Interactive Effect of Soil Pore Network Structure and Substrate Quality on Soil CO_2 Production: a Combined X-ray CT Incubation Experiment

Steven Sleutel[1], Liesbeth Bouckaert[1], Denis Van Loo[2], Luc Van Hoorebeke[2] and Stefaan De Neve[1]

[1]Department of Soil Management, Faculty of Bioscience Engineering, Ghent University, Coupure links 653, 9000 Gent, Belgium [email: Steven.Sleutel@UGent.be]

[2]UGCT Ugent Centre for X-ray CT, Ghent University, Proeftuinstraat 86, 9000 Gent, Belgium [email: UGCT@UGent.be]

Keywords: Soil, pore network, soil aggregate, soil organic matter

ABSTRACT

The role of soil structure in organic matter (OM) stabilization has been primarily investigated through physical fractionation studies operative at the scale of aggregates and smaller organo-mineral particles. By narrowing down soil structure to an arrangement of mineral and organic particles, the majority of studies did not explore the spatial organization of the soil pore network, the actual habitat of microorganisms. In a lab experiment we incubated a sandy loam soil (with application of ground grass or sawdust) in 18 small aluminum rings (Ø 1 cm, h 1 cm). Bulk density was adjusted to 1.1 or 1.3 Mg m^{-3} (compaction) and 6 rings were filled at a coarser Coarse Sand:Fine Sand:Silt+Clay ratio.

1.   INTRODUCTION

The pore structure of soil can have a significant impact on soil processes like OM decomposition by excluding OM from micro-organisms in small pores, by regulating the diffusion of substrates and metabolites and by regulating aeration and presence of moisture. Our aim was to investigate the complex interactions between soil pore structure, soil biota and decomposition of added OM substrates. We report on a lab incubation experiment in which CO_2 respiration from soil cores was monitored (headspace GC analysis) and an X-ray CT approach yielded soil pore size distributions. We set up a controlled incubation experiment with a reconstituted and the original sandy loam soil, which was also scanned afterwards by X-ray CT to quantify the pore size distribution. We looked into the influence of i) soil compaction or ii) artificial change in particle size distribution in combination with iii) application of distinct substrate types. We hypothesize that different changes in pore size class volumes caused by these manipulations would affect C mineralization differently. Possible interactions between substrate type and artificial pore structure changes were evaluated.

2.   EXPERIMENTAL

2.1.   Experimental set-up

An artificial soil with no particulate organic matter was then reconstituted by mixing these size fractions. In total nine different soil mesocosm treatments (each in 3 replicates) were constructed. The nine treatments were: a reference treatment, and treatments with i) compaction, and ii) artificial changes in soil texture in combination with addition of 2 different substrates or no substrate applied at all. (Sleutel et al., 2012). The reference treatment had a coarse sand, fine sand and silt+clay (CS:FS:S+C) ratio of 10:40:50, 0.035% N and 0.448% C. The artificial texture treatment had a modified CS:FS:S+C-ratio of 20:60:20, 0.014% N, 0.179% C and pH 6.3. Twenty seven (9 treatments with 3 replicates) aluminium cylinders (Ø=1.2 cm; h=1.2 cm) were filled with 1.2 g of soil. Both the reference and manipulated texture treatments were compacted in a vertical plane with a cylinder to obtain a target bulk density (BD) of 1.0 g cm^{-3} (h=0.99 cm). Water content in all treatments was adjusted to 28% water filled pore space (WFPS) by adding water.

2.2.   Soil incubation and carbon mineralization

The 27 pre-treated repacked soil columns were incubated at 20±1°C for 35 days to promote

aggregation, and thus in pore network structure development. The SOM and substrate derived C mineralization was monitored during incubation by sampling the headspace above the soil cores with a syringe. The CO_2 concentration was measured with a GC fitted with an ECD detector (Thermo Electron Trace GC Ultra).

A parallel first- and zero-order kinetic model was fitted to the C mineralization data for each soil core, and this model was used to calculate the cumulative C mineralization over 35-days ($C_{cum,35}$).

2.3. X-ray CT derived pore size distribution and visualization of OM

Two out of 3 replicates per treatment were scanned with a high resolution X-ray tomography set-up at the UGCT (www.UGCT.Ugent.be). The experimental conditions for the micro-focus CT scans consisted of a directional target microfocus X-ray tube operated at 100 kV and 80 µA (8 W), an amorphous silicon flatpannel detector (Varian Paxscan 2520) with 1400 ms exposure time per projection and 1400 projections per scan of 40 minutes. The raw data was reconstructed with the in-house developed reconstruction software Octopus (Vlassenbroeck et al., 2007) to a dataset of 1500x1500x1500 cubic voxels with a 10 µm voxel pitch for each soil column. Automated sequential scanning of up to 10 stacked soil cores was enabled by programmed movement of the rotational motor in a vertical plane and automated X-ray CT acquisition.

