Interactive comment on "The role of microorganisms on the formation of a stalactite in Botovskaya Cave, Siberia – palaeoenvironmental implications" *by* M. Pacton et al.

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We thank the reviewers for their thoughtful comments and changed the manuscript accordingly.

Comment: "In response to S. Verheyden (p. C1933), the authors claim that they have analyzed "several 13C profiles using SIMS, and subsequently analyzed them using SEM. Additionally, we analyzed the section using the incremental milling of powder for IRMS", and then go on to briefly describe the results. However, these data are not included anywhere in tables, graphs, or images. These supporting results should be included, at least as part of the supplementary materials, if they are to support the main

conclusions. In particular, it would be interesting to compare stable isotope transects across the porous cracks in the continuously milled vs. the original discretely drilled IRMS sequences alongside the SIMS data. This is an essential technical addition to the paper."

Authors response: We analyzed part of the sample using SIMS along with SEM (Fig. 8) and using IRMS (Fig. 9). Isotopic and microscopical data (Figs 8-9) are from the same area (including the porous crack). According to the reviewers suggestion, we added additional supplementary data showing i) another δ13C and δ18O SIMS profile including a porous crack from another part of the sample (supp Fig. 1); and ii) IRMS data from different parts of the sample (supp Fig. 2). All these new data confirm that the 13C depletion is typically associated with the porous cracks, which are characterized by small Low-Mg calcite rhombs and locally microbial filaments.

Comment: As it stands, the rationale for the microbial experiments lack completeness. Like the other reviewers, it seemed initially surprising that bright incubation conditions were used to mimic microbial textures found in aphotic caves, and I was likewise unsure about the origin of the biofilm and why that one was selected for this study. Future readers of this paper would benefit from additional descriptive context when introducing the microbial experiments. For example, a few sentences describing the biofilm origin (as in C1926-27), and your rationale for using the existing EPS mat (active or dead?) from Brazil for comparison to a Siberian stalagmite would be most helpful. Furthermore, the link between this experiment and the passive mineralization hypothesis could be more clearly articulated: is the Brazilian biofilm dead? If not, why are you so certain that the structure is facilitating mineralization, rather than live microbial mediation?

Authors response:

According to the reviewers comment, we add a description of the biofilm origin in the revised manuscript (see below). Because we found EPS closely associated with Fe-oxide rosettes, we selected a sample containing large amounts of EPS without a significant

influence of microbial metabolism. Previous TEM examination of this biofilm showed a dominance of EPS over bacteria and the latter in a dying state, thus confirming that this biofilm was suitable for our laboratory experiment.

Biofilm origin: Biofilms were cultured from water samples collected in the cave. The biofilms have been produced from a microbial mat from Lagoa Vermelha, Brazil (Vasconcelos et al., 1995) under stress-controlled conditions, i.e., hypersalinity in order to produce a significant amount of EPS. Prior to the Fe-experiments, the biofilm was analyzed using SEM, TEM (embedding in Epoxy and cut in ultrathin sections), and XRD in order to validate the absence of any mineral phase (carbonates, Fe-oxides, amorphous Mg-Si phases, etc.) and the abundance of microbes. TEM data indicate a complete absence of permineralization within EPS and very few bacteria. The abundance of EPS over isolated microorganisms is supported by DAPI-staining (very few fluorescent bacteria) and Gram coloration, suggesting that photosynthetic organisms were unlikely to have played a role in mineral formation. Moreover, TEM examinations of bacteria show cell disruptions with loss of intracellular materials (suppl. Fig. 3) that would suggest that they are dying (e.g., Diaz-Visurraga et al., 2010). Based on the TEM and DAPI-staining results we exclude light-favoured metabolically driven Fe-oxide formation.

