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Short communication

Ants alter molecular characteristics of soil organic carbon determined by pyrolysis-chromatography/mass spectrometry

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ABSTRACT

Colonization by ecosystem engineers, such as ants, have important but occasionally unnoticed roles in regulating soil carbon cycles. However, little information is available pertaining to ant effects on changing molecular features of soil organic matter. In this study, we characterized total soil organic carbon (SOC), total nitrogen, water extractable organic carbon (WEOC) as well as molecular characteristics of humic acids (HAs) of ant mound soils ($Soil_{Ant}$) and the surrounding soils ($Soil_{Con}$) using pyrolysis-chromatography/mass (py-GC/MS). SOC, total nitrogen and WEOC contents of $Soil_{Ant}$ were significantly lower than those of $Soil_{Con}$. $Soil_{Ant}$ samples had significant higher nitrogen containing compounds proportions in pyrolysis products of HAs as opposed to $Soil_{Con}$ samples. Another remarkable characteristic was that no polysaccharides, lignin, and phenols were identified in HAs of $Soil_{Ant}$ samples. It indicated that ant colonization significantly changed the molecular composition of soil organic matter.

1. Introduction

Soil is the third largest carbon pool on Earth, doubling that present in the atmosphere and is about three-fold larger than that in the biosphere in terrestrial ecosystems (Post et al., 1982; Scharlemann et al., 2014; Schlesinger and Andrews, 2000). Organic matter represents about 70% of soil carbon pools. The stability of soil carbon pool is affected by many factors, such as climate, hydrology, vegetation, as well as bioturbation, which is important but often ignored (Conen et al., 2007; Mau et al., 2015; Neff et al., 2002). Bioturbation has important roles in soil and its heterogeneity formation (Jouquet et al., 2007), also could alter soil carbon contents, mineralization rate and distribution in spatial (Muller-Lemans and Van, 1996). Most recent researches focus on changes of soil physical properties, element distribution and microflora after ant colonization, and most research were about the total amount of soil organic carbon (SOC), carbon dioxide emission and nutrient contents (Ohashi et al., 2012; Wills and Landis, 2017). Many soil fauna representatives, mainly earthworms, ants and termites, act as soil engineers (Jones et al., 1997). They could modify soil properties through their mechanical activities and nesting behaviors, resulting in movement and redistribution of organic and inorganic materials within the soil profile (Dauber and Wolters, 2000; Nkem et al., 2000). For instance, ant-derived extensive replacement of the 0- to 10-cm horizon with subsoil reduces its organic matter content by about 50% (Levan and Stone, 1983). Ant nest-mounds are potential hot spots for soil nutrient cycling (Domisch et al., 2006). Previous studies reported that the mounds of three ant species, *Myrmicascabrinodis, Lasiusniger* and *L. flavus,* tended to form a mosaic of microbial hot spots as evidenced by high carbon mineralization rate (Dauber and Wolters, 2000). This was demonstrated by the work of Wu et al. (2013) who reported that average carbon dioxide emissions from ant mounds were 1.08–3.09 times higher than those from the surrounding soils. Average total nitrogen, as well as total and available phosphorus concentrations in ant mounds of *Formica sanguinea* Latreille and *Lasiusflavus* Fabricius were higher than those in the control soil, while organic carbon, dissolved organic carbon, nitrate and ammonium in *F. sanguinea* mounds were higher than in the control soil, but not for *L. flavus* mounds (Wu et al., 2010). However, the contribution of ants' respiration to carbon emission from their nests was not considered and separated from the total emissions (Wu et al., 2013).

Molecular compounds of SOC and their interactive transformation have important effects on regulating soil carbon pool stability and its biogeochemical circulation (Grandy and Neff, 2008). Ant colonization might change stability of soil carbon pools by affecting the inherent

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recalcitrance, accessibility to microorganisms, and interaction with stabilizing substances of organic compounds (Cerda and Jurgensen, 2011; Domisch et al., 2008; Drager et al., 2016). Channels of ant nests could introduce more oxygen into soils of ant mounds than the surrounding soils, which was hypothesized to accelerate soil organic matter degradation (Ohashi et al., 2005; Sousa-Souto et al., 2012). Some ants, especially termites, feed on soils. Molecular characteristics would be changed when soil organic matter pass through ants' guts featured by alkaline conditions with soil particles, which could induce labile organic carbon loss and resistant-decomposition parts remaining in soils (Brune, 2014). During gut transit, Soil organic matter is strongly modified in terms of nature and organization (Brauman, 2000). Components like peptides, proteins, and polysaccharides of humic substances were generally selectively digested, and aromatic components were apparently not assimilated by soil-feeding termites (Ji et al., 2000). After colonization by soil-feeding termites, molecular weights of residual carbon components in soil are shifting towards to low from high in the gut of termites, and an accumulation of small molecules in the termites bodes. It presented a strong evidence that structural polysaccharides from plants or bacteria are carbon and energy sources for soil-feeding termites, and easily consumed by termites (Brauman, 2000; Ji and Brune, 2001). Some resistant-decomposition components like lignin and cellulose also could be degraded in ant guts because of numerous kinds of microbiota including bacteria, fungi, flagellates, spirilla and actinomycetes (Brune, 2014). Microbial diversity in ants' guts would greatly alter molecular characteristics of soil organic matter. It is important to decipher roles of ants on changing molecular characteristics of SOC, and this would promote our understanding of relations between soil carbon circulation and bioturbation (Filser et al., 2016). However, it is still unclear how these components change at molecular levels after ant colonization. Therefore, the objective of the present work was to compare SOC, water extractable organic carbon (WEOC), and the corresponding molecular composition characteristics between ant mound soils ($Soil_{Ant}$) and the surrounding soils ($Soil_{Con}$) based on pyrolysis-chromatography/mass (py-GC/MS) analysis. We hope to achieve further understanding on how ant nesting changes soil organic carbon molecular features, and to further reveal the role of fauna on carbon cycling.

