SOIL Discuss., author comment AC2
https://doi.org/10.5194/soil-2021-86-AC2, 2021

Reply on RC2

Hassan Al Majou et al.

Author comment on "Effect of freezing on the microstructure of a highly decomposed peat material close to water saturation when used prior to X-ray micro computed tomography" by Hassan Al Majou et al., SOIL Discuss., https://doi.org/10.5194/soil-2021-86-AC2, 2021

Reply to Reviewer #2

We thank the reviewer #2 for his comments which were very helpful in improving the manuscript submitted for publication.

The work presented by the authors is very important. It states the effect that freezing has on the structure of the material, and shows how such technique is not suitable for the circumstances in which the microstructure needs to be investigated. Therefore, some statistical analysis on at least three subvolumes needs to be presented on subvolumes having the same size and same initial properties (e.g., density, depth of extraction, water content). Additionally, The authors should provide some perspectives on how the presented results can be confirmed, and above all, how the other scientist could avoid freezing the subsamples. The methodology presented lack in many details that the authors need to provide before accepting this paper for publication.

Major comments:

- Lines 25-27: I am not sure that the pores detected post-freezing were not detected pre-freezing. Perhaps, they have been created in the freezing process.

The text is probably not clear enough. Yes, they may or may not have been created during the freezing process. If they were already present before freezing, they were not air-filled as they state after freezing or they were already both present and air-filled but small enough to be not detected by using X-ray CT. Because of their 3D distribution as shown by X-ray CT 3D images which reveal that many of these very small pores are distributed consistently with short sections of very fine tubular pores, we think that they were present before freezing because freezing cannot the short sections of tubular pores observed in the 3D images. They would be present, either already air-filled before freezing but too small to be detected or large enough to be detected but water-filled and so not distinguishable from the water saturated organic porous matrix.

Consequently, the last paragraph of the Discussion section and the Conclusion were modified, as well as the Abstract to take into account the comment of reviewer #2.

- Subsection 2.1: The readers would be interested in more details about the sampling. For instance, why these types of peats, why extracted in 25-40 cm depth, and finally, why stored at 3-4 degrees? Please spend few words on arguing more the field sampling methodology.

We selected a highly decomposed peat material close to water saturation because it was potentially highly sensitive to the creation of structure artefact during freezing as revealed for highly hydrated clayey materials when studied using freeze-drying process in scanning electron microscopy during the 90s.

We avoid the first top 25 cm horizon of the soil because of its high heterogeneity due to the heterogeneous distribution of the Molinia roots.

The samples was stored at 3 – 4 degrees to limit biological activity.

The text was modified as it follows:

".. Leroy et al. (2018, 2019a and 2019b). A highly decomposed peat was selected for study because it is potentially highly sensitive to the creation of structure artifacts during freezing due to its high water content and low fiber content. Large undisturbed samples (15´15´25 cm^3) were collected between 25 and 40 cm depth to avoid the heterogeneity of the top 20 cm due to Molinia roots. They were collected when the groundwater table level was close to the soil surface. The samples were stored at 3–4 °C to limit biological activity and in sealed plastic bags to avoid water loss."

- Subsection 2.2 - Line 111: can you please specify the correct size of the undisturbed volume? A few centimeters can be a couple or a dozen.

Yes, the text was not specific enough: this a dozen.

Text modified as it follows:

"Bulk density and particle density were determined by using undisturbed peat samples a dozen cubic centimeters in volume and the kerozene method developed by Monnier et al. (1973)."

- Why is the physico(physical)-chemical analysis relevant?

They are relevant to make easier use of our results for other peat materials according to the similarities or differences with peat material that we studied.

- Line 122: how is the sample hermetically sealed?

It was hermetically sealed with a screw cap.

Text was modified as it follows:

"… which was then hermetically sealed with a screw cap to avoid water loss."

- Line 127: In the authors' opinion, this water loss of 0.1g is relevant or can be considered as negligible? And why?