The scanned images were analyzed for total porosity (TP) and pore size distribution (PSD) using Morpho+ (Brabant et al., 2011). The equivalent sphere diameter ($\varnothing_{eq}$), i.e. the diameter of a circular sphere having the same volume as the pore, was calculated for each pore. A PSD resulted from the cumulative pore volumes of a number of $\varnothing_{eq}$ classes. All 18 images were processed identically to allow comparison of the calculated PSDs. Firstly, a volume of interest (VOI) was selected to decrease image processing time. These VOIs of 350x300x300 voxels were then filtered twice by a 3D median (26x26x26) filter and once by a 3D bilateral (2,2) filter. Following segmentation, the binary image was then used as input for pore labelling, which assigns a label to every discrete object. Next, a distance map calculation of the pore space was then executed as the basis for a watershed based separation of the pore space. A distribution of voxels according to $\varnothing_{eq}$ was then obtained and this was used to calculate the PSD. Total X-ray CT visible pore volume (%) was calculated from the ratio of the total volume (µm³) of segmented pore voxels and total volume (µm³) of the scanned CT volume.

3. RESULTS AND DISCUSSION

3.1. Carbon mineralization

Compaction from BD 1.0 to 1.3 g cm^{-3} resulted in a reduction of net grass- and sawdust-derived $C_{cum,35}$ (Figure 1). When expressed as a % of the C present in the soil, cumulative relative C mineralization decreased by more than 50% by in the compacted grass and sawdust amended soils compared to the non compacted soils upon compaction, (reduction from 4.7 to 2.2 and 3.6 to 1.8% of total C addition for grass and sawdust amended soils, respectively). This demonstrates the a strong effect of compaction on substrate derived C mineralization. Artificial change in texture did not affect net modelled $C_{cum,35}$ from grass while it strongly reduced net $C_{cum,35}$ of sawdust compared to the reference sawdust amended soil. Overall these results point to a specific interactive effect from both soil pore network structure and substrate type.
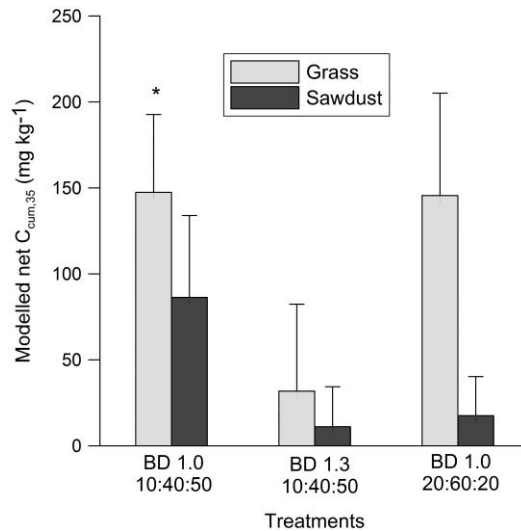
Figure 1: *Net substrate derived 35-days cumulative C mineralization ($C_{cum,35}$). Treatments indicated by
* have a statistically different (P<0.05) net C mineralization than the reference treatment (BD1.0 with
either grass or sawdust application) according to Dunnett's post hoc test*

3.2.   X-ray CT total porosity and pore size distribution

X-ray CT derived pore volumes (expressed relatively as a volume%) of 5 pore $\varnothing_{eq}$ classes were
calculated. Compaction significantly reduced the percentage of pore volume in the >800 µm class
while the pore volume of the 200-400 µm class was significantly increased in the unamended soils.
Soil compaction also reduced the percentage of pore volume in the >800 µm class when grass or
sawdust were added, but only significantly for sawdust. Artificial change in texture did not significantly
influence the pore volume in any $\varnothing_{eq}$ pore class for unamended, grass amended and sawdust
amended soils, nor the summed volume of 210-800 µm classes. The comparatively high volume
percentage of pores with $\varnothing_{eq}$ of 210-400 µm after soil compaction points to an increase in pores of this
class (only significant for the unamended soil).

