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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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Comment: "This paper deals with the still open question of potential active participation of bacteria in the deposition of speleothems. New techniques bring indeed some new and more precise data. Microbial studies in caves may open a different, until now less studied environment, therefore interesting. The fact that the paper combines different methodologies to investigate the minerals as well as the organic material and the combination of observations and experimental work is a rather robust methodological approach and certainly a step further than several former studies done in the cave environment. As previous comment, posted by Dr Bindschedler, my opinion is that the paper can be much ameliorated by taking the results with some more caution. Especially following points: Lines 15 to 20. The studied sample is an asymmetric stalactite and authors assume that it is 'likely the result of preferential water flow down one side.' However, the asymmetric forms in stalactites may also be due to a higher CO2 degassing rate at that side (if towards the upstream side of cave airflow) and, in an extreme case, resulting to anemolites (a helictite in which the eccentricity is ascribed to the action of air currents). This has implications on the statement at p. 6579-line1-10 that the Mn-Fe mineralization precipitated where the water flow was minimal, since the water flow would be MAXimal, or at least the water film on the stalactite would be the thickest downstream the airflow. . .. The given reference of Lau and Liu concerns biofilm deposition in open channels, a very different environment and flow velocities than here on the stalactite."

Authors response: We agree with Dr. Verheyden that airflow can lead to asymmetric growth, in fact this is seen in many caves and explained as summarized by the reviewer. However, multiple expeditions at different seasons (normally winter, but also summer) never observed any cave wind/air current in this section (as well as many other sections) of Botovskaya cave. The reason is most likely the complex labyrinthic network structure with very narrow passages, which prohibits the development of air currents forced by barometric pressure gradients (between cave and surface). The shallow passages also prohibit the development of vertical airflow, as passages have most often dimensions of 30-100cm width and 0.4-2 m height.

Comment: "Page 6571, lines 4-6: "The hiatus between layers E and D is the last speleothem surface on which a microbial community was present. Two calcite layers and two hiatuses separate layer B and last period of the microbial activity.' I fully agree with previous comment by Dr Bindschedler that the authors cannot state that microbial activity was not present in the other layers. So I suggest that the authors change it to 'microbial community was observed' and 'last period where microbial occurrence was observed'. I would avoid 'activity' since it supposes activity in the speleothem deposition which is not proven here."

Authors response: We agree with the reviewer and we changed the sentence accordingly to “The hiatus between layers E and D is the last speleothem surface on which a microbial community was observed. Two calcite layers and two hiatuses separate layer B and the last period where microbial occurrence was observed (Fig. 2), suggesting that it occurred at least two glacial-interglacial cycles before MIS-13.”

"Page6574, lines 22-25. The authors state that ‘biofilms seem to help initiate layer formation on the stalactite via organo-mineralization processes characterized by small minerals.’. However, speleothem layers initiate through small crystals towards palisade crystals by competition, as well in a totally inorganic precipitation and do not need ‘organo-mineralization processes’; (Kendall and Broughton, 1978)."

Authors response: The reviewer is correct in that inorganic precipitation leads to speleothem deposition. We do not argue that organo-mineralization processes are crucial for speleothem growth initialization, but merely point to the fact that organo-mineralization can support and/or initiate growth. Similar small Mg-calcite crystals have been recently observed associated with microbial activity (Frisia et al., 2012). These authors also argue that biomediation can help in the formation of carbonates. It is likely of course that inorganic precipitation leads to the majority of speleothem growth. In some cases – and there is too little information on a global scale – organic support can be the driving factor, which might be of importance for the search for reasons of isotope equilibrium/disequilibrium growth conditions, thus meriting further investigation.

Comment: "Chapter 5.3. d18O and d13C : If I understood well, the conclusion linking depleted d13C values and porous layers (putative biogenic) is based on a single stable isotopic profile where the d13C is depleted at the proximity of a crack. Replicate profiles should be done to confirm this. Laser-ablation isotopic measurements linking depleted d18O with the 8.2ka cold event was published and the depletion turned out to be due to the crack (See McDermott et al., 2001). Even if not the same methodology, porosity and cracks can probably have some influence on the measurement. I agree with the authors that if the results are confirmed this is an argument for microbial calcite deposition activity."

Authors response: We agree that repeated (and if possible independent) profile analyses are more robust then a single profile. Therefore, we analyzed several δ13C profiles using SIMS, and subsequently analyzed them using SEM. Additionally, we analyzed the section using the incremental milling of powder for IRMS (the common method for stalagmite sampling for climate time series). Depleted SIMS δ13C values (from -10 to -15‰ are always associated with low-Mg calcite and microbial-like structures (see images in Fig. 1 below). In the presented case, the abiotic precipitation does not explain these depleted δ13C values. The correlation between low-Mg calcite crystals and associated EPS-like morphologies, and depleted δ13C values has been observed at different locations in the stalactite. The extent of all these features is about 200 μm, whereas the cracks are "only" 10 to 50 μm-wide. Therefore, we argue that porosity and cracks are unlikely to have created the observed depleted δ13C values in the studied sample.

Comment: "p. 6575 line 9-12. Concerning the fact that the rosette-like arrangement of the oxides in the stalactite is unexpected, Cañaveras et al.1999 and Cuezva et al., 2009 related rosette- or nest-like aggregates in Altamira cave to bacterial CaCO Âz ÌĞ deposition. They also show the interest of tracking Vaterite in the samples because bacterial activity seems to be necessary to form vaterite spheroid elements, similar to and a possible precursor of the observed CaCO3 spheroid elements (see also; Sanchez-Moral et al., 2003; Rodriguez-Navarro et al., 2007). So, I wonder if the rosette-like structures could be carbonate deposited simultaneously or in alternation with the oxide in the stalactite presented in this paper."