Comment: In the authors response to S. Verheyden (p. C1932), they mention the dearth of global data regarding inorganic vs. biomediation of carbonate speleothem mineralization. Although the authors hint at a framework for understanding the circumstances in which "organic support can be the driving factor", nothing is mentioned in the conclusions of article regarding how this study fits in with an emerging larger picture of biotic vs. abiotic speleothem growth. While I agree with the other reviewers that it is important to interpret results cautiously, in my opinion this conservatism must be balanced when appropriate with the audacity to envision the broad implications of new directions and discoveries in research. Science proceeds not only by sharing observations and the results of experimental tests, but also by suggesting new ideas for further testing. In this paper, the latter could be strengthened. Such a statement about

the scope of biomediation's importance would of course be somewhat speculative; yet, I would like to see a mention in the conclusions about how this study fits in with this larger picture.

Authors response: We thank the reviewer for this remark. According to the existing literature, it seems that microbes are encountered in a wide variety of caves from cold (this study) to temperate and tropical conditions (e.g., Frisia et al., 2012; Jones et al., 2011). However, to our knowledge this is the first study that constrains $\delta13C_{carb}$ depletion along with detailed microscopical investigations, therefore not permitting to discuss broader (geographically) implications. The discussion of broad-view implications would possibly be suggestive in the sense that such records might occur in other climatic settings. Given the lack of samples we would rather be cautious with extrapolating our results. Similar investigations by combining microscopy and isotope geochemistry (SIMS) are definitely required in caves from different climatic zones in order to constrain the role of microbes in speleothem formation related to hiatuses and/or carbonate disruptions. We modified the conclusion accordingly.

Comment: For example, I wonder whether microbial or biofilm calcite deposition mediation might be an important factor in calcite initiation for cave systems near the extremes of speleothem deposition, such as near the permafrost line as in this cave site? If so, such information would be essential to any future high latitude/high altitude speleothem studies of glacial-interglacial cycles tied to dating the timing of periods of calcite growth initiation and cessation. For example: could the frequency of colonization of speleothem surfaces be an essential control on how quickly calcite deposition resumes following a climatic return to moist, speleothem-friendly conditions inside the cave? Could radiometric dates of the timing of such calcite growth periods have different sensitivity for abiotic vs. microbially-mediated speleothems? Would EPS structures induce calcite deposition more quickly or more slowly? Obvious, most of these questions are beyond the scope of this study, but this article could do with a few more lines pointing the way forward. Again, although any such broad interpretation or hypothesis

would be speculative, I would encourage the authors to include a brief discussion about the potential meaning of this line of research, which in my opinion would make this a more influential paper.

Authors response: Microbial carbonate formation can be mediated under different climatic conditions including cold and dry climate especially related to permafrost occurrence, e.g., Pellerin et al. (2009). However, the timing of periods of calcite growth initiation and cessation cannot be fixed with certainty. For example, carbonate lamination in stromatolites is interpreted to record the periodic response of a microbial community to daily, seasonal, or yearly environmental forcing and also of a regional climate forcing (Petryshin et al., 2012), but the timing of carbonate lamination formation can vary considerably depending on geological settings (e.g., Chivas et al., 1990; Paull et al., 1992; Font et al., 2010; Petryshin et al., 2012).

Comment: The inference of a former perched peat bog above the cave site from the observed intervals of Mg and Fe oxide deposition on this stalagmite seems plausible, particularly given the lack of recent instances in the cave which could point to a bedrock source. However, one can imagine random pockets of sulfide-rich material in the bedrock which would have altered the stalactite geochemistry only during the period in which it was weathering. Is there a reason why the peat bog model is less speculative than this sort of bedrock heterogeneity? Could the peat bog hypothesis be tested in future studies using biomarkers or fluorescence characteristics (if present in such ancient material)?

Authors response: According to Kadlec et al. (2008) and Fillipov (2000) no Fe-bearing minerals are present in the host rock above the cave. However, it seems that early geological studies in the vicinity of the cave found sparse sulfide-rich deposits on the top of the cave (Odintsev et al. 1946, 1947; Egorov 2011), but the reason why no Fe-oxides were recorded since then is still unknown. If Fe came from the oxidation of pyrite, this could have been achieved by reaction with either oxygen or ferric iron. The overall reactions are (1) $FeS_2 + 15/4 O_2 + 1/2 H_2O \rightarrow Fe^{3+} + 2SO_4^{2-} + H^+$ (2) FeS_2

