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Investigations of relationships among aggregate pore structure, microbial biomass, and soil organic carbon in a Mollisol using combined non-destructive measurements and phospholipid fatty acid analysis

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ABSTRACT

Limitations of traditional measurement methods have impeded progress in understanding the role of soil aggregation in protecting soil organic carbon (SOC) from decomposition by soil microbes living in pore spaces. In this paper, we used the Scanning Electronic Microscope (SEM) and X-ray micro Computed Tomography (micro-CT) to study the relationships of the aggregate pore structure and microbial distribution in the interior and exterior of soil aggregates, and thereby gained an insight into protection of carbon within macroaggregates of an undisturbed Mollisol in northeastern China. There were close relationships between soil pore structure and distribution of soil microbes and soil organic carbon (SOC), but they were different on the exterior and interior of soil aggregate. On the exterior of macroaggregates, there were negative relationships between soil porosities, the number of pores and SOC, especially for soil pores in the 10–30 μm and 30–100 μm classes, indicating these two pore sizes are unlikely to help sequester C. In contrast, there was a positive correlation between soil pores > 100 μm and SOC. Furthermore, soil pore structure had no impact on soil microbial biomass and density or on SOC contents in the interior of soil aggregates. This study provides a new method by combining SEM with micro-CT technology for linking soil structure and soil microbial properties with C sequestration and SOC changes.

1. Introduction

Soil structure is not only an important indicator of soil quality (Mueller et al., 2013), but also an important, albeit unclear, factor influencing soil C storage, fluxes, and even the global C cycle (Davidson and Janssens, 2006). It influences water holding capacity, infiltration, root penetration, organic matter turnover and soil respiration (Monga et al., 2008). Under the interaction of soil biota, mineral and organic components (Tisdall and Oades, 1982; Chenu and Cosentino, 2011; Garbout et al., 2013) soil particles are organized into aggregates, which are structural units. The quality of soil structure is often expressed as the degree of aggregate stability (Bronick and Lal, 2005). Aggregates limit the access of decomposing microorganisms and their enzymes to organic carbon (Schmidt et al., 2011; Ananyeva et al., 2013), and thus are considered to be an important factor for soil carbon stabilization.

Aggregates are often grouped into macroaggregates (> 250 μm) and microaggregates (< 250 μm) (Tisdall and Oades, 1982). Macroaggregates contain higher C contents and newer C than microaggregates (Six et al., 2000). Moreover, the turnover of macroaggregates affects the formation of microaggregates where physical protection is enhanced by physicochemical and chemical processes in microaggregates (Six et al., 2000; Denef et al., 2001; Ananyeva et al., 2013). Consequently, macroaggregate turnover can control soil carbon stabilization by changing soil physical structure and soil microbial activities (Denef et al., 2001; Kravchenko et al., 2013; Mangalassery et al., 2013). Soil pores are one of the most important physical features of a soil: they control gas diffusivity, water potential and microbial accessibility, and thereby strongly influence the dynamics of soil carbon quality and quantity (Lugato et al., 2009). Because soil physical structure and microbial communities both vary from the exterior to the

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interior of aggregates, there are significant differences in SOC concentrations between these two locations in the aggregates (Horn et al., 1994). In addition, soil aggregate structure is not stable and the dynamics of aggregate structure formation and reformation affect soil microbes and soil C distribution (Six et al., 2000). The mechanisms of how soil aggregates protect SOC, how soil pore structure affects soil carbon storage, or how the C cycles during the process of soil aggregation, are not clearly known.

Traditionally, micromorphological methods, such as photomicrographs of impregnated soil thin sections or soil blocks combined with digital image analysis, were used for direct observation and quantification of soil aggregate structure (Vogel et al., 1993; Vogel, 1997; Pagliai et al., 2004). These methods can provide detailed information of the constitution of soil aggregates and valuable information on the quality of the material, but they are time consuming and restricted to two-dimensional images only (Zhou et al., 2012). Recently, computed tomography (CT) has been used for this type of study (Bouckaert et al., 2013; Kravchenko et al., 2015). CT is a non-destructive testing technique and can quickly explore soil structure in three dimensions (3D) (Young et al., 2001). It is a unique tool for 3D visualization and quantification of soil structure. There are different types of CT, including clinic CT, X-ray micro-CT and synchrotron based X-ray micro-CT. The X-ray micro-CT technique has characteristics of higher resolution, stronger contrast, and faster scanning, and is widely used for studying microstructure of soil aggregate (< 10 mm in diameter) (Papadopoulos et al., 2009; Wang et al., 2011,2012; Zhou et al., 2012; Kravchenko et al., 2011, 2013; Dal Ferro et al., 2013). In addition, the Scanning Electronic Microscope (SEM) is becoming a recommended method for in-situ studies on soil microbial distribution (Schurig et al., 2015). The combination of these two in-situ methods may greatly improve our ability to assess C turnover as affected by soil pore structure and soil microbial activity. Kravchenko and Guber (2017) proposed that future progress would undoubtedly enable in-situ intact observations of soil chemical and physical characteristics via micro-CT, and join with micro-scale measurements of microbial presence and enzyme activity.