The water loss was indicated as being smaller than 0.1 g in the text (Line 127). It was actually less than 0.03 g which corresponds approximately to less than 0.03 % of the water amount in the sample 112 cm^3 in volume studied. Such a water loss was considered as being negligible.

Text was modified as it follows:

"… was weighed at the different steps of the process, i.e. prior and after each X-Ray m-CT imaging, to check the absence of water loss during the acquisition of the projected 2D images. Measurements showed that the weight variation between two successive steps and between the first and last step was <0.03 g for the two samples studied. This amount loss was considered negligible."

- Line 122 and 124: I believe that 'submitted' is not the correct verb to use. I suggest finding a more appropriate term. Suggestion: "The sample was first imaged/observed/analysed through x-ray and then frozen.."

The text was modified using "They were first imaged to X-ray µ-CT …" as suggested by the reviewer #2.

- Line 136: why the authors decided to convert the 16 bit image in 8bit? This choice implied a huge loss of data passing from a greyscale range of 65536 values to only 256. Such reduction could have definitely improved the measurements presented in the following sections.

The tomograph detector records images in 16 bit, i.e. divided into 65536 grey levels. It should be noted that the acquisition parameters (voltage, current, acquisition time, etc.) are always optimized to obtain a signal (projections) over the widest possible range of grey levels. Despite this, the "interesting" distribution of light energy is in a range narrower than 65536 grey levels. Indeed, for the samples studied in this paper, the "useful" signal representative of the transmission (and thus of the absorption) by the sample is distributed on approximately 2000 grey levels. The other grey levels correspond to noise, the sample holder or the direct signal of the X ray source. Thus, we cannot say that the original signal being on 65536 grey levels and that there would be a strong loss of information by "reducing" to 256 grey levels.

On the other hand, the tomographic reconstruction is done in 16 bit because all the other acquisition data (offset and gain corrections for example) are also in 16 bit. After reconstruction, we finish by recording the images in 8 bit (256 levels of grey) by always checking that (i) the histograms of the 16-bit and 8-bit images are identical and (ii) that the images are visually identical (to the eye of course). During this process and the recording, we increase the dynamic range of the image by spreading the histogram on all the possible range between 0 and 255 levels of grey (cf figure 1). This is a classical operation in image analysis which makes the segmentation step easier.

To conclude, converting grayscale images form 16 down to 8 bit depth does not change the shape of their grey level distribution and consequently the threshold value on the histogram. Moreover, this process from 16 to 8 bit leads to a very strong reduction of the size of the images (in Go) and thus makes possible to perform the numerical calculations which ensue bearable, while having a loss of information which is finally negligible.

The text was modified as it follows:

"… Samples were placed in the chamber and rotated by 360 degrees during acquisition. The samples were centered and waxed on a sample holder (circular plate) whose axis of rotation was collinear to that of the tomograph chuck. An operating voltage of 120 kV and a filament current of 100 µA were applied. The distance between the X-ray source and the sample and between the X-ray source and the detector was 300 and 500 mm, respectively, giving a voxel size of 60 µm. The tomograph detector recorded 2D projections in 16 bit, i.e. divided into 65536 grey levels. The resulting projections were converted into a 3D image stack using a microcluster of four personal computers (PCs) with the Phoenix 3D reconstruction software. A filtered backprojection algorithm was used according to Feldkamp et al. (1984). The reconstruction software contained several

different modules for artifact reduction (beam hardening, ring artifacts) to optimize the results. After reconstruction, the images were recorded in 8 bit (256 levels of grey) by always checking that the histograms of the 16-bit and 8-bit images were similar and that the images visually indistinguishable. During this process, we increased the dynamic range of the image by spreading the histogram over the entire range between 0 and 255 levels of grey. This facilitated the subsequent segmentation step. The 2000 projection images (angular increment of 0.18°) were acquired during sample rotation (with an acquisition time of 4 hours) for every sample before freezing and post-defreezing. As the cone beam geometry created artifacts, the first and the last 76 cross-sectional images were removed (Le Trong et al., 2008; Rozenbaum and Rolland du Roscoat, 2014). …'

- Line 140: How the authors preserved the freezing status of the samples during the 4 hours scanning time? What were the scanning conditions (e.g., temperature and humidity)?

