

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: *My main concern that must be addressed before the manuscript is published is the method of pore size characterization used in the study. It appears that the researchers used an object identification algorithm to identify individual pores and then used volumes and a shape factor of the identified pores as one of the main tools in characterizing them. I am afraid I have to say that this approach is quite meaningless, and probably some of the lack of pore effects reported in the study is just reflecting the fact that inadequate criteria of pore characterization were used. This approach*

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completely ignores pore diameters and tortuosity - that is, the pore characteristics that are most relevant to their functioning. Say, we identified two pores with approximately the same volumes. One of them could be a thin and long tortuous pore, the other can be a large round cavity. Their functioning in terms of water, air, microbes, SOM decomposition, anything, will be completely different, yet in the classification system of this study they will be lumped in the same size class. While the distance from the pore component of the study is valid and interesting, the components that are based on the object-based pore identification (Figs. 4 and 5) should not be included in the manuscript.

Response: Our aim was to compare the overall size and shape characteristics of the nine aggregates and we did this with the two sets of analyses presented in the original manuscript. We agree that analyses of both pore diameter and tortuosity could be useful in terms of understanding pore functioning and we have computed these data using: i) the Fiji plugin *AnalyseSkeleton* for tortuosity index (computed as length of pores divided by Euclidean distance between their furthest ends) and ii) pore diameter using the *thickness* function in the *BoneJ* package. We have included an interpretation of these data in the manuscript. We disagree with the reviewer when he/she suggests that the data on pore size and shape should not be included (Figures 4 & 5). We consider these data are essential in the characterisation of the pores in the nine aggregates and we would wish to include them in the final version along with the new information. We have updated our interpretation to include the pore tortuosity and thickness data in relation to soil heterotrophic respiration.

Comment 2: *Minor items: p.3 l. 6 -the part regarding representative volume does not seem to fit with the rest of the study.*

Response: Inclusion of this reference to representative elementary volumes was in error - it will be removed from the final version of the manuscript.

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Comment 3: *p.3 l. 10 - something is missing after 'and'*

Response: The 'and' has been omitted from the final version and this now makes the sense clear.

Comment 4: *The experimental part seems to be very thoroughly conducted. I am curious - what was the need in using glass beads? Not having them would simplify the authors life a lot in terms of creating aggregate masks.*

Response: We included the quartz beads to prevent the aggregates from fragmenting. The greatest risk of this was at the freeze-drying stage, when the aggregates were subject to forces that could cause them to move and fragment inside the vials; and also during transport to and from the synchrotron. We could not select aggregates with diameters that were exactly the same as the vials (fixed diameter) and so any movement of the vial could cause collisions between the dry aggregates and the wall of the vial, causing them to fragment. The quartz beads acted as an inert supporting medium reducing the forces of fragmentation on the aggregates, ensuring their structure was maintained prior to synchrotron X-ray CT scanning. If we repeat this experiment, we may consider a more dense supporting material, such as stainless steel beads, to avoid the problems associated with making masks of the aggregates where surrounding material is of a similar density. However, it is possible that the larger density steel beads could also lead to fragmentation of the aggregates during transit. This needs to be tested further.

Comment 5: *Are all these details in describing how the aggregate masks were created really needed? A lot of the steps talk about in-house R codes or macros and, without those provided as part of the manuscript, this procedure description is not something that anybody from the audience can even try to reproduce*

Response: The stages and macros we refer to in this section could be reproduced

quite quickly in Fiji or R by any other researcher. If we did not provide these details they would not be able to do this and we considered it important that others could reproduce our workflow. We therefore chose to leave these detailed instructions in the final version of the manuscript and provided the scripts as supplementary material (see comment by reviewer 2).

Comment 6: *I think it is unfortunate that the authors decided to aggregate the image data. Why not just use the subsections of the original 3 micron resolution data sets?*

Response: We did not wish to aggregate the data. However, we chose to do so because of the difficulties in analysing the data in terms of computer memory and processing time. To undertake various analyses of the 3D numeric array at the original resolution ($2544 \times 2544 \times \text{ca. } 1200$) was extremely challenging even using a high performance computing cluster. Preliminary tests showed that some analytical steps would take a number of days to complete and given this was to be repeated for 9 aggregates we took the decision to reduce the resolution so we could complete our study in a timely manner. Once reduced in resolution (by a factor of 8) each 3D numeric array was 340 Mb in size which we found to be manageable in terms of reading and writing from memory.