The hypothesis of this study is that there is a relationship between aggregate pore structure, microbial distribution in aggregates, and concomitant C protection. We further hypothesize that this relationship varies between interior and exterior of aggregates. Taking advantage of availability of non-destructive measurements, we aim to analyze the relationships among SOC, soil pore structure, soil microbial biomass and density in undisturbed soil aggregates using the SEM and micro-CT technology, and Phospholipid fatty acid (PLFA) analysis.

2. Materials and methods

2.1. Soil sampling and soil characteristics

The soil samples were taken from an undisturbed natural site in Laodaodong Village (43°20.808′N, 125°50.548′E), Shuangyang County, Jilin province of Northeast China. The site lies in the temperate zone with a continental monsoon climate. The mean annual temperature is 5.5 °C and the mean annual precipitation is 629.6 mm (Zhang, 2013). The soil type is Mollisols with a clay loam texture (27.2% clay, 36.9% silt and 35.9% sand). Soil is neutral or slightly acidic with an average pH of 6.9. Natural vegetation in the region was secondary forest and grassland. Soil samples from this area were chosen in this study because of minimal anthropogenic disturbance; there were no treatments imposed. Four soil samples were taken at random locations to a depth of 0–10 cm in October, 2013. Firstly, a pit of 20 cm depth was dug and soil profile was obtained. Then a $25 \times 20 \times 10$ cm rectangular container was vertically pushed into the soil by hand, and carefully dug out from the side of exposed profile to get an undisturbed soil sample. Soil samples were sealed and transported to the lab. Each of the four samples was subsampled, and measurements on aggregates sieving and associated SOC, phospholipid fatty acid (PFLA), soil pore structure, and soil microbe observation were made on the subsamples. Each of these measurements was replicated three times on different subsamples from each sample, and the means were calculated for each sample.

2.2. Aggregates and associated SOC analysis

Subsamples of soils were dry-sieved and aggregates in 1–2 mm size range were collected. The aggregates were weighed and then gently abraded using a shaker with a sieve of 0.25 mm to peel off concentric layers from the aggregates for subsequent SOC analysis (Park and Smucker, 2005). During the abrading process, the aggregates on the sieve were periodically weighed to determine the portion of material that had been abraded. The swarf or “peelings” from the aggregates corresponding to the exterior, transitional and interior layers were collected consecutively with each layer corresponding to one third of total aggregate weight. Whole aggregates, and the saved peeled materials from exterior and interior layers were ground for SOC analysis; the transitional layer SOC was not analyzed in this study. The SOC content was measured using a FlashEA1112 Elemental analyzer (Thermo-Finnigan, Milan, Italy). Since the soil was free of carbonates, SOC was assumed to be equivalent to total C.

2.3. Soil microbe observation

The aggregates (1–2 mm in diameter) from fresh soils were kept moist at 4 °C and then dehydrated by successively submersing them into acetone-water solutions with increasing acetone concentrations of 25%, 50%, 75%, 90%, 95% and 100% for 24 h each (García-Martínez et al., 2005). The fixed aggregates were air-dried and then cut in half with a scalpel to expose the cross sections of the aggregates. We chose three aggregates for each soil sample as replicates. The aggregate exterior and cut faces were examined with an S-4800 scanning electronic microscope (SEM, Hitachi, Japan) to observe microbes. We scanned the entire surface at the lower resolution, and then zoomed in (Fig. 1) to obtain 10 μm high resolution scans (Monga et al., 2008). Ten high resolution SEM images were obtained at equal intervals for each sample following a circular path in a clockwise direction. The ImageJ 1.47 software (National Institutes of Health, Bethesda, MD, USA) was used to calculate the percentage of area occupied by microbes in each image. Hereinafter, the percentage of area occupied by microbes will be referred to as microbial density. Here microbes refer to all microbes without classification into the species level. We identified bacteria and fungi from microbial dimensions and morphology in the images obtained by SEM. Most bacteria fall between 1 to 10 μm in size. The bodies of fungi are bigger than those of bacteria, and can be differentiated under optical microscopy. As for microbial morphology, bacteria include *Rickettsia* bacteria, rod-shaped bacteria, coccus-shaped bacteria and Mycoplasma bacteria. Cells of microscopic fungi exist in two basic morphological types: hyphae and yeasts. Hyphae are long with diameter of 2 to 10 μm, threadlike cells that make up the bodies of filamentous fungi. A yeast cell is distinguished by its round to oval shape. Details on microbial dimensions and morphology are given by Talaro and Chess (2012). All the process of distinguishing soil microbes within images were done by naked eye and by the same observer.

2.4. Phospholipid fatty acid (PLFA) measurement

The PLFA analysis was conducted to characterize soil microbial biomass following the procedure of Bossio and Scow (1998) and Wu et al. (2011). Lipids were extracted from the whole moist aggregates with a chloroform-methanol-citrate buffer mixture. The lipids were fractionated in a solid-phase extraction column (Supelco Inc., Bellefonte, PA, USA) and separated into the neutral lipids, glycolipids, and polar lipids. The polar lipids were trans-esterified to the fatty acid methyl esters by mild alkaline methanolysis. 150 μL methyl-