$+ 14 Fe^{3+} + 8H_2O \rightarrow 15Fe^{2+} + 2SO_4^{2-} + 16H^+$ Equation (1) usually occurs in acidic environments, where acidophilic microbes such as Thiobacillus ferrooxidans catalyze the oxidation process with oxygen. Under anoxic condition, equation (2) may be more predominant. Intermediate sulfur compounds are expected to occur during pyrite oxidation, a process called the "thiosulfate pathway" (Luther and George, 1987; Moses et al., 1987; Schippers and Jorgensen, 2001). The intermediate sulfur compounds in this process of pyrite oxidation are then either oxidized chemically by ferric iron or biologically by sulfur oxidizing microorganisms to sulfate (McGuire et al., 2001; Schippers and Sand, 1999). As a major ion in most groundwater systems, sulfate can be converted to sulfide by bacterial sulfate reduction when coupled with organic matter under anoxic conditions at a circumneutral pH. Ferric ion produced by the oxidation of solid or aqueous phase Fe (II) with oxygen can be precipitated and thereby immobilized as hydroxide, oxide, phosphate or sulfate, or, if bound to soluble organic ligands, will be converted to soluble complexes and dispersed from its source. However, no acidic conditions (leading to carbonate dissolution) or anoxic deposits (leading to OM accumulation) in the top of the cave have been found, which could support this interpretation. Indeed, Botovskaya cave was in the vadose (unsaturated) zone during stalactite deposition (stalactite formation can only happen in air-filled voids). Therefore it is highly improbable to create anoxic or acidic conditions in carbonate rock above the cave. Therefore, the most likely hypothesis to create such conditions remains the presence of a local peat bog at the time of deposition above the cave and seeping of acidic/anoxic water from there into the rocks fissures above the cave. âĂİWe cannot strictly rule out the sulfide-rich deposits hypothesis at this time, based on our data. Further studies are required to characterize any Fe-rich minerals in the host rock. Biomarkers could reveal (i) the presence of branched glycerol dibiphytanyl glycerol tetraethers (bDGTs) that are widespread in soils and peat bogs (e.g., Liu et al., 2010; Peterse et al., 2010; Weijers et al., 2011) or (ii) the n-alkane distribution that can be typical of Sphagnum species (Bingham et al., 2011), if there is enough amount of OM.

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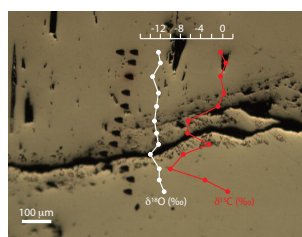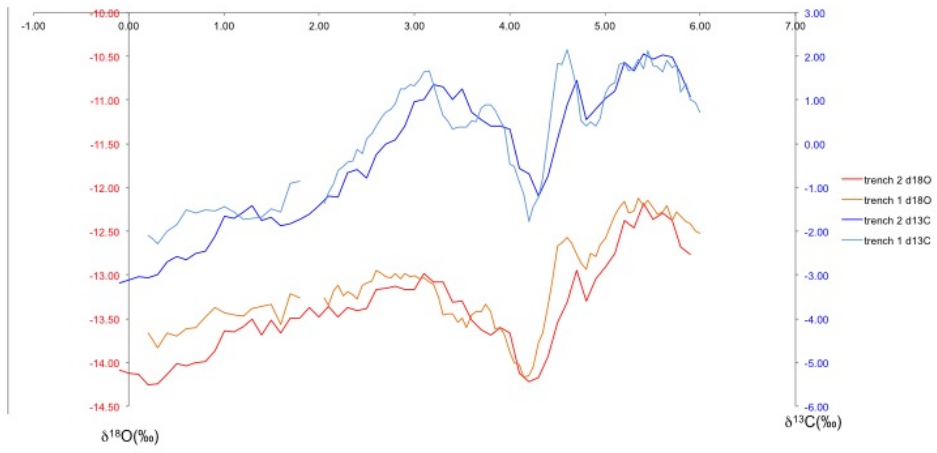
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Fig. 1. supplementary Figure 1

Fig. 2. supplementary Figure 2

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