



Root litter inputs exert greater influence over soil C than does aboveground litter in a subtropical natural forest

Xiaofei Liu · Teng-Chiu Lin · Matthew A. Vadeboncoeur · Zhijie Yang · Shidong Chen · Decheng Xiong · Chao Xu · Yiqing Li · Yusheng Yang

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Abstract

Aims Changes in plant net primary production due to climate change can influence aboveground and belowground litter inputs to forest soils. We aim to examine the effects of such changes on soil carbon in subtropical forest ecosystems where these effects have not been thoroughly investigated.

Methods We manipulated aboveground litter inputs and excluded roots in a factorial design, and measured the effects of each treatment and their interactions on soil carbon (C) and soil microbial community structure.

Results After only 3 years of treatment, aboveground litter addition and root exclusion respectively caused 9% and 21% reductions in soil C concentration in the 0–

10 cm soil, likely through different mechanisms. The reduction of soil C with aboveground litter addition was attributed to a priming effect, while reduced root-derived C inputs were likely the cause of the C reduction associated with root exclusion. PLFA analysis showed that both aboveground and belowground litter manipulations reduced Gram-positive bacteria biomass (by 30%–58%) compare to the control, but only root exclusion significantly reduced the actinobacteria biomarkers (by 46%–58%). Fungi, arbuscular mycorrhizal fungi, and ratios of Gram-negative to Gram-positive bacteria and bacteria to fungi did not differ among treatments. **Conclusion** Our results show that root-derived C inputs exert a stronger control on soil C concentrations and microbial community structures than aboveground litter does in subtropical natural forest soils. Our study also highlights that that both increases in aboveground litter and decreases in belowground C input to soil can lead to reduced soil C.

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X. Liu · Z. Yang · S. Chen · D. Xiong · C. Xu · Y. Li · Y. Yang

Key Laboratory for Subtropical Mountain Ecology, School of Geographical Science, Fujian Normal University, Fuzhou 350007, China

T.-C. Lin (✉)

Department of Life Science, National Taiwan Normal University, No. 88, Section 4, DingChow Road, Taipei 11677, Taiwan
e-mail: tclin@ntnu.edu.tw

M. A. Vadeboncoeur

Earth Systems Research Center, University of New Hampshire, 8 College Road, Durham, NH 03824, USA

Y. Li

College of Agriculture, Forestry and Natural Resource Management, University of Hawaii, Hilo, HI 96720, USA

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Introduction

Globally, soils store more organic carbon (C) than vegetation and the atmosphere combined (Pan et al. 2011). Soil C storage is mediated by microbes that use net primary production (NPP) from above- and

belowground litter and soil organic matter (SOM) as sources of C. Therefore, alterations in the quality and quantity of aboveground and belowground litter inputs to soils have profound effects on soil C dynamics (Lajtha et al. 2014a, 2018; Bowden et al. 2014). Global change has the potential to affect NPP and its allocation. For instance, increases in atmospheric CO₂ concentration, nitrogen deposition and air temperature are likely to promote plant productivity and increase litter inputs to soil, via increases in canopy N content and longer growing seasons (Drake et al. 2011; Hickler et al. 2008; Keenan et al. 2014). In addition, CO₂ addition is likely to increase belowground C allocation while nitrogen deposition might decrease it (Finzi et al. 2007; Drake et al. 2011). In contrast, in drought events generally decrease productivity since suppression of photosynthesis during drought (Gatti et al. 2014; Doughty et al. 2015) but increase the proportional allocation of C belowground (Hasibeder et al. 2015).

A large number of manipulative studies have examined how changes in litter input affect forest soil C dynamics (Leff et al. 2012; Xu et al. 2013; Lajtha et al. 2014a; Liu et al. 2017; Chen and Chen 2018). Although experimental additions or removals of aboveground litter have been widely used to explore its role in soil C dynamics, belowground litter manipulations are typically limited to removal. In general, litter removal, both above- and belowground, reduces soil C storage, while the effect of aboveground litter addition on soil carbon storage is variable (Xu et al. 2013; Bowden et al. 2014; Lajtha et al. 2014a; Pisani et al. 2016; Chen and Chen 2018), ranging from decreasing (Lajtha et al. 2014a; Pisani et al. 2016), no effects (Bowden et al. 2014) to increasing (Leff et al. 2012; Lajtha et al. 2014b). A meta-analysis showed that subtropical forest soils are more responsive to changes in litter production than other ecosystems (Xu et al. 2013).

Counter-intuitive net losses of soil C following increases in aboveground litter inputs have been attributed to positive priming effects (i.e., labile carbon in fresh litter provides energy to micro-organisms, which increase activity and in turn stimulate the decomposition of existing soil organic carbon). However, some studies have shown that priming occurs only when roots are present, possibly due to the important role of root exudates on soil microbe activities (Subke et al. 2004; Schaefer et al. 2009). Root exclusion is commonly applied in litter manipulation studies to separate autotrophic and heterotrophic respiration. However, few in-

situ litter manipulation studies have directly investigated the effects of root exclusion on priming. In addition, few studies of litter manipulation have been conducted in tropical and subtropical plantation forests despite their important role in the global C cycle (Cusack et al. 2018; Liu et al. 2017; Wang et al. 2017). Due to differences in SOC storage, forest floor properties, litterfall and fine root dynamics between different regions and between natural and plantation forests (Yang et al. 2009), the response of soil carbon concentration to aboveground and belowground litter manipulation may be different between these forests.