Correlation coefficients were calculated between the modelled $C_{cum,35}$ (mg C kg^{-1}) and volume% of 5
different $\varnothing_{eq}$ pore size classes, namely 10-200, 210-400, 410-600, 610-800 and >800 µm (Figure 2).
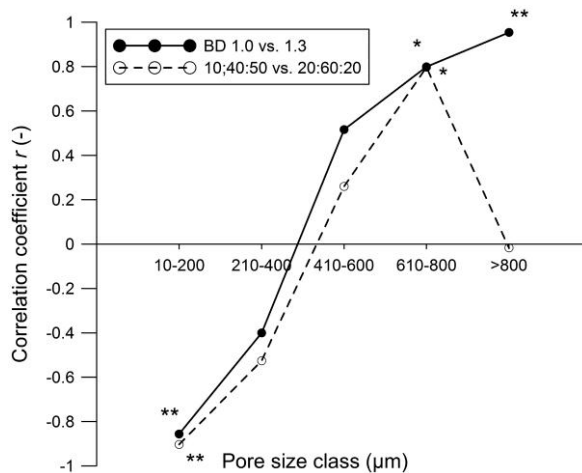

Figure 2: *Correlation analysis between net $C_{cum,35}$ and the X-ray CT derived volumes of pore size
classes plotted against the equivalent sphere diameter $\varnothing_{eq}$.*

For both manipulation of soil pore network structure treatments (change of BD 1.0 to 1.3 g cm^{-3} and of
CS:FS:C+S10:40:50 to 20:60:20), the correlation maps between net modelled $C_{cum,35}$ and percentage
of pore volumes in pore size classes switched from negative to positive with increasing $\varnothing_{eq}$. For both
treatments, net modelled $C_{cum,35}$ was negatively and significantly (both at $P<0.01$) correlated with
percentage of pore volume in the smallest pore size class (10-200 µm).
Enhanced soil aeration with increasing volumes of these larger pore size classes could be the main
reasons for increased bulk soil C mineralization. However, as these larger pores are mostly air-filled,

very fine water films (<10nm) are the only water source available in these larger pores. Second, as the added grinded grass and sawdust materials had comparable size as these pores, C mineralization of the added substrates could have actually only occurred inside these pores. Significant negative correlations observed between $C_{cum,35}$ and the volume of the 10-200 µm $\varnothing_{eq}$ pore size class for grass and sawdust amended soils, may have been an indirect consequence of the inverse relation with the volume of >410µm pores. In general, the significant negative and positive correlations observed between the substrate net C mineralization and the volume percentage of $\varnothing_{eq}$ pore class 10-200 µm (for both treatments) and 610-800 µm (for both treatments) and >800 µm (for the bulk density treatment only) respectively, imply a profound influence of PSD on C mineralization in the amended soils.

In future experiments, simultaneous measurements of the microbial community, C mineralization and visualization of organic matter and water in the pore space will facilitate interpretation of results of experimental incubation studies like the present one. Although the simultaneous visualization of OM and water in the pore space is very challenging, in parallel first work were able to successfully stain particulate OM in X-ray CT images of an artificial sand- particulate OM mixture. The enhanced X-ray CT contrast of the particulate OM compared to the pore space and the soil matrix in their study appears to be very promising for future separate visualization of SOM in soil cores.

4.   CONCLUSIONS

Pore size distribution in the small soil cores can be successfully quantified from 3D X-ray CT scans and computer image analysis. In both grass and sawdust amended soil, a clear trend of the correlation coefficients from negative to positive with increasing pore size class suggested a positive influence of macro porosity on C mineralization of freshly added substrates. The specific effect of this study's artificial modifications of the soil pore structure on substrate C mineralization was, however, found to be highly dependent on substrate quality. Explanations could involve changes in aeration, moisture availability, shift in habitable pore space etc. A more in depth research is required to improve an insight on this matter. Controlled incubation studies in combination with X-ray CT and PLFA analysis would seem to be fit for this purpose.

5.   ACKNOWLEDGEMENTS

6.   REFERENCES

Brabant, L., Vlassenbroeck, J., De Witte, Y., Cnudde, V., Boone, M., Dewanckele, J., Van Hoorebeke, L. (2011). Three-Dimensional Analysis of High-Resolution X-Ray Computed Tomography Data with Morpho+. Microscopy and Microanalysis, 17, 252-263.

Sleutel, S., Bouckaert, L., Buchan, D., Van Loo, D., Cornelis, W.M., Sanga, H.G. (2012). Manipulation of the soil pore and microbial community structure in soil mesocosm incubation studies. Soil Biology & Biochemistry, 45, 40-48.

Vlassenbroeck, J., Dierick, M., Masschaele, B., Cnudde, V., Hoorebeke, L., Jacobs, P. (2007). Software tools for quantification of X-ray microtomography at the UGCT. Nuclear Instruments & Methods in Physics Research Section a-Accelerators Spectrometers Detectors and Associated Equipment 580, 442-445.