



# Does short-term litter input manipulation affect soil respiration and its carbon-isotopic signature in a coniferous forest ecosystem of central China?



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## ARTICLE INFO

### Article history:

Received 2 December 2016  
Received in revised form 20 January 2017  
Accepted 21 January 2017  
Available online 9 February 2017

### Keywords:

Carbon isotope  
Litter inputs manipulation  
Microbial community structure  
Soil respiration  
Substrate availability

## ABSTRACT

Global change greatly alters the quality and quantity of plant litter inputs to soils and further impacts soil respiration. However, it is not fully understood how soil respiration may change with future shifts in litter input. The Detritus Input and Removal Treatment (DIRT) experiment were conducted in a coniferous forest (*Platycladus orientalis* (Linn.) Franco) ecosystem of central China to investigate the impact of above- and belowground litter input on soil respiration and the carbon-isotopic signature of soil-respired CO<sub>2</sub>. Short-term litter input manipulation significantly affected soil respiration. Based on annual flux values, soil respiration decreased by 31.9%, 20.5% and 37.2% in treatments with no litter (NL), no roots (NR) and no roots and no litter (NRNL), respectively, compared to the control (CK) treatment. Conversely, the double litter (DL) treatment increased soil respiration by 9.1% compared to the CK treatment. The recalitrance index of carbon (RIC) and the relative abundance of fungi increased under NL, NR and NRNL treatment compared to the CK treatment. The carbon-isotopic signature of soil-respired CO<sub>2</sub> was enriched under NRNL treatment and was slightly depleted under DL treatment compared to the CK treatment. The soil respiration rate and its carbon-isotopic signature exhibited similar seasonal patterns among treatments with higher soil respiration rates and lower δ<sup>13</sup>C values of soil-respired CO<sub>2</sub> in the summer compared with other seasons. Basal soil respiration was positively related to labile C and microbial biomass and negatively related to RIC and the fungi-to-bacteria (F:B) ratio, whereas the δ<sup>13</sup>C value of soil-respired CO<sub>2</sub> was negatively correlated with soil temperature and water content. Our results suggest that short-term litter input manipulation can affect soil respiration by altering substrate availability and microbial community structure and can impact the carbon-isotopic signature of soil-respired CO<sub>2</sub> possibly due to changes in the components of soil respiration and soil microclimate.

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## 1. Introduction

Soil respiration, the largest source of carbon (C) flux from terrestrial ecosystems to the atmosphere, is very important in regulating climate change, as well as the global ecosystem C balance (Luo and Zhou, 2006; Bond-Lamberty and Thomson, 2010). Soil respiration can be greatly affected by abiotic and biotic factors, such as soil temperature and moisture, the microbial community and the substrate supply; thus, it is very susceptible to global change (Bond-Lamberty and Thomson, 2010; Zhou et al., 2016; Sawada et al., 2016). Even a small change in soil respiration can have a large effect on atmospheric CO<sub>2</sub> with potential feedbacks to climate change (Heimann and Reichstein, 2008). Although in

recent decades numerous studies have related soil respiration to global change (e.g., van Groenigen et al., 2011; Giardina et al., 2014; Xue et al., 2016), uncertainty remains about how abiotic (e.g., soil temperature and moisture) and biotic (e.g., the microbial community and substrate supply) factors interactively affect soil respiration under controlled field conditions.

Soil respiration is largely influenced by substrate supply derived from litter and roots and by soil organic matter (SOM) (Sayer et al., 2011; van Groenigen et al., 2014). Alterations in the quality and quantity of plant litter inputs to soils have profound influences on SOM dynamics and soil respiration (Schlesinger et al., 2015). Increased inputs of fresh organic matter may result in a “priming effect,” which can in turn enhance the soil’s respiration (Fontaine et al., 2007; Kuzyakov, 2010). However, litter removal or root exclusion not only directly reduces the decomposition of litterfall or root respiration but also indirectly affects the biological process

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in the underlying soil (Lajtha et al., 2014; Fekete et al., 2014). For instance, litter removal or root exclusion may reduce SOM decomposition by reducing the substrates available for microbes (Leff et al., 2012; Philippot et al., 2013). Meanwhile, the  $\delta^{13}\text{C}$  values of soil-respired  $\text{CO}_2$  are also affected by the change in litter input and substrate availability (Rousk and Frey, 2015). When a plant's litter input changes, the  $\delta^{13}\text{C}$  value of the substrates also changes (Yang et al., 2015). Thus, the  $\delta^{13}\text{C}$  value of microbial respiration may also deviate somewhat due to the use of different substrates (Bowling et al., 2008; Rousk and Frey, 2015).

Alterations in detrital inputs can also affect soil microclimatic conditions, especially soil temperature and moisture, both of which influence soil respiration and its carbon-isotopic signature (Sayer, 2006; Phillips et al., 2010; Fekete et al., 2014). For instance, litter addition enhances the thickness of the organic layer and can reduce the effects of extremes as well as moderate minimum and maximum soil temperatures (Fekete et al., 2016), which can benefit the microbial reparation processes of litter and SOM. Meanwhile, surface litter layer regulates soil water content by reducing evaporation from mineral soil while absorbing a fraction of the precipitation (Sayer, 2006), and root exclusion can increase soil moisture due to loss of transpiration (Fekete et al., 2016). Some studies have reported that the  $\delta^{13}\text{C}$  value of soil-respired  $\text{CO}_2$  is enriched during dry conditions and depleted when the water level is high (Phillips et al., 2010). Soil moisture may affect the production of ectomycorrhizal extramatrical mycelium (Ekblad et al., 2005), and the ectomycorrhizal fungi are approximately 2‰ enriched relative to their plant hosts (Ekblad et al., 2016), ultimately affecting the  $\delta^{13}\text{C}$  value of the substrates.