The information is given Line 124: ".. they were frozen at -10°C for 48 h, defrosted for 48 h at 20°C and imaged again by X-ray m-CT."

In order to avoid confusion as mentioned by the reviewer #2, the text was modified as it follows:

"… were acquired during sample rotation (with an acquisition time of 4 hours) for every sample before freezing and post-defreezing."

- Line 141: Until this point, I thought that the sample was scanned before the freezing status and after freezing, ergo, frozen. But as I understand in this section the sample was scanned the second time after being defrosted, am I right? If this is the case, I strongly suggest the authors to rephrase the previous sections and clearly express that the 'after freezing' correspond to a status post-unfreezing.

Yes, as indicated Line 124, the sample was scanned the second time after defrosting. This is indicated now again by adding information in the text (see response to comment #9).

- Lines 143-146: For the consistency of the measurements and the comparison of the two samples, the authors should have extracted the same volume for both. Why your two samples have two different volume size?

The sub samples were extracted to compare qualitatively the 3D distribution of the small air-filled ovoid pores and not for the purpose to quantify them and to compare their distribution before and after freezing. This was done indeed for qualitative comparison only with the aim of having less pores in the 3D images thus making easier that comparison. However, even if their size was different between the two samples studied, they were of the same size for each sample before and after freezing.

Now, concerning the quantitative comparison of the samples before and after freezing, the whole 3D images were used and results showed a dramatic difference of the pore volume distribution before and after freezing (Figures 5 and 6).

Figures 7 and 8 were improved as done for Figures 3 and 4. The volumes were indeed aligned and a scale was added to facilitate the use of the qualitative information contained in these images.

As a consequence, the text corresponding to the last paragraph of section 2.2 was modified.

- Line 150: What does 'well inside the sample' mean? Please indicate the exact distance from the edges and the reason behind this choice.

The expression "well inside the sample" was indeed not appropriate. Our 3D images correspond to the largest rectangular parallelepiped volume included in the cylindrical sample studied. So, such 3D rectangular parallelepiped images were much easier to analyze than 3D cylindrical images. The text was simplified and clarified as it follows:

"A region of interest that excluded the irregular sample boundaries and outside region was defined for every sample by identifying the largest rectangular parallelepiped image in the cylindrical sample studied. …"

- Line 155: How was measured the signal to noise ratio?

The signal-to-noise ratio is defined as the the ratio of the average of the gray levels of the image to their standard deviation according to Avcibas et al. (2002) (I. Avcibas, B. Sankur, K. Sayood, Statistical evaluation of image quality measures, 2002, J. Electronic Imaging, DOI:10.1117/1.1455011)

The text was modified as it follows:

"… to [12.5-15.0]. The moving average filter replaced each voxel of the original image by the average grey value of its neighbors over a window centered on it as this is considered the optimal method to remove random noise on grey-level images (Smith, 1997). The signal-to-noise ratio was computed as being the ratio of the average of the grey levels of the image to their standard deviation according to Avcibas et al. (2002).

- Moving average filter: how is this filter defined? How does it work? Why was this specific filter chosen, respect to other ones (e.g. anisotropic or bilateral filters?). In figure 1, you could add the limits of the threshold used for the segmentation. Such info would help the reader following the procedure.

The moving average filter replaces each voxel of the original image by the average gray value of its neighbors over a window centered on it. It is recognized as optimal to remove random noise on gray-level images (Steven W. Smith. 1997. The scientist and engineer's guide to digital signal processing. California Technical Publishing, USA).