Comment 7: *While I do not see it as a big problem for the current study I believe in future the authors should seriously consider the need to look not just at pores in general, but to keep in mind that depending on their diameters and other characteristics pores can function very differently. The authors expectations regarding pore-emission-SOM relationship.*

Response: No response needed here we believe.

Comment 8: *I agree that scaling CO2 emission by TOC makes sense, but just for "quality" check - was there a positive correlation between SHR and TOC? Because if*

everything worked as expected there should be one, and it would be nice to hear about it. If there was none, it is also important to report.

Response: Yes there was a positive correlation between SHR (CO₂ generated) and TOC with a Pearson correlation of $r=0.29$. We have reported this in the new version of the manuscript but we do not place too great an emphasis upon it because we consider scaling CO₂ to TOC content to be a more relevant measure.

Comment 9: *The discussion on differences between aggregate and bulk soil findings is a bit simplistic. It is a basic soil science knowledge that density of aggregates is typically greater than soil bulk density (simply put, soil bulk consists of aggregates and large pores among them). Much more interesting would be comparisons of porosity, density, etc. results of this study with literature data that were collected on the same spatial scale (i.e., based on aggregates).*

Response: We do not agree that this discussion is too simplistic. We felt it necessary to explain these differences; not all readers will be familiar with the relationships between aggregate and larger scale bulk density values. The problem with comparing bulk densities (BD) of aggregates (with data captured at a similar scale) is that there are many features that can influence BD (SOC content, texture, mineralogy, differences in soil formation processes) and making clear interpretations concerning them would be problematic. We do not propose to change the final version of the manuscript in this respect.

Comment 10: *In Table 1 and in other places that mention porosity it should be noted that here we are looking at image-based porosity that reflects volume of pores above certain threshold.*

Response: We agree with this comment and will make these changes to the final version of the manuscript.

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Comment 11: *Fig.6 - maybe do not show PP, OO, and MM values? They are not informative and without them the differences in other transition groups will be more visible.*

Response: We do not agree that the PP, OO and MM values should be removed from Figure 6. We consider it is helpful to show that the three phase transitions (for each phase) must sum to 1 and their inclusion makes this clear in the Figure. We have not made this change to this Figure in the final manuscript.

Comment 12: *I have to admit that what is shown on Fig.7 and its relationship to what is shown on Fig.6 eludes me*

Response: Figure 7 shows the individual aggregate transition probability values of the far right panel (OO-OM-OP) of Figure 6 (here expressed as percentages rather than decimal proportions). This change in presentation style may have caused some confusion so we have altered Figure 7 to make this clear by reporting the transitions as decimal proportions in the final version. We have altered the captions to make the relations clearer.

Comment 13: *Figs. 8 and 10 - even though the relationships are not significant, adding regression line, p -value and r^2 would be good*

Response: We refer the reviewer to an article on the use and misuse of regression by R. Webster (Webster, R. 1997. Regression and functional relations. *European Journal of Soil Science*, **48**, 557–566). Properly applied, regression is used to derive a predictive relationship of one variable from another or (in strictly limited circumstances) to calibrate a linear functional relationship. The regression line is not a suitable summary of the bivariate relationship between two variables unless one is measured without error. The regression line is therefore not a suitable decoration for the scatter plot. We can calculate the correlation coefficient, and report a p -value for the null hypothesis

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that is zero, but this will reflect little more than the small size of our sample.

Comment 14: *I understand the driving for reporting the probabilities as the main outcome of this study from the modeling perspective, but can this probability information be somehow presented in units of actual distances? I believe it would be of interest to greater audience.*

Response: The transition probabilities are reported for one voxel transitions and the scale of the voxels is of side length of 6.6 μm . We only report and discuss these one-step transitions; transitions over larger scales could be computed and presented and we assume this is what the comment is suggesting, but we are not certain. We have not undertaken these analyses to date and so we do not present them.

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