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Response of organic carbon mineralization and microbial community to leaf litter and nutrient additions in subtropical forest soils

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ABSTRACT

Microorganisms are vital in soil organic carbon (SOC) mineralization. The deposition of atmospheric nitrogen (N) and phosphorus (P), as well as leaf-litter addition, may affect SOC mineralization and microbial community structure by changing the availability of soil nutrients and carbon (C). In this study, we added leaf-litters labeled by ^{13}C (*Pinus massoniana* and *Michelia macclurei*) and nutrients (ammonium chloride and monopotassium phosphate) alone and in combination to soils collected from a coniferous forest in subtropical China. We aimed to investigate the effect of leaf-litter and nutrient addition on SOC mineralization and soil microbial community. CO_2 production was continuously measured during 120-day laboratory incubation, and CO_2 sources were partitioned using ^{13}C isotopic techniques. The addition of *P. massoniana* and *M. macclurei* leaf-litters increased SOC mineralization by 7.4% and 22.4%, respectively. N and P addition alone decreased soil respiration by 6.6% and 7.1%, respectively. Compared with P addition, N addition exerted a higher inhibitory effect on SOC mineralization induced by leaf-litter addition. Leaf-litter addition stimulated soil microbial activity and decreased the ratio of bacteria to fungi as a result of greater promotion on fungal growth. Moreover, 16:0 and 18:1ω9c phospholipid fatty acids (PLFAs) had greater amount of ^{13}C incorporation than other PLFAs, especially in nutrient-addition treatments. These results suggested that increased C input through leaf litter can stimulate SOC mineralization, whereas atmospheric N and P deposition can reduce this stimulatory effect and promote soil C storage in subtropical forests. Our results also illustrated that the use of ^{13}C-labeled leaf litter coupled with ^{13}C-PLFA profiling is a powerful tool for determining the microbial utilization of C.

1. Introduction

Carbon (C) stored in soil comprises approximately three-quarters of terrestrial C worldwide. This value is more than three times the amount of C in the atmosphere (Schlesinger and Andrews, 2000). However, the level of soil organic C (SOC) at a particular time is controlled by the balance between C input from litter and C output from SOC mineralization (Vesterdal et al., 2012). SOC mineralization is affected by microbial activities, which were controlled by the source of energy for microbes (Vanhala et al., 2008). In forest ecosystems, in addition to roots, leaf litter represents a major source of SOC inputs. Thus, leaf litter may influence SOC mineralization through the priming effect (Kuzyakov, 2010; Zhang and Wang, 2012).

A positive priming effect has been defined as a short-term increase in the turnover of SOC induced by the addition of an external organic substrate to the soil (Kuzyakov et al., 2000). The priming effect of adding plant materials or easily decomposable substances in order to simulate organic C input in natural ecosystems has been extensively studied (Hamer and Marschner, 2005; Potthast et al., 2010; Wang et al., 2013a). However, the directions of the priming effect reported in different experiments are inconsistent, showing positive priming (Fontaine et al., 2007; Zhang and Wang, 2012) and negative or no priming effect (Hamer and Marschner, 2005; Nottingham et al., 2009) induced by organic C addition. In forest ecosystems, leaf litter serves as the main source of SOC and alters the native SOC mineralization rate.

Nutrient availability may be important in explaining the tremendous differences in the extent of the priming effect observed in literature (Kuzyakov, 2010). Numerous studies have assessed the influence of nitrogen (N) availability by N addition on C mineralization. However, no general conclusion has been drawn yet, and increases (Cleveland and Townsend, 2006; Tu et al., 2013), decreases

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(Craine et al., 2007; Bradford et al., 2008; Mo et al., 2008), and no change (Thirukkumaran and Parkinson, 2000) in soil respiration after N addition have been observed. In China, the mean annual N deposition increased from 13.2 kg N ha^{-1} in the 1980s to 21.1 kg N ha^{-1} in the 2000s (Liu et al., 2013). This may increase soil N availability and consequently influence SOC mineralization and priming effect. N deposition can also aggravate phosphorus (P) limitation in plant and microbial processes, particularly in acidic soil (Vitousek et al., 2010), but this limitation may be weakened by P addition to soils. However, the results of studies through laboratory incubation on the effects of P addition on microbial activities remain controversial, showing stimulatory (Allen and Schlesinger, 2004; Bradford et al., 2008), inhibitory (Thirukkumaran and Parkinson, 2000), or no (Groffman and Fisk, 2011) effects on soil respiration. Moreover, the previous studies were mainly conducted in boreal and temperate forests, resulting in little information on response of SOC mineralization to combining addition of leaf litter and nutrients in subtropical forests.

The important role of biotic factors (e.g., microbial community structure and activity) in SOC mineralization is now being recognized (Strickland et al., 2009; Garcia-Pausas and Paterson, 2011; Tavi et al., 2013). Several experimental studies have demonstrated that external substrate addition can alter soil microbial community structure (de Vries et al., 2006; Moore-Kucera and Dick, 2008; Denef et al., 2009; Dungait et al., 2011), which may consequently affect the magnitude and direction of SOC mineralization and change C flow within the soil microbial community (Williams et al., 2006; Garcia-Pausas and Paterson, 2011; Yao et al., 2012). Recently, some studies have used ^{13}C stable isotopic technology to successfully trace C flow from ^{13}C-labeled substrates into soil microbial community in agricultural and grassland soils (Dungait et al., 2011; Yao et al., 2012; Zhang et al., 2013). These studies have provided important information on groups of microbes utilizing a given substrate through GC-C-IRMS analyses of individual phospholipid fatty acids (PLFAs). However, information on this issue is limited in forest ecosystems, particularly in subtropics, although some studies have been conducted in temperate forests (Moore-Kucera and Dick, 2008; Rubino et al., 2010).