The bilateral and anisotropic filters, apart from the fact that these are complex methods, are too elaborate and would not bring any significant benefit for grayscale images and pertain to the domain of photography and 3D rendered imagery. One pertinent alternative are morphological filters (J. Serra, Alternating sequential filters, Image analysis and mathematical morphology, 1988, Academic Press New York). They were tested but provided no significant improvements.

The text was modified as it follows:

"… to [12.5-15.0]. The moving average filter replaced each voxel of the original image by the average gray value of its neighbors over a window centered on it. It was indeed recognized as optimal to remove random noise on gray-level images (Smith, 1997). The signal-to-noise ratio was computed as being the ratio of the average of the gray levels of the image to their standard deviation according to Avcibas et al. (2002).

- Lines 158-159: Here I am having some trouble understanding. The 3D volumes of the sample should have been normalized, in order to have greyscale values coinciding for each phase within the scanned object, and to have an automatic threshold choice. A threshold is defined as a range. Is the range going from 0 to this value or from this value to 256, or else? This section is very important for all of your results are based on this technique. Please implement the information and clarify all the choices.

The objective was to separate (i) the air-filled pores and (ii) both the water saturated organic matrix and associated water saturated pores in every 3D image knowing that, between two successive 3D scans for a given sample (several days between the scan before freezing and that after freezing for the defrosted sample), the beam intensity can vary even if it is highly stable over the 4 hours of acquisition time. The objective being to determine the best threshold value of grey level between the air-filled pores and the water saturated organic matrix and associated water saturated pores in every 3D image, the threshold value was somewhat different between the 3D images recorded but its determination was optimized to obtain the best separation between the two phases whatever the difference of grey level distribution between the 3D images.

Furthermore, normalization would require to identify the phases before hand (to have their gray level match between samples), which is precisely the objective of the segmentation procedure. This is somewhere a chicken and egg problem.

Finally, the identification of the threshold is automatic. It is defined as the lowest single value of grey level between the two peaks, which can readily be (and in fact, is) determined automatically.

The text was modified as it follows:

"… and 68, respectively (Fig. 1b). The voxels with a grey level smaller than the threshold value were considered as being pore voxels while those with a grey level higher than or equal to the threshold value were considered as matrix voxels. This simple procedure has no adjustable parameter and therefore introduces no bias when comparing the images. Line 161: How are the pores identified? By 'scanning the image' is not an appropriate terminology. Is the identification done manually? Or otherwise?

"In each binary image, each pore (i.e. group of contiguous foreground voxels surrounded by matrix voxels) was identified by a voxel-by-voxel scanning of the image, and its volume (in terms of number of voxels) recorded by the following algorithmic procedure:

- *Consider each voxel during a raster scan of the image. Let v be the current voxel;*
- *If v is a matrix voxel, or has been marked as belonging to an already identified pore, proceed to the next voxel;*
- *If v is a pore voxel belonging to a yet unidentified pore, starting from v, perform a geodesic reconstruction of the pore (Lantuéjoul and Beucher, 1981). During the reconstruction of the pore, mark all its voxels as belonging to an identified pore, and keep count of their number. Once the reconstruction is complete, the number of voxels yields the volume of the pore. Proceed to the next voxel in the raster scan."*

- Line 178-181: What does this conclusion imply?

The text was clarified.

- Line 202-203: I would define the difference in grey scale values as darker grey (pores are not black, otherwise they would correspond to a unique value) for the pores and lighter grey for the organic material.

Yes, this was not correct.

The text was modified as it follows:

"These pores are in very dark grey in Fig. 2. The lighter dark grey background

corresponds to the highly decomposed organic material and related micro-porosity which was filled by water."

- Line 204-207: Please define better in the Figure and in the text this differentiation. Such statement is confusing and not well described.

The text was modified as it follows:

"These pores are shown in very dark grey in Fig. 2. The lighter dark grey background corresponds to the highly decomposed organic material and related porosity which was filled by water. For each pair of 2D X-ray m-CT images, comparison showed the presence of (i) pores recognizable on the images before freezing which were still present post-defreezing but exhibiting a different morphology, (ii) pores recognizable on the images before freezing which were not present post-defreezing, (iii) and the presence of pores recognizable post-defreezing and which were not present before freezing (Fig. 2)."