Authors response: When looking at high-magnification at the rosette structures (see figure 2 below), they appear to be composed of nano-fibers and no Ca was detected during elemental analysis. This is not the case of those reported by Canaveras et al.

(1999) and Cuezva et al. (2009), both showing platelets arranged in rosette structures. It seems clear that in our case, rosettes are formed through microbial activity related to Fe supply. It would be difficult to explain the absence of these features in the younger carbonate layers if the rosettes were assumed to be of inorganic carbonate origin (why would they then not be found elsewhere?). Therefore we argue that the hypothesis that rosette formation is closely related to oxide precipitation through microbial activity is the most realistic explanation.

Comment: "p. 6578, line15 to 18. The authors suppose that the source for the iron and the Manganese in the oxides is a now disappeared peat bog. However, manganese coatings are often encountered on pebbles in cave streams and iron can be present in the form of sulfides in the above lying limestone (see also Peck, 1986). The peat bog is therefore certainly not the only possible source. A more detailed (geochemical) description of the host rock can bring interesting elements to better understand possible sources."

Authors response: According to Kadlec et al. (2008), no Fe-bearing minerals are present in the host rock above the cave, but we agree that a detailed analysis of these would advantageous to ascertain the Fe origin. We proposed a former peat deposit as a possible hypothesis because no Fe-deposits/coatings are observed in the youngest part of the stalactite, or in other young (i.e. Holocene) speleothems from this cave. Therefore, if the host rock would be the source for iron, we would expect transport of iron into the cave during all speleothem growth periods (warm interglacials without continuous permafrost). As this is not the case the source must have disappeared eventually.

References cited in our response: Frisia, S., Borsato, A., Drysdale, R. N., Paul, B., Greig, A., Cotte, M.: A re-evaluation of the palaeoclimatic significance of phosphorus variability in speleothems revealed by high-resolution synchrotronmicro XRF mapping. Climate of the Past, 8, 2039-2051, 2012. Kadlec, J., Chadima, M., Lisa, L., Hercman, H., Osintsev, A., and OberhÂÍ ansli, H.: Clastic cave deposits in Botovskaya Cave

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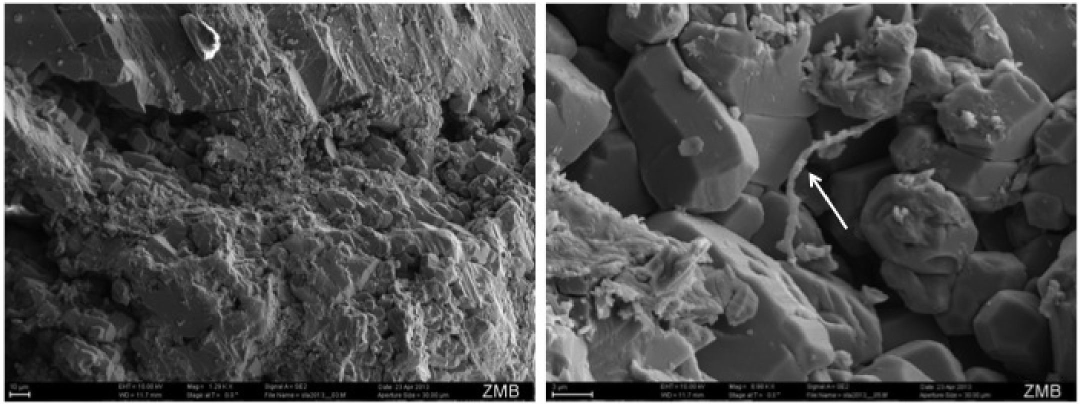

Fig. 1. Secondary electron images of the calcite rhombs in the cavities. Arrow indicates a microbial filament, which is associated with these rhombs.

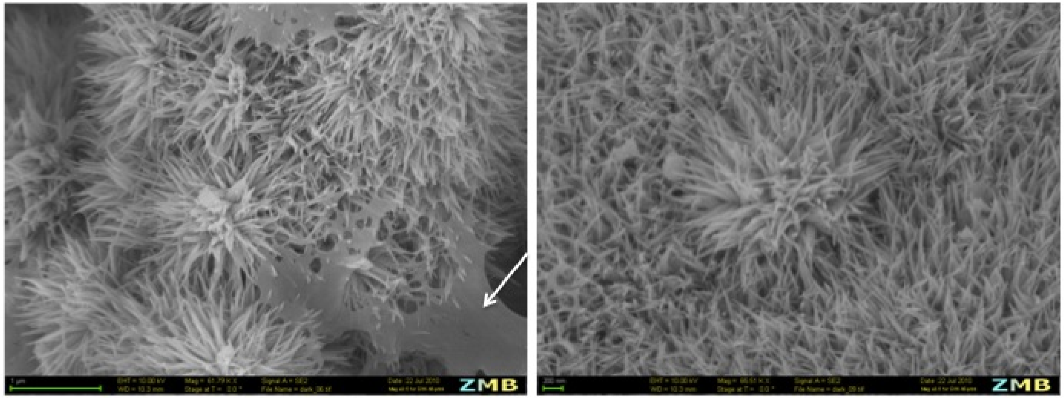

Fig. 2. High-magnification secondary electron images of the rosette structures, which are composed of acicular nano-fibers. The image on the left also shows EPS (see arrow).

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