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Fresh carbon and nitrogen inputs alter organic carbon mineralization and microbial community in forest deep soil layers

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ABSTRACT

In terrestrial ecosystems, deep soils are a major reservoir of organic carbon (C). Improving knowledge of how deep soil organic carbon (SOC) mineralization responds to fresh C supply and nitrogen (N) availability is essential to better understand whether this C pool will react to climate change. However, little is known about the effects of C and N inputs on SOC mineralization and microbial communities in forest deep soils. To quantify the effects of C and N inputs on SOC mineralization, we apply two species of ^{13}C–labeled leaf litters and ammonium chloride solution while incubating soils collected from 60 cm to 70 cm depth in a coniferous forest in subtropical China. The soil phospholipid fatty acid (PLFA) profiles are also determined to establish the effects of C and N supply on microbial community structure, and the $\delta^{13}C$ in PLFAs is used to establish pathways of leaf litter-derived C flux among microbial communities. The addition of leaf litters stimulates deep SOC mineralization, indicating that the stability of deep SOC is attributed to a lack of fresh C input, but the addition of *Michelia macclurei* litter with higher C:P ratio has a greater positive priming effect than adding *Pinus massoniana* litter. N addition reduces the magnitude of positive priming and alters the direction of priming in soils with *P. massoniana* litter addition, suggesting that N deposition may suppress deep SOC mineralization and favor the maintenance of SOC storage. Leaf litter addition enhances the biomass of individual PLFA and increases the fungi:bacteria ratio, suggesting that microbes are limited by energy and that soil microbial community composition is modified by C inputs. N addition decreases the fungi:bacteria ratio, but increases the Gram–positive:-Gram–negative bacteria ratio. The highest ^{13}C–enrichment and distribution of litter–derived C are found in 16:0 and 18:1ω9c PLFAs, but litter species and N addition do not affect total PLFA–C and litter-derived PLFA–C. These results support the views that a lack of fresh C supply and N deposition may prevent the mineralization of SOC pool in deep layers and that the utilization of labile substrate by 16:0 and 18:1ω9c populations promotes positive SOC priming.

1. Introduction

Deep soils (below 20 cm–300 cm), as a major reservoir of organic carbon (C) in terrestrial ecosystems, store approximately 1729 Gt C, which is thrice as much as the C stored in the top 20 cm (Jobbágy and Jackson, 2000; Fontaine et al., 2007). Small changes in the processes governing deep soil organic C (SOC) cycling may result in the large emission of CO_2 and consequently have significant effects on the global C cycle. Changes in land use and in the use of drought-resistant trees with deep root systems will alter the input of fresh C to deep soils (Jobbágy and Jackson, 2000; Lal, 2004), and an increase in nitrogen (N) deposition may alter N availability in soils (Liu et al., 2013). This condition likely changes SOC mineralization and stimulates the loss of deep SOC pool (Fontaine et al., 2007). Improving knowledge of how deep SOC mineralization responds to fresh C supply and N deposition is essential to better understand whether this C pool will react to climate change and increase the release of CO_2 to the atmosphere.

Several studies have recently demonstrated that deep SOC is more stable than surface SOC (Jobbágy and Jackson, 2000;

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Trumbore, 2000; Ewing et al., 2006; Schimel et al., 2011). However, the mechanisms for the stability of deep SOC remain unclear. The general explanations include chemical sorption, physical protection, and inappropriate conditions for microbes (e.g., oxygen limitation), resulting in the unavailability of C for soil microbes (Trumbore, 2000; Ewing et al., 2006). However, these factors could not explain the observations of some recent studies (Fontaine et al., 2007; Kramer and Gleixner, 2008; Schimel et al., 2011). For instance, Kramer and Gleixner (2008) found the occurrence of old C in microbial phospholipids from deep soil, indicating that deep SOC is biologically available. A new theory has recently been proposed that the stability of deep SOC is attributed to a lack of fresh C for soil microbes (Fontaine and Barot, 2005; Fontaine et al., 2007). However, whether the limitation by fresh C observed in a grassland deep soil will also be the dominant control of SOC stability in other soils (e.g., forest soils) remains unclear.

Adding fresh C or N to soil may change the turnover of SOC, referred to as the priming effect (PE) (Kuzyakov et al., 2000). This phenomenon is universal for surface soil (Fontaine et al., 2004; Wang et al., 2013a). However, whether the PE occurs in deep soils is less known, particularly in forest ecosystems, although Fontaine et al. (2007) reported that the amount of SOC-derived CO_2 increased after cellulose addition in grassland deep soils. N availability in surface soils affects the magnitude and direction of the PE (Treseder, 2008; Hartley et al., 2010; Kuzyakov, 2010; Liu and Greaver, 2010; Garcia-Pausas and Paterson, 2011; Zhang and Wang, 2012), but whether these observations hold in deep soils is unclear. By contrast, in surface soils, no consistent results are available on the effects of N availability on the PE (Hartley et al., 2010; Zhang and Wang, 2012).

Microorganisms regulate C and nutrient cycling through SOC and litter decomposition. Several studies have used ^{13}C isotopic technology to trace the C movement from ^{13}C-labeled substrates into microbial communities in surface soils (Phillips et al., 2002; Waldrop and Firestone, 2004; Williams et al., 2006; Moore-Kucera and Dick, 2008; Rubino et al., 2010; Zhang et al., 2013). These studies may help identify the types of microbes involved in SOC and litter decomposition. However, few previous studies have quantified the contribution of different microbial groups to C cycling, particularly in deep soils. Microbial groups have a preference for utilization of C with different forms of organic C (Fierer et al., 2003; Kramer and Gleixner, 2008). Therefore, if C sources change with depth, microbial groups may also be altered. Given that microbial activity has a strong impact on soil C cycling, more knowledge on microbial substrate usage and transformation of soil C is needed to understand SOC dynamics better.