The Detritus Input and Removal Treatment (DIRT) experiment was established to examine feedbacks between plants, microbes, and SOM through long-term manipulation of above- and below-ground litter inputs in forest ecosystems (Bowden et al., 1993; Nadelhoffer et al., 2004; Veres et al., 2015). In addition, this experiment provides a unique opportunity to understand the influence of abiotic (e.g., soil temperature and moisture) and biotic (e.g., microbial community and substrate supply) factors on soil organic C dynamics. Here, our overall objective was to assess the effect of different litter input manipulation on soil respiration and its carbon-isotopic signature in a coniferous forest (*Platycladus orientalis* (Linn.) Franco) ecosystem in the Northern Subtropics of Central China. To achieve this goal, a DIRT experiment in coniferous forest ecosystem was established to test the two following hypotheses. First, we hypothesized that the increase in soil respiration induced by litter addition would outpace the decrease in soil respiration by litter removal due to priming effect. Second, we hypothesized that the carbon-isotopic signature of soil-respired  $\text{CO}_2$  would be more enriched in litter removal or in no input treatment and more depleted in litter addition treatment due to corresponding changes of substrates and soil microclimate under these treatments.

## 2. Materials and methods

### 2.1. Study site

This study was conducted at the Wulongchi Experiment Station (32°45'N, 111°13'E; 280–400 m a.s.l.) in the Danjiangkou Reservoir area. The climate in this area belongs to the subtropical monsoon of the north subtropical zone. The mean annual temperature is 15.7 °C, with monthly averages of 27.3 °C in July and 4.2 °C in January. The annual precipitation is 749.3 mm, of which 70–80% occurs between April and October. The soil is yellow-brown with 11% sand, 41% silt, and 48% clay in the top 30 cm. Approximately 20 years ago, following a reorganization of land use, a large

uncultivated area was converted to a woodland plantation with coniferous plants (*Platycladus orientalis* (Linn.) Franco).

### 2.2. Experimental design and soil sampling

The DIRT experimental plots were established in November, 2014. We randomly selected six 10 m × 10 m study sites. In each study site, five 1 m × 1 m plots free of trees and saplings were randomly selected, and above- and belowground plant C inputs were manipulated in a number of ways. The treatments included control (CK, normal annual aboveground litter inputs), double litter (DL, twice the litter inputs of the control plots), no litter (NL, annual aboveground litter inputs excluded), no roots (NR, plots trenched and root regrowth into plots prevented), and no input (NRNL, plots trenched and annual aboveground litter excluded). The surface solar radiation was approximately the same in all treatments, as the distribution and slope of land was not greatly different (average slope of 5°) and the site faced south. Therefore, the climatic effect was similar in all plots. Moreover, the plots were established at random, thus reducing the effects of incidental minor differences.

In September 2015, three soil cores (diameter = 5 cm) from each plot were collected from the top layer of soil (0–10 cm) after removing the litter and organic horizon. The cores were combined to yield one composite sample per plot. All soil samples were immediately sieved through a 2-mm mesh. A small subsample was removed from each soil sample and stored at –80 °C for Phospholipid Fatty Acids (PLFAs) extraction. Another subsample was removed and stored at 4 °C for incubation. The remaining soil samples were air-dried and stored in airtight plastic bags until analysis.

### 2.3. Soil respiration and its carbon-isotopic signature

Fluxes of  $\text{CO}_2$  were measured twelve times from January 2015 to December 2015 using static chambers and the gas chromatography technique. Static chambers were inserted into each debris input and removal treatment. The static chamber comprised two parts: (1) a cylindrical bottom pedestal (diameter = 0.25 m, height = 0.2 m) that was permanently inserted in the soil and (2) a removable cover (diameter = 0.25 m, height = 0.3 m) that was placed on top to ensure the chamber was enclosed during sampling and removed afterwards. On the top wall of each chamber cover, a battery-operated fan of 10-cm diameter was installed to mix the air in the chamber while the sample was collected. Generally, once the chambers were closed, 140-ml air samples were collected every 10 min using 100-ml plastic syringes. The samples were then injected into 150-ml pre-evacuated gas bags over half an hour. Simultaneously, the air temperature of each experimental plot was measured with a mercurial thermometer. Soil temperature and moisture were measured outside each chamber with a portable instrument that measured soil temperature and moisture (SIN-TH8, SinoMeasure, China). The concentration and  $\delta^{13}\text{C}$  value of  $\text{CO}_2$  were analyzed with a  $\text{CO}_2$  Isotope Analyzer (912-0003, LGR, America).

The  $\text{CO}_2$  fluxes were calculated using linear model regression analysis of the change in gas concentration in the chambers with time over a 30-min period with an average chamber temperature (Metcalf et al., 2007):

$$F = \Delta C / \Delta t * 273 / (273 + T) * 44 / 22.4 * V / A \quad (1)$$

where F is the  $\text{CO}_2$  flux ( $\text{mg m}^{-2} \text{h}^{-1}$ ), T is the air temperature inside chambers, 44 is the molecular weight of  $\text{CO}_2$ , 22.4 is the molar volume of an ideal gas at standard temperature and pressure ( $1 \text{ mol}^{-1}$ ), V is the chamber volume ( $\text{m}^3$ ) and A is the chamber area

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