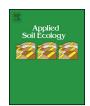
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Fate of Chinese-fir litter during decomposition as a result of inorganic N additions



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ABSTRACT

We conducted a controlled experiment to evaluate Chinese-fir litter decomposition and its response to the addition of inorganic N. Litter-derived CO_2 , microbial biomass carbon (MBC), and dissolved organic carbon (DOC) were monitored during an 87-d incubation of a mixed soil-litter substrate using the ^{13}C tracer technique. Litter C was mostly converted to CO_2 (47.4% of original mass), followed by MBC (3.6%), and DOC (1.0%), with 48% remaining unaltered in the soil. The litter decomposition rate significantly increased with the addition of inorganic N, although the effect depended on whether N was added as NH_4^+ or NO_3^- . Soil-derived CO_2 , MBC, and DOC also increased following the combined addition of litter and N. The results showed that only a small percentage of litter C was retained as MBC or DOC and that the conversion rate depended, in part, on the form of inorganic N added to the Chinese-fir plantation soil.

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1. Introduction

In forest ecosystems, plant litter plays an important role in maintaining soil fertility by regulating the nutrient cycle during decomposition (Fioretto et al., 2003; Pandey et al., 2007). Therefore, understanding the decomposition dynamics of plant litter and its controlling factors is critical. Consequently, numerous studies on plant litter decomposition have been conducted using the litterbag technique. These studies confirmed that plant litter decomposition is controlled by initial litter chemistry (Aber and Melillo, 1982; Melillo et al., 1982), external nutrients (Hobbie, 2000; Magill and Aber, 1998), and climate (Berg and McClaugherty, 2008). However, the fate of plant litter during decomposition has not been well evaluated because the decomposition rate is simply expressed as mass loss in the litterbag technique. Most plant litter C is released as CO₂ into the atmosphere (Chapin et al., 2002). However, the proportions of the CO₂ produced and other fates of plant litter-C (such as MBC and DOC) compared with the total mass loss are unknown (Troyer et al., 2011).

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Microorganisms play a critical role in the carbon transformation in forest soil (Nottingham et al., 2009; Hu et al., 2011), and the microbial biomass serves as an important pool for plant litter during decomposition (Garcia and Rice, 1994). In agricultural soils with different histories of maize cropping, John et al. (2003) found that after 40 years, about 23–46% of the microbial biomass carbon (MBC) is derived from maize carbon. The newly formed MBC and the altered microbial community composition in response to plant litter accelerates the decomposition of plant litter and the native soil organic carbon, which potentially influences the transformation of soil carbon and nitrogen in forest ecosystems. Thus, understanding the proportion of plant litter C that is allocated as MBC is important for determining the fate of plant litter and because of its potential effect on soil ecological processes.

Dissolved organic carbon (DOC) is a small fraction of soil organic matter (Troyer et al., 2011); however, its importance in microbial metabolism and ecological processes could not be neglected because of its high bioavailability (Qualls and Haines, 1992). The DOC concentration in soil is controlled by the interaction of its production and degradation (Kalbitz et al., 2000). Different methods, including isotopic techniques (Troyer et al., 2011; Hagedom et al., 2004), have been used to identify the sources of DOC in soil. The DOC in the soil originates from recent plant litter, roots and native soil organic matter (Yano et al., 2005; Fröberg et al., 2003). Thus, determining the proportion of plant litter C allocated into DOC during decomposition is very important.

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As a main nutritional limiting factor in most ecosystems (Koerselman and Meuleman, 1996), the effects of nitrogen on ecological process, especially on plant litter decomposition (Magill and Aber, 2000) and soil respiration (Ramirez et al., 2010), were widely studied. A meta-analysis based on 24 individual studies (Knorr et al., 2005) showed that nitrogen positively affects plant litter decomposition during the early stage of decomposition (<24 months), and negatively affects it in the latter stages (>24 months). In addition, the effect of nitrogen is related to plant litter quality, the nitrogen addition rates, and form of the nitrogen (Knorr et al., 2005). Considering the effects of the interaction of the different factors was not calculated in the meta-analysis, predicting the effect of nitrogen and the changes in the proportion of the different intermediates on plant litter decomposition (Liao et al., 2000).

Chinese-fir (*Cunninghamia lanceolata*) is an important coniferous timber species that has been extensively grown in Southern China for more than 1000 years (Chen and Wang, 2004). Chinese-fir litter is characterized by high C:N ratio and lignin concentration (Yang et al., 2004), thus its decomposition may be limited by a lack of inorganic N (Liao et al., 2000). Unsustainable forest management practices, such as successive planting, short rotation times, whole-tree harvesting, and poor site preparation, had significantly decreased soil fertility, e.g. soil nitrogen availability (Chen et al., 1990). Therefore, the high C:N ratio of Chinese-fir litter was supposed to interact with low nitrogen availability in soil to hinder the carbon cycling, such as litter decomposition and SOC mineralization, in Chinese-fir plantations (Liao et al., 2000).

To understand better the litter and SOC decomposition in Chinese-fir plantations, we conducted a controlled experiment under laboratory conditions using ^{13}C -labeled Chinese-fir litter and the addition of inorganic N. The specific objectives of the study are as follows: (1) to quantify the different fates (CO $_2$ production, MBC and DOC) of Chinese-fir litter during decomposition; and (2) to examine the effects of NH $_4$ -N and NO $_3$ -N addition on the fate of Chinese-fir litter during decomposition. Given that microbes preferentially take up inorganic nitrogen as NH $_4$ -N (Lavelle and Spain, 2003) and cost more energy to uptake NO $_3$ -N, we hypothesize that the addition of NH $_4$ -N accelerates Chinese-fir litter decomposition compared with the addition of NO $_3$ -N.

