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SOIL Discussions

Interactive comment on "Three dimensional soil organic matter distribution, accessibility and microbial respiration in macro-aggregates using osmium staining and synchrotron X-ray CT" *by* B. G Rawlins et al.

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Comment 1: *Abstract should focus on the key message in concise form. The last paragraph of the introduction section should be brief with clear objectives. Discussion section should include validity of the experimental approach, justification of results obtained (i.e. porosity, pore shape, SOM volume, accessibility and soil respiration).*

Response: We consider that the original version of the abstract does focus on the key message, and does so concisely with clear objectives. In order to be self-explanatory the abstract requires some context for the study and not just results. The discussion

section in our original version of the manuscript focussed on the wider implications of our findings, putting them in the context of other work and exploring options for further analyses of our data. We have included as part of our new discussion section consideration of how we might proceed to determine the location and quantities of finely disseminated organic matter sorbed onto mineral surfaces as suggested by reviewer 1 in other comments below.

Comment 2: *The authors followed largely the staining and scanning protocol published by Peth et al. (2014). The authors haven't provided any experimental data to demonstrate that Os was preferentially taken up by SOM only not adsorbed on mineral matrix of the soil. The authors used aggregates from a Clay soil for their experiment. Low diffusivity of clay soil could preclude the flow of Os vapour to SOM but increase the chance of adsorption of the vapour on clay surfaces. Moreover, Os can also react with clay-SOM complex not only the particulate SOM (POM) in the aggregates. From the Figure 3 it is not at all clear (resolution is too coarse) whether Os adsorbed on mineral matrix or SOM or POM. In my view, much better presentation could be a thresholded image slice showing pores and SOM alongside with greyscale scanned image of that slice. It will be nice to see if the authors could separate the 3D distribution of SOM adsorbed on clay surfaces and POM.*.

Response: Peth et al. (2014) demonstrated using the same methodology that Os was preferentially adsorbed to organic matter rather than clay minerals. We do not consider it necessary to repeat the same verification steps as Peth et al. (2014). In addition, as we freeze-dried our samples we consider there is more scope for the Os vapour to diffuse into finer pores than may have been the case with the Peth et al approach in which small quantities of moisture would have remained in the finest pores following their use of air-drying. The separation/identification of SOM adsorbed on clay surfaces is beyond the scope of this paper and we do not state it as one of our objectives. The osmium retention by clay was addressed in our original manuscript by basing the threshold for organic matter classification – using differences between Os absorption

above and below the adsorption edge – on the inferred volumetric organic matter content of the aggregate. This requires that SOM adsorbs Os more than does clay, not that there is no adsorption of Os by clay. See further response to this in comment 5 below. In the final (modified) version of the manuscript we present a thresholded image for the same slice as shown in the original Figure 3, as suggested by the reviewer.

Comment 3: *Another concern, POM and adsorbed SOM both contain carbohydrates, will this affect Os reaction with SOM? I think the methodological approach followed in this work requires a calibration/verification protocol. Authors could use X-ray spectroscopy to verify the SOM distribution they found in an image slice using Os staining and scanning. A standard sample with known distribution of SOM or POM can also be used to verify the method presented in this paper.*

Response: In their paper, Peth et al. (2014) stated: 'We selected osmium as a staining agent as this reacts with unsaturated C-bonds of organic compounds ....including finely disseminated organic matter often absorbed onto clay mineral surfaces and not visible as discrete organic particles (Chenu and Plante, 2006). They showed that they could detect Os-staining of both POM and finely disseminated SOM and validated the method using SEM-based EDX X-ray analysis (see Figures 3 and 5 in Peth et al (2014)). Therefore we do not consider it necessary to undertake another validation of the approach.

Comment 4: *Authors presented that SOM occupied $>$50% of total aggregate volume, although %SOM was 4-7%, which is very difficult to grasp and warrant a validation of the approach used.*

Response: The approach we developed to estimate the volume of organic matter in each aggregate was based on sound physical principles and accurate estimates of constants (such as the density of mineral matter (2.65 g cm^{-3})). The reviewer does not state on what basis he considers this approach to be flawed and without further

detail we contend that our approach is justified and does not require further validation.

Comment 5: *Authors also need to present concentration of POM and SOM on silt+clay particles in their aggregates to justify the 3D distribution of SOM.*

Response: Some of this was also addressed in response to comment 2. We consider this to be beyond the scope of our paper. To our knowledge such an analysis has not been undertaken before and would require careful development in terms of the approach. We would need to identify an upper threshold size/volume/shape for sorbed organic matter and rules governing the size and shape of neighbouring mineral particles to select SOM on clay or silt particles. We have not modified our manuscript in this respect.

Comment 6: *Authors also need to present a thresholded image and greyscale scanned image to demonstrate their stepwise approach of image segmentation.*

Response: We have updated Figure 3 to show the thresholding, stepwise approach in the revised version of the manscript.

Comment 7: *Authors need to describe how the pores and stained SOM separated during phase segmentation of the image slices. Since the volume of SOM was calculated by subtracting volume of mineral phase from total volume of soil solid phase, accuracy of the image thresholding is very important.*

Response: We gave a detailed description of the segmentation of the image slices in the original version of the manuscript. We first separated the pores from the solid phase using a two component mixture algorithm. We then computed the the volume of organic matter and the differences in the adsorption values above and below the osmium adsorption edge to differentiate organic matter from mineral phases. The use of image thresholding based on the two-component mixture algorithm has a clear theoretical basis for its application.