- Line 220-225: As pointed out before, how is defined the quantification of the pores? This is very unclear.

See comment #16

- Line 237: Why have the authors chosen two different volume sizes for the two samples. How can you make a comparison between the two cases, if you are using a different volume size?

As indicated in response to "Comment #11", the subsamples were extracted to show the 3D distribution of the small air-filled pores ("ovoid pores" in the first version of the text) and not for the purpose to quantify them and to compare their distribution before and after freezing. However, even if their size was different between the two samples studied, they were of the same size for each sample before and after freezing.

- Line 239: air-filled ovoid pores, why did you use the term 'ovoid'?

Yes, the term 'ovoid' was not appropriate and somewhat confusing. It was removed from all the text.

- Line 265: How is this volume of water - equal to 8.7% - quantified? This seems to be an important step in your methodology and the description is missing.

Yes, this not clear enough in the text.

Between 20°C (specific volume of water: 1.0018 cm^3 g^{-1}) and -10°C (specific volume of water: 1.0891 cm^3 g^{-1}), the specific volume of water increases by 8.7%. This is clarified in the text and a reference is given (Harvey, Allan H., 2017. Properties of Ice and Supercooled Water. In Haynes, William M.; Lide, David R.; Bruno, Thomas J. (eds.). CRC Handbook of Chemistry and Physics (97th ed.). Boca Raton, FL: CRC Press.)

The text was modified as it follows:

"The increase in the specific volume of liquid water by 8.7% when it turns to solid from 20°C to –10°C (Harvey, 2017) increases the porosity of both the pores in the organic matrix and the tubular pores fillled with water before freezing to a porosity post-defreezing …"

- These results need to be implemented. I suggest the authors to run the same analysis on other sub-volumes and present a statistical comparison.

See comment #11.

- Line 290-293: unclear statement.

We tried to clarify the sentence as it follows:

"Finally, the increase in the specific volume of water because of freezing may also be responsible for the alteration of the already air-filled tubular pores >500 voxels before freezing as shown by the 3D binary images (Figs. 3 and 4) and the pore volume distribution (Figs. 5 and 6) because of deformations in the structure of the surrounding porous organic matrix during freezing.

- Eq7 and Eq 8: what does 1.087 represent exactly?

The value 1.087 is the coefficient by which the volume of water is increased when it turns from liquid (20°C) to solid (-10°C). As indicated for comment #23, between 20°C (1.001798228 cm^3 g^{-1}) and -10°C (1.089087345 cm^3 g^{-1}), the volume of water increases by 8.7 %.

The text was modified as it follows:

"… to the water-filled tubular pores post-defreezing, and 1.087 the coefficient by which the volume of water is increased when it turns from liquid (20°C) to solid (–10°C)."

- Line 302: As asked in previous comments, why the authors state that small pores were detected only after freezing, but perhaps these pores was produced by the freezing process. Why is this hypothesis not taken into account?

The question was quite relevant (see answer to comment #1, major comments).

- Line 307: pores calculated theoretically, how?

Yes, the term "theoretically" was indeed inappropriate.

The text was modified as it follows:

"… The volume of these pores newly occupied by air post-defreezing was measured using X-ray m-CT and their cumulated volume was found to be consistent with the one calculated by taking into account the thermal expansion of water from 20°C (liquid) to –10°C (ice)."

- Conclusions: The work presented by the authors is very important. It states the effect that freezing has on the structure of the material, and shows how such technique is not suitable for the circumstances in which the microstructure needs to be investigated. Therefore, some statistical analysis on at least three subvolumes needs to be presented on subvolumes having the same size and same initial properties (e.g., density, depth of extraction, water content). Additionally, The authors should provide some perspectives on how the presented results can be confirmed, and above all, how the other scientist could avoid freezing the subsamples.

