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Increasing molecular structural complexity and decreasing nitrogen availability depress the mineralization of organic matter in subtropical forest soils

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ABSTRACT

The degradation of soil organic matter (SOM) is determined by its compositional and structural properties, but the properties of the remaining SOM can be modified during the mineralization process. In this study, 12 soils (Ultisols, Alfisols and Inceptisols) from subtropical forests were incubated under laboratory conditions (25 °C, aeration) for 90 days. The mechanisms underlying the changes of these properties were investigated by analyzing pre- and post-incubation soils using UV absorption, fluorescence spectroscopy and acid hydrolysis methods. The turnover rate of SOM decreased with increasing soil depth. The amount of CO_2 released ranged from 90 to 620 mg CO_2-C kg^{-1}, representing c. 0.9–3.1% of soil organic C (SOC). The proportion of nonhydrolyzable (recalcitrant) N in total N (R_N) increased with soil depth, whereas it was not apparent in relation to the proportion of nonhydrolyzable (recalcitrant) C in total C (R_C). Furthermore, a negative correlation was observed between SOM mineralization and R_N ($P < 0.05$) rather than R_C, implying that the availability of N was a limiting factor in SOM decomposition when compared with C. The decrease in E_2-to-E_4 ratio and fluorescence intensity (FI) of water-extractable organic C (WEOC) with increasing soil depth suggested that WEOC in the deeper horizons was probably rich in electron-withdrawing groups (COOH) but poor in electron-donating groups (OH, NH_2), and it was composed of components with larger molecular size, higher humification degree and structural complexity. These could be responsible for the lower SOM mineralization rates in deeper horizons. After 90 days of incubation, a blue shift of wavelengths in the synchrotron-scan spectra, and an obvious increase of FI values, together with the increase in E_2-to-E_4 ratio of WEOC implied a reduction in molecular size and the degree of aromatic condensation and humification, resulting in a shift to less complicated structures of WEOC.

1. Introduction

Soil organic matter is quite important for its impact on the chemical and biological processes that control the resilience, productivity and sustainability of the forest ecosystems. Excessive mineralization of SOM may result in the degeneration of soil quality and the release of large amounts of greenhouse gases (Duarte et al., 2013). The mechanisms regulating the decomposition of SOM, especially the relationships between SOM mineralization and its chemical characters have received increasing attention (Breulmann et al., 2014; Pausas et al., 2008).

The decomposition rate of SOM is assumed to be influenced by its quality, as defined by the chemical composition and relative proportions of its constitutive organic compounds. The quality of SOM chemically depends on the distribution between labile and recalcitrant pools, which can be determined by the acid hydrolysis procedure (Olk and Gregorich, 2006). The labile pool with a small size can be easily mineralized while the recalcitrant fraction with a large size has a lower turnover rate. Pelz et al. (2005) studied the isotopic composition of several SOM fractions and found that the nonhydrolyzable residues showed the lowest penetration of recent C. Despite an important indicator of SOM quality, N availability has received less attention in comparison to dynamics of soil organic C. The reduction in respiration rate with soil depth was previously reported, and N increased its recalcitrance with soil depth while no

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obvious difference was detected in C quality through the soil profile (Rovira et al., 2007). Higher N mineralization was also found to coincide with higher C mineralization in agricultural soils with different fertilizer practices (Franzluebbers, 1999), suggesting that N limitation may be a restraint to SOM decomposition.

Dissolved organic matter (DOM), another fraction worthy of special attention, is one of the most biologically active due to its solubility and liability. Incubation studies in the laboratory showed that 4–93% of soil-derived DOM can be depleted (Filep and Rékási, 2011; Kalbitz et al., 2003a,b). DOM is a mixture containing low molecular weight substances and organic components with complex molecules, therefore intrinsic DOM properties may influence its biodegradation (Cioce and Peterson, 2015). UV-Vis and fluorescence spectroscopy analysis indicated that DOM rich in aromatic structures and complex molecules but poor in sugars was less decomposed (Kalbitz et al., 2003a,b). Saadi et al. (2006) reported that dissolved sugars and amino acids were preferentially utilized by microorganisms. Evaluation of changes in the composition of DOM during degradation could offer deeper insights into mechanisms that control DOM biodegradation. The synthesis of microbial extracellular metabolites and the accumulation of microbial autolysis compounds during SOM turnover may lead to the decrease in aromaticity of DOM (Malcolm, 1990). However, an increase in aromaticity of DOM after the incubation of DOM solutions was also reported (Kalbitz et al., 2003a,b). Additionally, another major determinant of SOM turnover is soil enzymes which are closely related to the quantity and quality of SOM (Baldrian et al., 2008). Enriched SOM coincided with higher enzyme activities, such as β-glucosidase, which facilitated SOM turnover (Lakhdar et al., 2011).