Comment 8: *Authors also referred 2.65 g cm^{-3} as bulk density of the mineral matter but should be written as particle density of the mineral particles. Moreover, the term density of organic matter is much preferable than 'bulk density' of organic matter.*

Response: We agree with this comment and we have amended the final version of the manuscript to reflect this.

Comment 9: *The figures presented in the article are not clear enough to show the distribution of pore geometry in the aggregates. The naming of 9 aggregates in Tables and Figures is not clear.*.

Response: We believe this comment is directed at Figures 4 and 5. We needed a way to summarize the features of the pores (size and shape factor) and we consider that the boxplots presented do this effectively. We have undertaken further analyses of pore tortuosity and thickness/diameter and we present these new data in the final version of the manuscript (see response to comment 1 by reviewer 1).

Comment 10: *A graph with multiple lines showing pore volume against pore diameter in different aggregates, I think would be much more informative than presenting Figure 4 as boxplots.*

Response: This analysis is not possible based on the outputs from the 3D objects counter function from the BoneJ package which is routinely used for pore analysis. We consider that the boxplots in Figures 4 and 5 are informative as they summarize the data for each aggregate. We have also computed pore diameters for all 9 aggregates in response to comment 1 of reviewer 1 and we include these data in our modified version of the manuscript.

Comment 11: *Figure 5. Is it possible to extract images of different pore shapes of aggregates using threshold pore images? Authors can use threshold images to demonstrate the variation in pore shape and then distribution of different shapes in aggre-*

gates.

Response: We described how we applied the 3D objects counter function in BoneJ (see section 2.5.4) to extract pore volume and surface area to compute pore shapes for each pore structure from a regular block within each aggregate. This is based on the pore:solid phase threshold images. Our aim here was to summarise the overall features of the pore size and shape for each aggregate so they could be compared and we consider that this was achieved effectively.

Comment 12: *Figure 6: Authors should focus on transition between SOM and pores. I feel it would much better if the authors could translate transition probability values in a form understandable for wider audience.*

Response: We do focus on the transitions between organic matter and pores in Figure 7 (see next comment). We describe how to compute transition probabilities in our Methods section and we consider that the majority of readers would be able to understand this based on the mathematical notation which is not particularly complex.

Comment 13: *Not clear why Figure 7 is included in the text*

Response: Figure 7 presents, in a more detailed form than Figure 6, the transition probabilities between organic matter centred voxels (O) and the other phases. We consider this plot is useful as the reader can see clearly how these important properties, which have never been computed at the aggregate scale before, vary between the nine aggregates. Note we have improved this Figure based on a comment by reviewer 1.

Comment 14: *Figure 8 and 10: Dull scatter plots, a simple regression equation with R2 value can covey the massage.*

Response: See our response to Reviewer 1's comment 13. We are sorry that the

reviewer finds the scatter plot dull, but the use of regression lines for decoration (except in some circumstances which do not apply here) is statistically unsound. We do include the correlation coefficient, however. We have not changed the final version of the manuscript in this respect.

Comment 15: *If possible calculate pore connectivity from the dataset and plot it against SHR.*

Response: We consider this to be beyond the scope of the current paper but could be addressed in a subsequent analysis.

Comment 16: *Table 3: not clear why this table is needed. Authors need to present variogram model graphs showing the spatial variability of SOM in the aggregates. The graphs are more informative than the presented box plots in Figure 9.*

Response: We disagree with the reviewer on this point. We chose to focus on the range parameter of the variogram models because this is the main feature of the spatial variation. We considered that an effective way to summarize the range data for each of the nine aggregates and three phases was by presenting a boxplot of the data and we consider that these present the data very effectively. Individual models for each region of each aggregate would confuse the reader in our view. We have not changed the final version of the manuscript.

Comment 17: *Authors incubated aggregate samples in 37C for 24 hours and then measured the CO2 concentration of the headspace. The temperature was bit high to measure soil respiration and I suppose it gradually made the aggregates dry over 24 hours, which would affect the respiration rate.*

Response: Based on the literature we considered 37 °C to be an appropriate temperature for incubation. As the vials were sealed during the incubation phase we do not expect the soils would have dried substantially over this period.

Comment 18: *The authors wrote in many instances they used custom wrote scripts/macros in R and Fiji without presenting the codes. Authors may present the codes in supplementary material of the manuscript.*

Response: Yes we can provide these as supplementary materials.

References:

Chenu, C., Plante, A.F., 2006. Clay-sized organo-mineral complexes in a cultivation chronosequence: revisiting the concept of the 'primary organo-mineral complex. European Journal of Soil Science 57, 596-607.

Peth, S., Chenu, C., Leblond, N., Mordhorst, A., Garnier, P., Nunan, N., Pot, V., Ogurreck, M., and Beckmann, F. 2014. Localization of soil organic matter in soil aggregates using synchrotron-based X-ray microtomography, Soil Biology and Biochemistry, 78, 189 – 194.

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