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Interactive comment on "Unravelling the enigmatic origin of calcitic nanofibres in soils and caves: purely physicochemical or biogenic processes?" *by* S. Bindschedler et al.

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Thank you for the comments regarding the methodologies used. The images appearing in this manuscript are only a few chosen from about six thousand images. During this research, different imaging (High-vacuum-, Low-temperature (LT)-, and Environmental (Wet mode)-SEM) and dehydration (ethanol series, critical point and freeze-drying) techniques have been compared. 1) Regarding imaging of natural samples, wet-mode SEM has not been used because the imaging resolution is far too low regarding the sizes of nanofibres and NFC. Then, the observations of similar natural samples using both high-vacuum and low-temperature SEM did not show any significant differences that could alter the conclusions drawn from the images. Therefore, we have used both methods. 2) Regarding dehydration techniques, we have chosen the ethanol method for organic samples because it led to a better sample preservation than with critical-point drying. For natural samples (which are a mixture or nanofibres, NFC and various organic features and therefore, with a larger proportion of mineral matter), we have chosen freeze-drying as this was the only way to dehydrate samples without dissolving CaCO3 phases and disturbing the 3D arrangement of the sample.

Remark 1: We would like to briefly explain why we have used two different methods for preparing laboratory and natural samples. Laboratory samples were first fixed in glutaraldehyde and Osmium tetroxide, and further dehydrated in ethanol series with a final step in TMS. We have chosen this method mostly because this is used as a standard method for organic samples, and it provided good images for samples containing almost only organic matter at this resolution (under 1μm). However, it was not possible to use the same methodology for natural samples, because of CaCO3 dissolution and samples dispersion in the liquid phase. Our choice was therefore matrix-dependant. We are aware that this difference in treatment between both types of samples can lead to artefacts. However, technically and logistically, it was the only way to cope with the nature of our samples.

Remark 2: There were two reasons for not using LT-SEM on laboratory samples. First, there was a logistics problem. At our institute, the LT mode is available only once a couple of weeks per year, and needs to be booked in advance. Unfortunately, this was not compatible with the schedule of our experiments, which needed some time to be set up. Second, LT-SEM with highly hydrated samples gives better results on cut sections. The network of organic nanofibres obtained after enzymatic dissection are located on the sample surface.

To conclude on methodology aspects, we are totally aware that using two different preparation techniques is not ideal when one wants to compare images. However, after comparing different preparation methods, it appears to be the only way to achieve

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useful and informative imaging of our samples with their different characteristics (i.e. organic versus mineral). Moreover, after having observed numerous samples with different techniques, we are confident that even though artefacts may exist, they are less significant than the gain in resolution resulting from optimized preparation techniques for each type of sample.

L66: The organic signature is based on results published in Bindschedler et al. (2010), using an Osmium labelling method. This has been clarified in the manuscript (P3, L69).

L88: We totally agree with this note. First, depending on the definition, there are many different levels of influence of biotic processes on mineralization. Furthermore, these processes are sometimes difficult to disentangle from other physicochemical parameters. In this paragraph (L87-105), we did not discuss this point in detail in order to keep the introduction as simple as possible. However, considering that, for the origin of nanofibres, we are likely dealing with a passive biological influence, introducing the term organonimeral might have been more appropriate. The term organomineralization is actually used in L272-276 when discussing the processes of organic nanofibre mineralization.

L396: “iii)” changed to “ii)” in L405.

L398: See answer in the methodology remarks.

L421: The use of Osmium to label organic matter is originally adapted after the publication of Pearson et al. (2004). We have modified their protocol for the purpose of our samples (see Bindschedler et al. 2010).

Fig 1D : We are not sure if understood the comment right, as the scale is more or less the same. Possibly, there was confusion between NFC and nanofibres, so we added an arrow on the image 1D to help identify both features: Figure 1D (and captions; P33, L915-916) is modified with arrows pointing at nanofibres (white arrow) and needle fibre calcite (NFC; black arrow).

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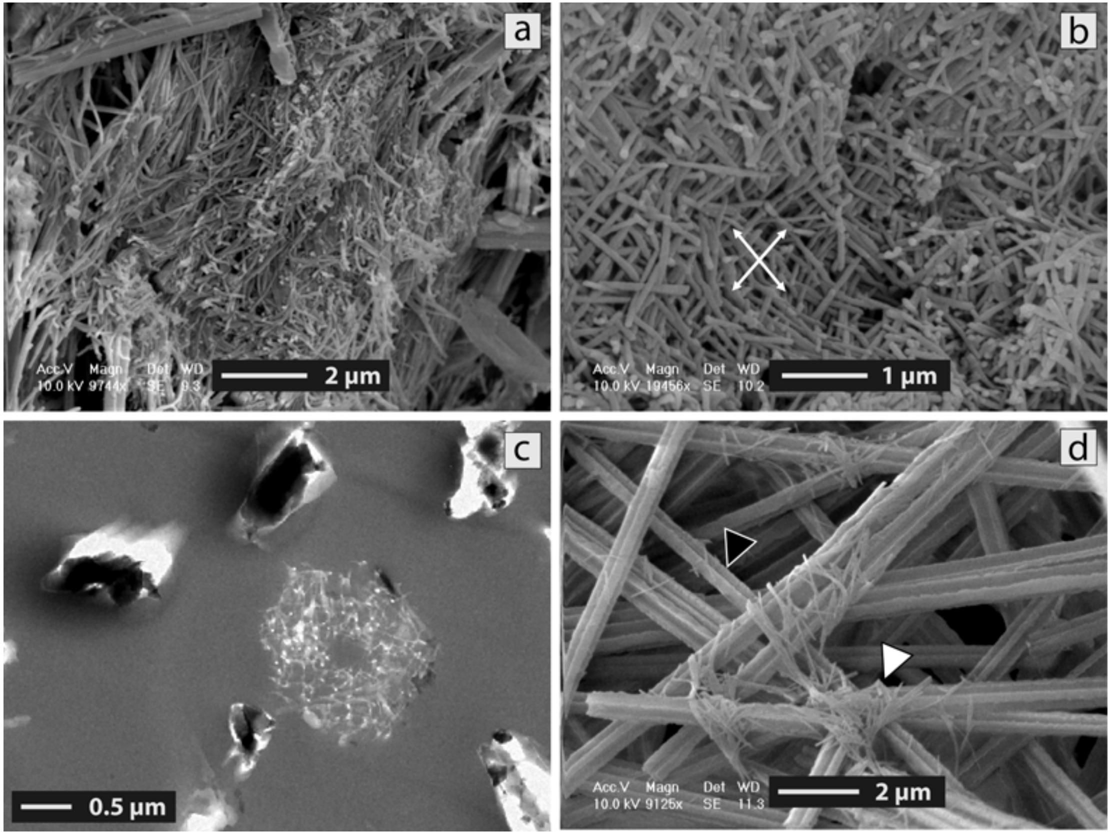

Fig. 1.

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