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Comparison of the effects of self-assembly and chemical composition on humic acid mineralization

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ABSTRACT

The purpose of this study was to quantitatively assess the effect of organic matter self-assembly on its resistance to microbial mineralization. Humic acids isolated from leonardite, two peats and a mineral soil were used as organic matter samples because they provide a broad range of variability in terms of origin and the nature of their organic components. Using a benzene-methanol extraction the original humic acid samples were disassembled into humic components and a humic-lipid composite. The composite was further disassembled by using an alkaline aqueous extraction into humic amphiphilic and lipid components. Mixtures that reproduced the composition of self-assembled samples were prepared by mixing the solid individual fractions in the exact proportions that they were present in the original material. The original humic acid and their corresponding mixtures were added as the sole carbon source in separate aerobic cultures containing a microbial consortium isolated from a mineral soil. After incubation for 125 days mineralization of the self-assembled samples was shown to be higher by as much as 70% compared to their corresponding physical mixtures. The extent of mineralization of the self-assembled samples was not correlated to chemical composition base on the carbon-type distribution or hydrophobicity index derived from ^{13}C solid-state NMR spectra. Mineralization of the physical mixtures and fractions did vary with chemical composition and was accompanied by preferential mineralization of alkyl carbon. These results suggest the microbial mineralization of humic acids is related to their self-assembly.

1. Introduction

Natural organic matter represents the largest pool of carbon in terrestrial ecosystems; soil organic matter (SOM) represents more than four times as much carbon as the biotic carbon pool (Jobbagy and Jackson, 2000; Guo and Gifford, 2002). The release of CO_2 to the atmosphere by SOM mineralization is one order of magnitude larger than anthropogenic emissions (Schlesinger, 1997; Lal, 2008). This impact has resulted in increased interest in the persistence of SOM and resulted in the development of a growing number of soil carbon cycling models to predict SOM persistence in soils and surficial environments (Manzoni and Porporato, 2009). However, the ability of these models to predict SOM's long term response to climate change is limited by the current status of the understanding of the molecular processes that control SOM's persistence (Kleber et al., 2011; Schmidt et al., 2011). Most of these models describe SOM's participation in carbon cycling by using at least three carbon pools (Trumbore, 1997; Davidson and Janssens, 2006; Trumbore, 2009; Von Lützow and Kögel-Knabner, 2009) defined by their biological stability (labile, stabile, refractory and inert), their decomposition rate (fast-active, slow-intermediate and very slow/passive) and their turnover time (short, long, very long). These models rely on recalcitrance as a major sequestration mechanism by assuming that organic matter components are resistant to decomposition mainly as a consequence of its chemical structure. Recent research challenges the recalcitrance concept and introduces a new conceptual framework to explain the persistence of soil organic matter (Schmidt et al., 2011). This emerging view emphasizes that molecular structure alone can not explain the persistence of SOM and the interactions between the organic matter and its environment should be considered.

Recent SOM molecular aggregation models (Wershaw, 1994; Piccolo, 2001; Kleber et al., 2007; Chilom and Rice, 2009) are also part of this conceptual framework. They all emphasize the interactions among the organic matter components, and with mineral matter, rather than the individual chemical characteristics of

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these components. In the "membrane model" Wershaw (1994) described SOM as ordered aggregates that exist as micelles in solution and as bilayer membranes coating mineral grains in soils and sediments. Piccolo (2001) proposed a "supramolecular" model that describes SOM as supramolecular associations of relatively small molecules with the aggregate's conformation being determined by intermolecular forces and the complexity of the multiple non-covalent interactions. Kleber et al. (2007) introduced a "zonal model" that describes SOM as a mixture of components of variable amphiphilicity that sorbs on mineral surface in a discrete sequence. Chilom and Rice (2009) have described SOM as an emergent, hierarchical self-assembling or self-organizing system in which its components form ordered structures by non-covalent or weak interactions like van der Waals, hydrogen bonding and hydrophobic interactions. The environment can influence the self-assembly process by modifying the interactions among components (Chilom et al., 2013). The comparison of heat capacities measured for humic acid in its self-assembled and "disassembled" states has provided direct evidence for self-organization in SOM (Chilom and Rice, 2009).

One of the characteristics of self-assembly is that the resulting assembled materials have different properties and behave differently than the substances that they originate from. For example, Salloum et al. (2001) fractionated natural organic matter samples and found the organic carbon-normalized sorption coefficient (K_{OC}) values of 1-naphthol for the fractions differed from those of the source material. There are no data correlating the role of SOM organizational state and its resistance to microbial degradation, and this could be a crucial property for determining the stability of organic matter in the environment.

The objectives of this paper are to provide a quantitative estimate of the effect of organic matter's self-assembly on its microbial mineralization by separating the effects of organization from that of organic matter's chemical composition. The strategy applied here is to compare: i) mineralization of samples that are in their original self-assembled state with a physical mixture of the samples' components with the same composition as the original material to determine the effect of self-assembly, and; ii) the relative importance of chemical composition and self-assembly as variables impacting on mineralization. This approach will provide information about how the organizational state of organic matter affects the SOM stability and provide clues about how this understanding may be used to increase the residence time of organic carbon in soil.