However, a majority of studies on SOM decomposition focus on topsoil in forest floor or agricultural land under different management conditions (Rovira et al., 2007; Haynes, 2005), and the mechanisms controlling the decomposability of SOM in subtropical forest soils with a continuous profile remain less explored. For these reasons, we conducted a soil incubation experiment under laboratory conditions, covering different soil types and horizons to investigate how inherent characters of SOM influence its turnover and what may happen to the SOM quality after incubation. We hypothesized that (I) the availability of N may be more crucial to that of C in SOM mineralization; (II) compared with topsoil, lower SOM mineralization rate in the subsurface horizons may be due to DOM in the deeper layers with higher degree of aromatic condensation and humification; (III) DOM may be less humified after incubation.

2. Materials and methods

2.1. Site description and soil sampling

The study site was in the subtropical forest landscape (29°19′–29°26′ N, 114° 27′–114°43′ E) from central China, with a mean annual precipitation of 1681 mm and a relative humidity of 80%. Samples were collected along the altitude gradient (600, 1200 and 1500 m a.s.l.) for their differences in soil types which include yellow red soil, yellow brown soil and meadow soil. Soils were classified according to the Soil Taxonomy Classification (USDA). The geological substrates were granite and gneiss, whereas the main vegetation in the three sites varied from bamboo (*Phyllostachys pubescens* J.Houz.) to pine (*Pinus taiwanensis* Hayata) and herbage (*Imperata cylindrica* L.Beauv.). The details of the study sites are presented in Table 1. Samples of each site were collected from the whole soil profile (0–60 cm), sieved to 2 mm and stored at 4 °C for analyses of organic carbon mineralization, microbial biomass C (MBC) and WEOC. The remaining samples were air dried and stored for further use.

2.2. Soil incubation and kinetic models

Previous to the incubations, three replicates of 100 g dry-equivalent soils were placed in 1000 cm^3 flasks with lids that were perforated to allow gas diffusion. Soils were adjusted to 60% of water holding capacity and incubated at 25 °C in the dark for 5 days to activate soil microorganisms. After this, samples were incubated in the same condition for 90 days. Moisture contents were maintained by weighing each sample and spraying distilled water to compensate for any water loss. Caps of the bottles were opened to replenish the oxygen supply except for regular CO_2 collection (Rey et al., 2008). Head-space gas was analyzed for CO_2 concentration using gas chromatography (Agilent, G7890A) at 13 time intervals. The potential C mineralization rate was calculated and expressed as mg of CO_2-C kg^{-1} of dry soil.

Carbon mineralization kinetics was fitted with two models. The first plus zero order model separates the mineralizable organic C into fast and slow pools (Ameloot et al., 2014). This model assumes an initial size of the easily mineralizable C pool according to first-order kinetics and a more resistant fraction according to zero-order kinetics.

$$C_t = C_0(1 - \exp(-k^*t)) + h^*t \quad (1)$$

where C_t is the cumulative organic C mineralized (mg CO_2-C kg^{-1}) at time t (d); C_0 and k represent organic C content (mg CO_2-C kg^{-1}) and C mineralization rate constant (d^{-1}) of the fast pool; h stands for the C mineralization rate (mg CO_2-C kg^{-1} d^{-1}) of the slow pool.

The double exponential model (Turrión et al., 2012) also includes labile and recalcitrant pools and can be presented as:

$$C_t = C_f(1 - \exp(-k_1^*t)) + C_s(1 - \exp(-k_2^*t)) \quad (2)$$

where C_f and C_s represent organic C content (mg CO_2-C kg^{-1}) of the fast and slow pools, respectively; k_1 and k_2 are two decomposition rate constants (d^{-1}) accordingly.

2.3. SOM fractionation and labile organic carbon analysis

At the end of incubation, soils were homogenized and split into three subsets for analysis. The SOC was determined by wet oxidation with potassium dichromate, total nitrogen (TN) concentration was measured by the Kjeldahl method (Bremner, 1996; Nelson and Sommers, 1996). Furthermore, the acid hydrolysis protocol recommended by Rovira and Vallejo (2002) was adopted to determine labile Pool I, Pool II and the recalcitrant C pool. Pool I was composed of non-cellulosic polysaccharides, either of plant or microbial origin, whereas Pool II only was composed of cellulose, mainly of plant origin (Belay-Tedla et al., 2009).

The WEOC was extracted with deionized water (Carrillo-Gonzalez et al., 2013) and MBC was extracted with 0.5 *M* potassium sulfate with and without chloroform fumigation (Wagai et al., 2013). All extracts were centrifuged (10 min, 10414 g), passed through the 0.45 μm membrane filter and quantified for organic C (Vario TOC, Elementar, Germany). MBC was estimated from the increase in the sulfate-extractable C before and after fumigation using the correction factor of 0.45. To investigate the composition of WEOC, fluorescence spectra of WEOC before and after incubation were recorded using a Jasco luminescence spectrophotometer.