In this study, we incubated deep soils from a subtropical forest with two species of ^{13}C-labeled leaf litters and inorganic N. We then measured the amount of CO_2–C respired and the corresponding $\delta^{13}C$ to test whether the supply of fresh organic C and N to deep soils stimulates SOC mineralization. We also measured soil microbial community and $\delta^{13}C$-phospholipid fatty acids (PLFAs) to trace the C flow of ^{13}C-labeled leaf litters into the microbial PLFA fractions. The objective of our study was to determine if the supply of fresh C and N can affect deep SOC stability and whether C from *Pinus massoniana* and *Michelia macclurei* litters with different C:P ratios might direct the movement of C into different groups of soil microbial community. We hypothesized that leaf litter addition would stimulate SOC mineralization and that leaf litter and N addition would modify soil microbial community structure and change ^{13}C distribution among PLFAs. To our knowledge, this study is the first to assess the effects of fresh C and N supply on PEs in deep soils and on the distribution of litter-derived C in the soil microbial community during litter decomposition in forest ecosystems. This finding represents an important step toward a deeper understanding of the litter–soil biogeochemical continuum.

2. Materials and methods

2.1. Soil and ^{13}C-labeled leaf litters

The soil used in this experiment was collected from the 60 cm–70 cm layer in a *Cunninghamia lanceolata* forest located at the Huitong National Research Station of Forest Ecosystem (26°40′N and 109°26′E) in Huitong County, Hunan Province. The soil samples were immediately taken to the laboratory and then passed through a 2 mm sieve, and roots and visible residues were removed manually. Soil total C and N contents were 4.78 and 0.71 g kg^{-1}, respectively. Soil mineral N (NH_4^+–N and NO_3^-–N) was 8.6 mg kg^{-1}. Soil pH was 4.46. The sand, silt, and clay contents in soils were 8.3%, 47.2%, and 44.5%, respectively. Soil bulk density was 1.38 g cm^{-3}. A pulse-chase technique was used to label seedlings of *P. massoniana* and *M. macclurei* with $^{13}CO_2$ gas with 99.9% abundance in a growth chamber (Wang et al., 2013a). At the end of the three-month labeling period, seedlings were harvested and separated into needle, stem, and root parts. After labeling, the concentrations of C, N, P, Ca, and Mg were 477.1, 19.2, 1.51, 1.61, and 2.64 g kg^{-1} in *P. massoniana* leaf litter, and 476.7, 22.2, 0.70, 4.00, and 3.37 g kg^{-1} in *M. macclurei* leaf litter, respectively. Moreover, the $\delta^{13}C$ was 1318‰ and 2107‰ in *P. massoniana* and *M. macclurei* leaf litters, respectively.

2.2. Experimental design and soil incubation

Approximately 6.0 kg collected soils was pre-incubated for 5 d in a bucket containing a beaker with distilled water to avoid desiccation, and a beaker with sodium hydroxide (NaOH) solution was also used to trap the evolved CO_2. The experiment was set up to have six treatments with three replicates. The treatments included soil without addition (Control, CT), soil with N addition (N), soil with *P. massoniana* (PM) or *M. macclurei* (MM) litter addition, and soil with combined addition of *P. massoniana* litter and N (PM + N) or *M. macclurei* litter and N (MM + N). For incubation, 240 g soil (dry weight) for each replicate of each treatment was placed in a 500 mL Mason jar. Then, ground ^{13}C-labeled leaf litter (1.43 g C kg^{-1} dry soil) and ammonium chloride solution were added to and mixed homogeneously with the soil according to the experimental design. Finally, soil water content was adjusted to 60% of water holding capacity by adding distilled water. The released CO_2 was measured using alkali-trapping techniques (Wang et al., 2013a) at 1, 3, 6, 12, 23, 41, 62, 83, 102, and 120 d after incubation. Briefly, a glass vial containing 20 mL of 0.2 M NaOH solution was placed in each Mason jar to trap evolved CO_2 from the soil, and the Mason jars were sealed for 1, 2, 3, 6, 11, 18, 21, 21, 19 and 18 days before removing for analysis, respectively. All the Mason jars with soil were incubated in the dark for 120 d at 28 °C. Three additional Mason jars with a beaker containing 20 mL of 0.2 M NaOH were sealed, serving as controls to account for CO_2 trapped from the air. To determine the $\delta^{13}C$ of released CO_2–C, 10 mL of NaOH solution was correspondingly collected from the glass vial containing 20 mL of NaOH solution. The remaining 10 mL of NaOH solution was used to determine the amount of released CO_2.

The CO_2 evolved from soil was measured by titration with 0.1 M HCl. The evolved CO_2 from the soil sample was calculated from the difference in the value of evolved CO_2 in the Mason jars with and without soil. The $\delta^{13}C$ of CO_2–C in NaOH solution was measured using a stable isotope–ratio mass spectrometer (Picarro G2131) with 0.1‰ analytical precision.

2.3. Partitioning sources of CO_2 and quantification of the PE

To calculate the amount of CO_2–C derived from litter and SOC decomposition under incubation, the following equation was used: