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Manipulation of the soil pore and microbial community structure in soil mesocosm incubation studies

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ABSTRACT

Soil pore structure exerts a profound influence on distribution of moisture, O_2 and micro-organisms, thereby potentially controlling organic matter (OM) decomposition in soils. Although pore space is the habitat for soil micro-organisms and the actual location of soil biochemical processes, to date, very few studies looked into this relation mainly because of practical constraints. New experimental designs need to be developed which allow specific investigations of the relation between soil pore network structure, the microbial community and OM decomposition. We therefore subjected a sandy loam soil to a number of artificial manipulations namely i) compaction, ii) artificial change in particle size distribution, iii) addition of different substrates and iv) change in soil pH to manipulate soil pore structure and the decomposer community for use in lab incubation set-ups. Moisture retention data showed that compaction and artificial change in particle size distribution decreased volumes of large (9–300 μm) and small (<0.2 and 3–9 μm) pore size classes, respectively. PLFA signature analysis showed that acidification promoted fungi, while an effect of application of either sawdust or grass on the decomposer community was smaller. Acidification significantly reduced C mineralization and microbial biomass C. Surprisingly, the largest shift in microbial community (with promotion of fungi and protozoa relative to bacteria) over all treatments was observed in the treatments with artificially changed particle size distribution. We conclude that it is possible to 'tailor' soil pore structure and the decomposer community in soil mesocosm incubation experiments by such manipulations. However, non-targeted effects on microbial community structure, microbial biomass and gross C mineralization seem unavoidable.

1. Introduction

Distribution patterns of decomposer organisms in the soil do not generally match the allocation patterns of soil organic carbon (SOC) (Ekschmitt et al., 2008). Soil organic matter (SOM) may be physically protected from microbial decomposition by its spatial separation from decomposers which are limited in their motility. For example, microfauna (i.e. mainly nematodes and protozoans) are bound to the water film covering soil, and mesofauna (mainly enchytraeids, springtails and mites) depend on existing macropores in soil for movement (Ekschmitt et al., 2008). Next to confinement of organisms to habitable pore spaces, the microstructure of soil has a significant impact on soil processes by protecting micro-organisms from predation, by inducing spatial heterogeneity in moisture content, pH and O_2 availability and by regulating the diffusion of substrate to and metabolites from microbial cells (Nunan et al., 2006). Despite the growing body of evidence showing the importance of pore structure for microbial processes such as decomposition of organic matter (OM) (Strong et al., 2004), there is insufficient data for quantification of the relative importance of physical stabilization mechanisms of OM to SOM decomposition.

Ample research over the last decades has focused on interactions between soil structure dynamics and soil OM turnover (for example Six et al. (2000) and Elliott (1986)). Most research efforts have primarily relied upon physical separation of soil aggregates and associated OM into 'occluded' and 'free' fractions with differing conceptually allocated degrees of physical protection. However, narrowing down soil structure to soil aggregation is an oversimplification (Young et al., 2001) and study of aggregates can only provide indirect information on the complex interactions between soil micropore structure and OM turnover.

Because of the ever-present co-occurrence of a spectrum of factors influencing SOM decomposition, studying the complex relationship between soil pore network structure and decomposition requires specifically designed experiments. Ideally, influence of variation in the soil pore structure should be studied in isolation,

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with all other factors influencing SOM decomposition constant. To study the relationship between pore size distribution and OM decomposition Strong et al. (2004) used natural spatial variation in both parameters by taking samples along a field transect. Hassink et al. (1993) compared pore size distributions and microbial biomass pools in grassland soils with different textures. However, this approach also yields unavoidable variation among samples in SOM composition and soil mineralogy. Several other authors (De Neve and Hofman, 2000; Franzluebbers, 1999) showed compaction reduced C mineralization at different moisture levels and shifted the water-filled porosity at which C mineralization was maximized. Yet, compaction mainly reduces macro porosity (Van der Linden et al., 1989) and as such little work has been done on relations between smaller pores and SOM decomposition. Thomsen et al. (1999) added clay to soil to study the influence of clay content on OM decomposition and, although unintended, by doing so they must have altered fine porosity as well. Other than this, we have no further knowledge of specific studies on OM decomposition with manipulation of smaller pores.

Our objective was to investigate a number of artificial operations to manipulate soil pore structure and the microbial community for use in soil incubation studies focused on the relationship between the soil pore structure, soil micro-organisms and C mineralization. We set up a controlled incubation experiment with a reconstituted sandy loam soil, from which particulate organic matter was removed. We looked into the influence of i) compaction, ii) artificial change in particle size distribution, iii) substrate type and iv) soil pH. We hypothesize that specific pore size classes may be targeted through different manipulations such as compaction and artificial change in particle size distribution. In addressing this hypothesis, we were interested in identifying potential non-targeted effects on microbial community structure. Second, we hypothesize that addition of a selected substrate or a change in pH by dilute acid addition can be used to alter the decomposer community structure. Relative to our second hypothesis, we also looked for potential non-targeted effects on the pore size distribution.

2. Materials and methods

2.1. Experimental set-up

The soil used for the incubations was a sandy loam soil (7% clay, 42% silt, 51% sand) with 0.797% SOC and 0.061%N, and a pH_{H_2O} of 6.3. A 20 kg soil sample was taken from the 0–30 cm depth layer of a cropland field, situated in Lendelede (Belgium), with a spade. Particulate organic matter was removed as follows. The bulk soil was dry sieved at 2000, 200 and 53 μm sieves, then the >2000, >200 and >53 μm fractions were dispersed by shaking in 50 g l^{-1} sodium metaphosphate (1:3 w/v ratio). The dispersed slurries were passed once more on the respective sieves, then followed by extensive rinsing with deionized water. In this way three size fractions were obtained namely, coarse sand (200–2000 μm), fine sand (53–200 μm) and silt + clay (<53 μm). The coarse and fine sand fractions were placed in a muffle furnace at 500 °C for 5 h to remove the (particulate) organic matter. An artificial soil with no particulate organic matter was then reconstituted by mixing these size fractions. In total 8 different soil mesocosm treatments (each in 3 replicates) were constructed. The 8 treatments were a reference treatment, and treatments with i) compaction, ii) changes in particle size distribution, iii) addition of different substrates, and iv) changes in soil pH. Treatments i) and ii) were aimed specifically at manipulating the soil pore size distribution, whereas treatments iii) and iv) were expected to change the soil microbial community composition.

The reference treatment had a 10:40:50 coarse sand:fine sand:silt + clay ratio (CS:FS:S + C-ratio), 0.035 %N, 0.448 %C and pH 6.3. One hundred gram of the reference soil was filled in 5 cm Ø PVC tubes. The soil columns were slightly compacted in a vertical plane with a cylinder to obtain a target bulk density (BD) of 1.3 Mg m^{-3}. The compaction treatment i) consisted of 3 tubes filled with the 10:40:50: CS:FS:S + C reference particle size distribution, compacted to a BD of 1.6 Mg m^{-3}. The particle size distribution treatments ii) consisted of six tubes filled at two different CS:FS:S + C-ratios, namely at 15:50:35 and 20:60:20, both in 3 replicates. The removal of particulate organic matter prevented variation in the soil OM quality after mixing the soil with alternative CS:FS:S + C-ratios. The substrate addition treatment iii) consisted of three tubes filled with soil as in the reference treatment, mixed with 0.25 g of ground sawdust (promotion of fungal controlled degradation expected) and three tubes with soil mixed with 0.25 g of dried and ground grass (promotion of bacteria controlled degradation expected). The pH or acidification treatment consisted of 6 tubes filled with soil as in the reference treatment, with addition of 6 and 15 ml of 0.01 M HCl to reach pH 5.3 and 4.3, respectively. Initially, the water content in all treatments was adjusted to 50% water-filled pore space by adding deionized water (taking into account bulk density differences and added 0.01 M HCl).

2.2. Soil incubation, carbon mineralization and microbial biomass carbon

The 24 pre-treated repacked soil columns were incubated at 20 ± 1 °C for 35 days to achieve aggregation, which will be mirrored in pore network structure development. This approach was also used by Denef et al. (2002) and De Gryze et al. (2006) who incubated mixed dispersed soil to generate a variety in aggregate size distributions. Denef et al. (2002) (working with a silt loam Kastanozem) found that most macro-aggregate formation occurred within a 2 week period and De Gryze et al. (2006) (working with sandy loam to clay loam Luvisols) found leveling-off of further aggregate formation after 3 weeks. Consequently, a 35-day lab incubation period was considered to be sufficient to achieve pore network development in the reconstituted soils.

The SOM and substrate derived C mineralization was monitored during incubation as a measure of biological activity. The CO_2 evolved from soil was monitored by placing the tubes in airtight closed jars. The total initial weights of the jars with soil filled tubes were recorded. Small vials containing 15 ml of 0.2 M NaOH solution were placed in jars to trap the evolved CO_2. Amounts of evolved CO_2 were regularly measured by titration of the NaOH with 1 M HCl to pH 8.3 in the presence of $BaCl_2$ (Anderson, 1982). After removal of the vials containing NaOH, the glass jars were left open for 2 h to allow replenishment of oxygen. Soil moisture content was adjusted, fresh vials containing NaOH were added, and the jars were sealed again to continue the C mineralization measurements. A parallel first- and zero order kinetic model:

$$C(t) = C_f\left(1 - e^{-kft}\right) + k_s t \quad (1)$$

was fitted to the C mineralization data of both unamended and amended soils, expressed as C (mg C kg^{-1}), with $C(t)$, the cumulative amount of substrate (i.e. carbon) mineralized at time t (day). With k_f (day^{-1}) and k_s (mg C kg^{-1} day^{-1}) the mineralization rate constants of a fast carbon pool (C_f) and of the slow C pool, respectively. The parameters C_f, k_f and k_s were determined through non-linear regression using SPSS 15.0. At days 15 and 25 the water loss by evaporation was checked by weighing the tubes and deionized water was added accordingly.

At the end of the incubations the soils were removed from the tubes and mixed manually. Soil microbial biomass OC was determined immediately with the fumigation–extraction method