2. Materials and methods

2.1. Organic matter samples

The source materials used in this study were the IHSS leonardite (Lot no. BS104L), the IHSS Pahokee Peat (Lot no. BS103P), a peat soil classified as a Cryohemist that was collected in central Colorado (Kohl, 1999), and a mineral soil classified as fine-silty, mixed Udic Haploborolls (Malo, 1994). These latter two soils are referred to as the Guanella Pass (GP) peat and the Poinsett silt-loam soil, respectively. These materials were chosen because the humic acids extracted from them display a range of chemical compositions (e.g., the NMR spectrum of the leonardite humic acid reveals that is comprised almost exclusively of aromatic and aliphatic carbon while the Poinsett silt-loam humic acid displays a typical humic acid carbon-type distribution).

Self-assembled organic matter was extracted from these materials in the form of humic acid by a traditional alkali extraction method (Stevenson, 1994). When used with no further treatment these original humic acid samples are referred to as HA_0. Following the procedure of Chilom et al. (2009) the HA_0 samples were disassembled by Soxhlet extraction with a benzene:methanol azeotrope (3:1, v:v) for 72 h yielding the composite L_0. After removing L_0, the extracted HA_0 samples are referred to as HA_1. The L_0 composite is then further disassembled by alkali extraction to obtain an amphiphilic component, referred to as HA_2, and a lipid component, referred to as L_1. The fractionation scheme is presented in Fig. 1.

A carbon mass balance was prepared for each sample and the abundance of each fraction in the starting material was calculated. Using the carbon mass balance, physical mixtures that reproduced HA_0 and L_0 were prepared by mixing their individual fractions in the exact proportions that they were present in the original, assembled material. The first mixture, intended to model HA_0, was prepared by mixing the three fractions HA_1, HA_2, and L_1. It is referred to as $HA_0/3$. The second mixture, made to model L_0, was prepared by mixing fractions HA_2 and L_1. It is referred to as $L_0/2$.

The organic carbon content of all the samples was determined using a Shimadzu TOC-VCSN. The instrument operates by catalytically oxidizing organic matter under a flow of CO_2-free air and measuring the amount of CO_2 produced via infrared absorption.

2.2. Mineralization experiments

A nutrient solution containing 3 g $NaNO_3$, 1 g KH_2PO_4, 0.5 g $MgSO_4 \cdot 7H_2O$ and 0.01 g $FeSO_4 \cdot 7H_2O$ in 1 L sterile deionized water was prepared (Filip et al., 1999). The organic matter samples described in the previous section served as the only carbon source in these experiments. Amounts of 70–100 mg dry organic matter were added to 100 mL of the nutrient solution in 250 mL Pyrex bottles. The solution contained predetermined amounts of 0.1 M NaOH so the resulting solutions were at pH = 7.5. The Pyrex bottles were closed with screw caps equipped with silicon/PTFE liners that had inlet and outlet holes fitted with PTFE tubing. The inlet tubing allowed the solutions to be flushed with CO_2-free air every day for 15 min while the outlet tube was open in order to maintain aerobic conditions during the incubation experiment. For the rest of the time the systems were close via 2-way valves.

A microbial inoculum was prepared from a fresh sample of Poinsett soil that was kept at 4 °C before use. The soil sample was suspended in 100 mL sterile deionized water and was vigorously shaken overnight on an end-over-end shaker. The suspension was

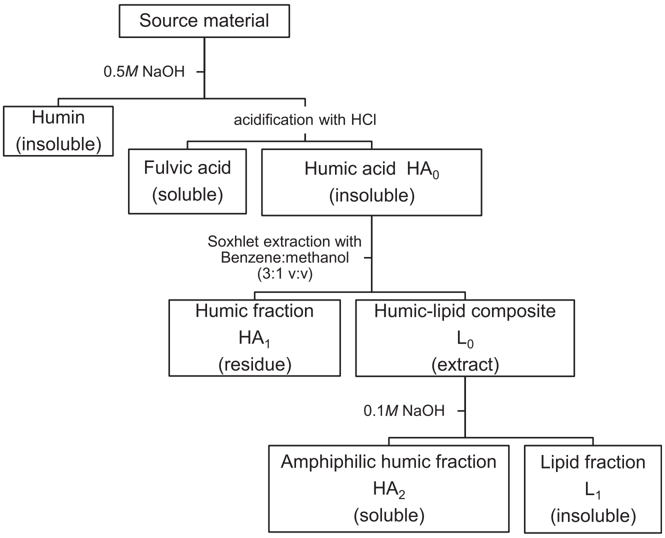

Fig. 1. Schematic diagram of the procedure to isolate humic acid (HA_0) and its fractions (HA_1, L_0, HA_2, L_1) from source materials.