In the present study, we used the ^{13}C-labeled *Pinus massoniana* (coniferous tree species) and *Michelia macclurei* (broadleaved tree species) leaf litter to investigate the response of native SOC mineralization and soil microbial community to the addition of leaf-litter, N, and P, alone and in combination, in a subtropical forest soil. We hypothesized that (1) an increase in native SOC mineralization occurs after leaf-litter addition, and this increase is greater in soils with leaf-litter addition at a high C:P ratio (*M. macclurei*); (2) N and P addition decreases the priming effect induced by leaf-litter addition; and (3) N and P addition changes the impact of leaf litter supply on the microbial community structure and the ^{13}C incorporation into different groups of microorganisms. This study aimed to investigate the effects of leaf-litter and nutrient addition on the mineralization of native SOC and how soil microbial community composition and ^{13}C flow within soil community respond to litter and nutrient addition. To the best of our knowledge, this study is the first quantitative research on the effects of N and P addition on the priming effect and ^{13}C flow with soil microbial community in subtropical forests, which favors to better understand effect of N and P deposition on the C cycle in forest ecosystems.

2. Materials and methods

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

The soil used in this experiment was collected at a layer of 0 cm–10 cm from a coniferous forest located at the Huitong National Research Station of Forest Ecosystem in Huitong county, Hunan province (latitude 26°40′ to 27°9′ N and longitude 109°26′ to 110°08′ E). The soil samples were taken to the laboratory, passed through a 2 mm sieve and root and other residues in soil samples were removed by hand. The total C and N concentrations in the soil samples were 17.5 g kg^{-1} and 1.45 g kg^{-1}, respectively. The soil mineral N (NH_4^+–N and NO_3^-–N) concentration was 11.2 mg·kg^{-1}. In the experiment, the used soil had low available P with 1.04 mg kg^{-1}, limiting the growth of plants and microbes. Soil pH was 4.35. The sand, silt, and clay contents in the soil were 11.2%, 46.1%, and 42.7%. The soil bulk density was 1.26 g cm^{-3}. A pulse-chase technique was used to label *P. massoniana* and *M. macclurei* seedlings with $^{13}CO_2$ gas with an abundance of 99.9% in a growth chamber. The seedlings were labeled with 99.9 atom % $^{13}CO_2$ at every 7 days. At the end of the three-month labeling period, the seedlings were harvested, rinsed with deionized water, dried, and separated into leaves, stems, and roots. These components had different $\delta^{13}C$. In this experiment, leaves are used and their chemical properties are shown in Table 1.

2.2. Experimental design and soil incubation

The experiment was set up to have nine treatments with three replicates. The details are provided in Table 2. In this experiment, *P. massoniana* and *M. macclurei* leaf litters had similar C concentration. *P. massoniana* litter had higher P concentration and lower C:P ratio. We expected that the difference in chemical quality of the two litters would result in different responses of SOC mineralization and soil microbial community.

For incubation, 240 g of soil (dry weight) for each replicate of each treatment was placed in a 500 mL Mason jar. The ^{13}C-labeled leaf-litter, ammonium chloride solution, and potassium dihydrogen phosphate solution were then added to the soil according to the experimental design. The grounded leaf-litter was evenly incorporated with the soil to make a homogeneous mixture with the soil. Finally, the water content of the soil in each treatment was adjusted to 60% of water holding capacity by adding deionized water. A glass vial containing 20 mL of 0.1 M NaOH solution was placed in each Mason jar to trap evolved CO_2 from the soil, and the Mason jars were then sealed. 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 0.1 M NaOH were sealed. These jars served as controls to account for the CO_2 trapped from the air. New beakers containing the 20 Ml NaOH solution were added on each collection date. The collected NaOH solution in each beaker was immediately transferred to sample flask and sealed with lid. Beakers with NaOH solution trapped CO_2 were collected at 1, 3, 6, 12, 23, 41, 62, 83, 102, and 120 d after incubation. To determine the $\delta^{13}C$ of released CO_2, 10 mL of NaOH solution was collected from the glass vial containing 20 mL of NaOH solution on each collection date. The remaining 10 mL of NaOH solution was used to determine the amount of released CO_2. The released CO_2 was measured using alkali-trapping techniques.

2.3. Soil chemical analysis

The concentrations of C and N in the soil and leaf litter samples were determined using an element analyzer. To measure the P concentration, 0.2 g of litter samples were digested in 10 mL of triacid mixture (nitric, perchloric, and sulfuric acids; 5:1:1), and

Table 1
Chemical properties of the labeled *Pinus massoniana* (PM) and *Michelia macclurei* (MM) leaf-litter used in the experiment.

	C (g kg^{-1})	N (g kg^{-1})	P (g kg^{-1})	C/N	C/P	Ca (g kg^{-1})	Mg (g kg^{-1})	$\delta^{13}C$ (‰)
PM	477.1	19.2	1.51	24.9	316	1.61	2.64	1318
MM	476.7	22.2	0.70	21.5	681	4.0	3.37	2107