2. Materials and methods

2.1. Soil and labeled litter

The soil used was collected from a 0 cm to 10 cm layer in a second-generation Chinese-fir plantation located at the Huitong Experimental Station of Forest Ecology, Chinese Academy of Sciences (109°36′E, 26°51′N), Hunan Province, China. The soil was classified as clay loam (25.12% sand, 45.53% silt, 29.35% clay) with a pH of 4.3. The soil bulk density was 1.4 g cm $^{-3}$. The soil C and N concentrations were 12.61 g kg $^{-1}$ and 1.18 g kg $^{-1}$, respectively, which corresponds to a C:N ratio of 10.7. The δ^{13} C of soil organic carbon was -27.8%.

Uniformly 13 C-labeled Chinese-fir leaves were obtained using 13 C-labeled carbon dioxide (13 CO₂)-C in a growth chamber for 3 months. The total C, N, P and dissolved organic C concentration of the Chinese-fir leaves were 465.1 g kg $^{-1}$, 8.11 g kg $^{-1}$, 0.99 g kg $^{-1}$ and 93 g kg $^{-1}$. The δ^{13} C of the Chinese-fir leaf was 243%.

2.2. Experimental design

The soil samples were taken to the laboratory and treated as follows. Approximately 12 kg of soil from the second-generation Chinese-fir plantation was passed through a 2 mm sieve and adjusted to water-holding capacity (WHC) of 40%. The soil was

pre-incubated for 15 d in a bucket containing a beaker with 100 mL of distilled $\rm H_2O$ to avoid desiccation, and a beaker with 100 mL of 0.1 mol $\rm L^{-1}$ sodium hydroxide (NaOH) solution to trap evolved $\rm CO_2$. The experiment included 2 litter additions (CK_F = no fir addition, FIR = 1.5 mg litter-C g⁻¹ soil) and 3 nitrogen additions (CK_N = no N addition, +NH₄ = 100 mg NH₄⁺-N kg⁻¹ soil, +NO₃ = 100 mg NO₃⁻-N kg⁻¹ soil) arranged in a 2 × 3 factorial design.

For the incubation, 15 replicates of 145 g of dry soil (conversion according to water content) were placed in 1000 mL incubation vessels for each treatment. Then, 465 mg of ¹³C-labeled Chinese-fir litter, 7.5 mL of 0.24 mol L^{-1} ammonium sulfate solution, and 15 mL of 0.24 mol L⁻¹ potassium nitrate solution were added to the soil. Lastly, the water content of each treatment was adjusted by adding distilled water to maintain a WHC of 60%, and the soil was thoroughly mixed. The 15 replicates of each treatment were divided into 3 groups. The first group included three replicates, and was used to measure the CO₂ released from soil. The jars were incubated in the dark for 87 d at 16.5 °C (the average annual temperature). Three additional jars with a beaker containing 10 mL of distilled H₂O and a beaker containing 10 mL of 0.1 mol L⁻¹ NaOH solution were sealed served as the controls to account the CO₂ trapped in the air. To collect CO₂ from respiration, a glass vial containing 10 mL of 0.1 mol L⁻¹ NaOH solution was placed in the incubation jars and the jars were sealed. The NaOH traps were replaced periodically before saturation. The CO₂ released from the soil was measured daily for the first 15 d. The second group contained three replicates and it was used to analyze ¹³C abundance. Soils were placed in 1000 mL glass jars containing a vessel with 10 mL of distilled H₂O and stored in the dark. After 15, 47, and 87 d, the gas in each treatment was sampled with syringe for ¹³C abundance analysis. After sampling, all flasks were opened, aired for 10 min to avoid completely anaerobic conditions, sealed, and then stored in the dark. The third group contained six replicates. Those jars also contained a vessel with 10 mL of distilled H₂O to keep soils moist, then opened and aired periodically to avoid anaerobic conditions. After 15, and 87 d, three jars were randomly selected from each treatment and were opened. The soil samples were then used to analyze inorganic N, soil microbial biomass carbon, and dissolved organic carbon.

2.3. Soil chemical analysis

The CO_2 trapped in NaOH was titrated with $0.05 \, \text{mol} \, \text{L}^{-1}$ HCl (Hu et al., 2006). Inorganic N was determined from $2 \, \text{mol} \, \text{L}^{-1}$ KCl extractions (1:5 soil solution ratio). NH_4^+ -N was colorimetrically determined using a spectrophotometer, and NO_3^- -N was determined by ion chromatography (Liu et al., 1996).

Soil microbial biomass C was determined in duplicate using the chloroform fumigation – K_2SO_4 extraction method, as described by Vance et al. (1987). Briefly, 25 g of soil was fumigated 24 h at 25 °C with 10 ml ethanol-free cholorform and extracted with 100 mL of 0.5 mol L⁻¹ K_2SO_4 for 1 h. The organic carbon in the extracting agent was measured via dichromate oxidation (Vance et al., 1987). The microbial biomass C was calculated using the following formula: $C_{MBC} = EC/Kc$, where EC is the difference in organic C concentration of the fumigated and unfumigated extracting agent and Kc = 0.38 (Lu, 1999). The organic C concentration in unfumigated extracting agent was used to express the DOC concentration (Dijkstra et al., 2006).

The remaining parallel extracts were combined and dried at $60\,^{\circ}\text{C}$ in a ventilated oven, and the dried K_2SO_4 were ground and used to determine the ^{13}C abundance using stable isotope-ratio mass spectrometers (DELTA <code>plus</code> XP). The analytical precision of the $\delta^{13}\text{C}$ measurements was 0.15‰.

The δ^{13} C value in unfumigated extracting agent was used to express the 13 C abundance of the DOC, whereas the 13 C abundance of the MBC was estimated using the following equation:

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