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Biogeosciences Discussions

Interactive comment on "In vitro formation of Ca-oxalates and the mineral glushinskite by fungal interaction with carbonate substrates and seawater" *by* K. Kolo and Ph. Claeys

K. Kolo and Ph. Claeys

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We would like to thank Referee #2 for his comments on our manuscript. We disagree with some of the comments of Referee #2 and welcome this opportunity to clarify our views.

General Comments

1. We strongly disagree with the Referee's comment that the major conclusion of this paper is just that fungi dissolve carbonates and precipitate oxalates. This comment is not representative of our conclusions. We present in our ms **11 conclusion points** that have not been previously documented. Referee #2 refers inappropriately to our opening *de facto* statement of the conclusions, which is certainly not our *major* and *only* conclusion.

2. Referee #2 claims that our work had already been done two centuries ago; he bases his claims on citation Braconnot (1825), and de Bary (1887). However, without citing a precise reference, such as a book title or a journal the list of references cited in this work and other works shows the fact that there is currently extensive research work on fungal-carbonate substrates interaction on topics such as: sediment diagenesis; bioremediation; biodegradation; biosorption; biomineralization; astrobiology... etc.

3. Referee #2 considers the precipitation of the Mg-mineral glushinskite for the first time as a minor result. We do not consider this as a minor result. Besides the importance of glushinskite as a biomineral that indicates the presence of life, magnesium and carbon are recycled from carbonate substrates through this mineral, bearing clear diagenetic implications. Its method of formation is related to fungi through circumstantial evidence (e.g. Wilson et al., 1980, Mineralogical Mag. 43, p. 837–840). Here the role of fungi in its formation is clearly demonstrated, not only from Mg-bearing carbonate substrates but also from Mg-liquid substrates. The mineral glushinskite was reported in 1980 (Wilson et al., 1980, Mineralogical Mag. 43, p. 43, 837–840) with a single crystal habit. We identified through SEM and Raman spectroscopy new and undocumented crystal habits of the mineral formed by fungal interaction with Mg-rich substrates.

4. Referee #2 considers that our experimental procedure is poorly thought-out and also adds that our results are "questionable at best" because the experiments were conducted without controlled parameters. However, it is stipulated in the paper that the experiment was done, on purpose, under uncontrolled conditions to evaluate and simulate as much as possible the "natural" interactions between fungi and carbonate/seawater substrates but with the possibility to monitor the results *in-situ*. The fungi grew from airborne spores, attacked and interacted with the substrates and produced new biominerals, new crystal habits and diagenetic effects. These are **material results** produced through the fungi-substrates inter-

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action. The players were the fungi and the substrates. We documented these material results through various analytical procedures.

5. Referee #2 finds that the work is based on SEM images. This is not true. We actually used beside SEM images, EDX, XRD, Raman spectroscopy and optical microscopy. Our comments in figures captions and in text are analytical, interpretative and documentary relating result to process, reason and cause. As far as we know, all published works dealing with fungi-substrates interactions are based on results documented by one or more of the previous analytical tools, to these we added Raman spectroscopy. Without these tools the results are meaningless.

6. Referee #2 finds our hypothesis “an unsubstantiated conclusion” that the observed zoned Ca-oxalates could form a precursor of diagenetic structures identified in calcium carbonates, similar to ooidal structures. It is indeed correct that we speculate on this issue, but at the same time we think we have the right within the scientific process to propose hypothesis to be tested, especially that, and to our knowledge, this is the first report on such type of microbial zoned crystals. We agree that neither the size of these crystals, maybe nor their abundance could form real input into sediments. We accept the argument of Reviewers #2 and have modified the text.

Answer to BGD questions:

1. We disagree with point 2 in the Referee’s #2 answer to BGD questions where he states that this paper presents no novel concepts or ideas. We consider that our work has documented:

1. The interaction fungi-carbonates substrates can be observed at the thin section scale, which provides in-situ analyses of the mineralogical changes and the identification of a sequence of mineral replacement. Our method can be applied easily to any surface, any substrate whether the experiment is using specific fungal

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cultures or is open to atmosphere and to evaluate the results of that interaction in situ.

2. According to our experimental procedure, these changes can easily be studied under the SEM, EDX and Raman spectroscopy, whether the changes are on the substrates or within the fungal mass.

3. The validity of using Raman spectroscopy to identify mineral phases at such scale and under such interaction.

4. The use of Mg-bearing liquid substrate for the fungal interaction with the demonstration that fungal interaction with this substrate extracted elements from seawater to precipitate new mineral phases such as glushinskite.

5. Moreover, we consider that the observations provided by our experiments must also be viewed in a broader sedimentological and diagenetic context as the dissolution and re-precipitation of carbonate substrates by fungal interaction creates a new mineral substrate that differs from the original lithology in term of minerals, texture and geochemistry and leading to the concept of “bio-stratification"

2. Referee #2 finds that the number and the quality of the references are not appropriate. We have cited published and available references with direct relevance to our work. Should we cite the reference of Braconnot (1825), we would gladly do it. We searched both the Royal Library of Belgium and the UC Melvyl catalogues without finding a book or article published by Henri Braconnot in 1825. We would thankfully welcome any suggestions from our colleague on this matter.

To finalize, we would like to highlight some of the novel aspects of this work:

1. The feasibility of in-situ investigation of fungal-carbonate and seawater substrates interactions.

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2. The usage of seawater as a source of Ca and Mg cations in fungally induced mineralization processes.
3. The *in-situ* Raman identification of single crystal biominerals with variable crystal habits and forms.
4. Clear demonstration of intra-hyphal mineralization.
5. The report of glushinskite formation.
6. The report of lamellar glushinskite. (Unknown so far).
7. Novel crystal habits of glushinskite.
8. The demonstration of sequential biomineral formation during the fungi-substrate interactions.
9. The presence of microbially zoned crystals of biominerals.
10. The presence of doubly-layered fungal hypha biomineralization.
11. The identification of new crystal habits of Ca-oxalates, reported here as “Greek Pillar".
12. The link with the diagenetic aspects: e.g. “biostratification" and replacement with their implications in geology, bioremediation, biodegradation and astrobiology and early life search.

The authors

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