

## ***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.***

### **Anonymous Referee #2**

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The article deals with 3D visualization and quantification of organic matter (SOM) in soil aggregates. It is an interesting topic to many SOM researchers. The authors stained SOM in aggregates with Osmium (Os) tetroxide and scanned the aggregate using synchrotron X-ray CT. In general the article is casually written with an elaborate method and out of focus discussion section. 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).

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

Authors also need to present a thresholded image and greyscale scanned image to demonstrate their stepwise approach of image segmentation. 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. Authors also referred 2.65 g cm<sup>-3</sup> 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.

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. 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. 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 aggregates. 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. Not clear why Figure 7 is included in the text. Figure 8 and 10: Dull scatter plots, a simple regression equation with R2 value can convey the message. If possible calculate pore connectivity from the dataset and plot it against SHR. 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.

Authors incubated aggregate samples in 37°C 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.

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.

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Discussion paper