2. Materials and methods

2.1. Sample site

Ant mound samples were taken from Iberia Parish, in Louisiana, USA. The Iberia site (29°57′14.9″N 91°42′38.9″W) was from a bermudagrass (Cynodon spp.) pasture field of more than 15 years situated in the Louisiana State University Agricultural Center Iberia Research Station. The average annual precipitation is about 1516 mm. The soil at the site was Baldwin silty clay loam (Fine, montmorillonitic, thermic Vertic Ochraqualfs).

2.2. Soil sample collection and preparation

Six pairs of soil samples were collected from rounded or elliptic ant mounds ($Soil_{Ant}$, roughly, about 50 cm in diameter, and 10 cm high) and the surrounding soils ($Soil_{Con}$) at each site in December 2015 (Fig. 1). Ants were Solenopsis invicta and all ant mounds occurred in grassland with no surface water. When sampling, ant mounds with similar sizes were chosen. $Soil_{Con}$ samples were collected within 0.5 m around the corresponding $Soil_{Ant}$ sites. These soil samples were all within 10 cm depth from the surface. In total, 12 soil samples were collected in six pairs.

For convenience, samples collected from ant mounds were labeled as $Soil_{Ant1}$ to $Soil_{Ant6}$, and the corresponding surrounding soil samples as $Soil_{Con1}$ to $Soil_{Con6}$, respectively. Soil samples were sealed in polyethylene bags and transported in a cooler full of ice. In the laboratory, soil samples were freeze-dried after stones and vegetation residues were picked out. After that they were ground to pass through a 500 μm nylon sieve and then stored in polythene bags until analysis.

2.3. Water extractable organic carbon determination

The water extractable organic carbon (WEOC) was extracted according to Dodla's work (Dodla et al., 2012b). In particular, 5.00 g soil was first mixed with 30.0 mL deionized water in Erlenmeyer flasks and shaken for 30 min. After shaking, the mixtures were centrifuged and filtered through a 0.45 μm filter. Filtrates was analysed for total organic carbon using a TOC-VCPH analyzer.

2.4. Humic acids extraction

Humic acids (HAs) extraction was subsequently performed according to Buurman et al. (2005) with minor modifications. About 25 g of dry soil was shaken for 24 h in 250 mL of 0.1 mol L^{-1} NaOH under N_2. Extracts were then centrifuged at 8000 rpm (Feige, Changzhou, China) for 30 min, decanted, centrifuged, and decanted again under the same conditions. The resulting extracts were acidified to pH 1 to 2 with 6 N HCl, and 3 mL of concentrated HF was added to dissolve silicates. The acidified solution was shaken for 24 h, dialyzed (6000-8000D) against ultrapure water until there was no Cl^- detected using $AgNO_3$ solution, and then freeze-dried before py-GC/MS analysis.

2.5. Pyrolysis-chromatography/mass spectrometry analysis

Pyrolysis-chromatography/mass spectrometry (Py-GC/MS) analysis was performed at 620 °C for 20 s using a CDS 5000 pyroprobe platinum heated filament pyrolyser (Chemical Data System Analytical, Oxford, PA), directly connected to a Varian 3900 gas chromatography system coupled to a Varian Saturn 2100T ion trap mass spectrometer (Dodla et al., 2012a). The temperature of the injector and transfer line were kept at 300 °C and 240 °C, respectively. Mass spectra were recorded in electron impact mode (70 eV) at 1 scan s^{-1} in the 45 to 600 m/z range.

The identification of pyrolysis products was based on a comparison of their mass spectra to those of standard compounds, the NIST 2005 mass spectral library, literature data and GC/MS characteristics. The AMDIS analysis software was employed to assist compound identification. The relative distributions of products, ratio of total ion current of special components to total ion current determined, were estimated using the peak areas of the total ion current pyrograms. Pyrolytic compounds were grouped, according to the work of Gonzalez-Perez et al. (2012), which was based on probable origin and chemical similarity, into alkyls, aromatics, lignin, nitrogen containing compounds, phenols, polysaccharides, and sulfur containing compounds.

2.6. Chemical analysis

Soil organic carbon (SOC), total nitrogen, total hydrogen and total sulfur were determined by dry combustion at 900 °C using an Elemental analyzer (Vario EL III, Germany). Negligible amount of $CaCO_3$ in soil samples were removed by adding 1 N HCl before total SOC analysis (Dodla et al., 2012b).

During the analysis, all samples were analyzed in duplicates to assure accuracy and precision. The differences between replicates were within 0.5% and 0.1% for SOC and total nitrogen, respectively. Analysis of Chinese standard reference soil (No. GBW07401) yielded recovery rates of 100% (w/w) for carbon and 96% (w/w) for nitrogen. All glass bottles used were acid washed in a 3 mol/L HNO_3 solution, rinsed with deionized water, and then oven dried before use. The chemical reagents used were all of analytical grade.

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