is the Finlay & Clemmensen paper on biogenic weathering? The title of the paper would suggest otherwise

*The paper is on carbon flow in relation to both decomposition and weathering.*

**p. 5, l. 14** Please provide a reference for the suggestion about the importance of horizontal gene transfer in such microbial consortia in EPS.

*Reference is now provided.*

#### **p. 7, l. 1**

Here I disagree. In such habitats, in case of a low pH, plants with cluster roots (or proteoid roots; I think they are the same) or dauciform roots produce carboxylates that *desorb* phosphorus from mineral surfaces. But desorption is not weathering, dissolution of minerals. Weathering would happen in the case of high-pH with calcium phosphates; in low-pH soils P is far too scarce to form substantial amounts of Fe- and Al-phosphates that are weathered.

*We agree with the reviewer and have re-written the text more carefully*

#### **p. 9, l. 14**

In the light of current criticisms of humic and fulvic acids as large molecules (Lehmann & Kleber claim these to be aggregates of essentially small molecules) this statement may need reconsideration in terms of underlying mechanisms.

*Agreed – we have altered the text to reflect this*

#### **p. 15, l. 21**

When introducing the Blum et al. hypothesis, they should also refer to contradictory data by Dijkstra and Smits (now only referred to on p. 17, l. 18-22; however I interpret that paper as showing that Blum et al.'s conclusion is grossly overstated – but I would love to see the opinion of the authors of this ms).

*Agreed – we have altered the text to reflect this. We originally discussed the Blum paper in more detail but removed the text because of this overstatement.*

**p. 17, l. 19**

Please provide a reference to that further study.

*The reference is now added – the comparable forest referred to is actually mentioned in the same study by Dijkstra & Smits.*

**p. 19, l. 17**

Note that exudation of carboxylates / organic anions can also have a major function in the desorption of iron-oxide bound soil organic matter and the subsequent acquisition of carbon (Keiluweit et al., Nature Clim. Change 5: 588-595. 2015) and nitrogen (Jilling et al., Biogeochemistry 139: 103-122. 2018) (and possibly phosphorus, as both inorganic and organic P are sorbed on such surfaces).

*Agreed – we have altered the text to reflect this*

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