We believe we understand the reviewer's concerns. However, the study of three sub-samples would not provide more information since there is more information in the entire samples. We could have done otherwise, for example, by multiplying studies of samples of much smaller size, but that is not what we have done. We have studied two relatively large samples and the recorded differences are so large that they do not allow any doubt as to the reality of the disturbances linked to freezing. On the other hand, as the reviewer *points out, it is important to specify that the peaty materials studied here were potentially very sensitive to the alteration of their structure, which our study confirmed. All peaty materials are undoubtedly not as sensitive to such alterations during freezing and this will need to be clarified during future studies.*

Minor comments:

- Figure 1: It would be easier for the readers to understand the difference in the grey value distribution if the before and after freezing curves are in the same plot, perhaps plotted with different color or line format.

Figure 1 was modified as suggested. The curves recorded before freezing andpost-defreezing are in the same plot with different colors.

The legend of Figure 1 is now:

Figure 1. Distribution of the grey level values in the 3D X-ray µ-CT images recorded before freezing (blue) and post-freezing (red) for sample A (a) and sample B (b). The grey level values corresponding to the threshold value between the air-filled pores and the water saturated porous organic matrix are also plotted.

- Figure 2: The arrows are not very clear. The authors should highlight in a different way the pores that appear before/after freezing. Maybe with a circle or shades of two different colors.

Yes, it was not clear enough. Numbers are now plotted with different colors. The legend of Figure 2 is now:

Figure 2. Pairs of 2D µ-CT images of samples A (a and b) and B (c and d) extracted from the 3D X-ray µ-CT images in grey levels showing air filled pores (very dark grey), the solid organic material with water filling the associated pores (dark grey) and particles of iron oxy-hydroxides (very light grey). The numbers identify pores which were present before freezing (a and c) and still present post-defreezing (b and d) but with a different shape or size (blue), present before freezing and not post-defreezing (red) and not present before freezing but present post-defreezing (yellow).

- Figure 3: The volumes are not aligned in the row direction. Scale is missing in the figure

Figure 3 was modified and the volumes are now aligned in the row direction. A scale was added.

- Figure 4: Same comment as Figure 3

As done for Figure 3, Figure 4 was modified and the volumes are now aligned in the row direction. A scale was added.

- Figure 7: The authors should modify this figure, showing the reader where this subvolume is located in the global volume. Why is this size of subvolume chosen?

Answer to these comments was already given above (see comment #11, major comments).

The subvolumes selected can be located easily by using the largest pores of the subsamples which are recognizable in the whole 3D images from which they were extracted.

The volumes of Figure 7 were aligned and a scale was added.

- Figure 8: Same comment as Figure 7

The volumes of Figure 8 were aligned and a scale was added.

- Table 2: How is this porosity measured in the images? Based on the segmented image? After reading the manuscript, this info is still unclear.

The total porosity was not measured on the 3D images but, as indicated in the 2.2 sub-section (Lines 113 to 114), by dividing the volume of water contained in a saturated sample by the known volume of the sample as described by Boetler (1976) and Nimmo (2013).

- Title: "Effect of freezing on the microstructure of a highly decomposed peat material close to water saturation when used prior to X-ray micro computed tomography". I am not sure about the second part of the title, 'when used prior to x-ray micro tomography'. I'd suggest the authors to clarify it.

The title is now:

"Effect of freezing prior to X-ray micro computed tomography on the microstructure of a highly decomposed peat material close to water saturation "

- Abstract: The specification of the tomography (lines 20-23) are irrelevant in the abstract.

This information was removed from the abstract as suggested by the reviewer #2.

- The Introduction can use some improvement in the English format.

Some improvement was done in the introduction (see text revised).

- Line 129: the authors already said that the sample depth corresponded to 30-37 cm in subsection 2.3, hence, no reason to repeat it here.

This was removed from the text as suggested by the reviewer #2.