Soil microbes play a key role in ecosystem processes through a large number of critical biochemical processes, including the mineralization of carbon and nutrients in litter (Paul 2014). Microbial community composition and function may respond rapidly to changes in litter inputs (Brant et al. 2006a; Yarwood et al. 2013). For example, an experiment in temperate forests in Oregon reported that root exclusion increased soil actinobacteria biomass and decreased fungal biomass (Brant et al. 2006a). In contrast, root exclusion increased the biomass of bacteria, fungi and actinobacteria, but decreased the Gram-negative to Gram-positive bacteria ratio and the bacteria to fungi ratio in a subtropical Chinese fir plantation (Wang et al. 2013). Our limited knowledge of the effects of belowground C inputs on microbial community structure hinders our understanding of how climate change may affect soil C cycling as microorganisms are known to mediate critical carbon transformations in soils (Nicolardot et al. 1994; Tveit et al. 2013).

Aboveground litter removal and addition could have opposite effects on microbial structures. For instance, increasing aboveground litter inputs decreased the abundance of putative oligotrophic soil bacteria (Acidobacteria) but increased putative copiotrophic soil bacteria (Alphaproteobacteria) in a lowland tropical rain forest (Nemergut et al. 2010). A laboratory experiment also reported increases in putative copiotrophic bacteria in response to C addition (Cleveland et al. 2007). In contrast, some studies showed that microbial community structure did not differ between the aboveground litter addition and the control plots in subtropical plantations and temperate deciduous forests (Nadelhoffer et al. 2006; Wang et al. 2013). In a nutrient-poor temperate pine plantation in China, litter removal had little impact on microbial biomass and community structure (Yarwood et al. 2013). The inconsistent results of litter manipulation experiments suggest that the effects of

altering litter input on soil microbial structure are complex and might be site- or ecosystem-specific. A thorough understanding of the mechanisms (e.g. priming) underlying the responses of soil C stocks and microbial community structure to litter manipulation is required to accurately predict the effect of changes in litter inputs on soil microbial community structure.

Climate change is likely to affect both above- and belowground litter input through the alternations on primary productivity (Liu et al. 2004), fine root turnover (Bai et al. 2010), and carbon allocation (Davidson et al. 2002). The multi-site Detritus Input and Removal Treatment (DIRT) experiment was designed to assess how rates and sources of above- and belowground litter inputs affect the long-term stability, accumulation, microbial community and chemistry of SOM in forest ecosystems (Nadelhoffer et al. 2006; Brant et al. 2006a; Lajtha et al. 2014a, 2018; Pisani et al. 2016). Many DIRT experiments have examined the effects of modifying aboveground or belowground litter on SOM properties (Nadelhoffer et al. 2006; Fekete et al. 2014; Lajtha et al. 2014a). However, few studies to date have simultaneously manipulated aboveground and belowground litter inputs to investigate the relative importance of aboveground and belowground litter to soil processes and soil microbial communities. The dynamics of SOM are simultaneously affected by aboveground and belowground litter inputs, both of which may be affected by global change drivers. However, it is difficult to add belowground litter without severely disrupting the soil and current increases in atmospheric CO₂ concentration are likely to alter aboveground litter input via affecting primary production. Therefore, we conducted a factorial experiment manipulating both aboveground litter input (doubling and excluding) and belowground litter input (excluding) to the soil in a natural forest dominated by *Castanopsis carlesii* (~200 years old) in subtropical China. The objective of the study is to examine how aboveground litter alternation and root exclusion affect soil C and soil microbial community structures independently and interactively.

Materials and methods

Study site

This study was conducted at the Forest Ecosystem and Global Change Research Station (FEGCRS) (26° 11' N,

117° 228'E, 386 m a.s.l.) in Sanming, Fujian Province, China. The study area has a humid mid-subtropical monsoon climate. Mean annual precipitation was 1630 mm with 75% occurring from March to August and annual mean temperature was 19.5 °C (9.4 °C in January and 28.4 °C in July) between 1956 and 2006. The soils, which generally exceed 1 m in depth, developed from sandstone and are classified as red soil according to the Chinese classification system, equivalent to Oxisol in the USDA Soil Taxonomy (State Soil Survey Service of China 1998; Soil Survey Staff 2014). The soils have a sandy texture, with 67% sand, 18% silt and 15% clay. Due to rapid litter decay associated with high temperature and moisture, there is no organic horizon besides a thin litter layer (Cusack et al. 2018; Fang et al. 2009). The soils have a pH (1:2.5 fresh soil/distilled water) of 3.8; total nitrogen (TN) of 2.34 g kg⁻¹, and soil phosphorus (TP) concentration of 0.48 mg kg⁻¹ (Liu et al. 2017).

The experimental site is a natural evergreen broadleaved forest dominated by *Castanopsis carlesii* (Fagaceae) (82% of basal area) and has not been disturbed by human activities for nearly 200 years. Other overstory trees include *C. kawakamii*, *Schima superba* (Theaceae), *Litsea subcoriacea* (Lauraceae), and *Elaeocarpus decipiens* (Elaeocarpaceae). The total biomass, stem density, mean tree height and mean diameter at breast height are 397.4 Mg ha⁻¹, 1192 tree ha⁻¹, 19.2 m and 22.13 cm, respectively (Lin et al. 2017).

Experimental design

We conducted a factorial aboveground and belowground litter manipulation in the *Catanopsis* natural forest. The experiment consists of six treatments (Table 1): control (CT), no aboveground litter (NL), double aboveground litter (DL), no roots (NR), no roots and double aboveground litter (NRDL), and no litter input (NI, both above- and belowground litter were excluded). Each treatment was randomly applied in three 1 m² plots located under forest canopy. Aboveground litter was excluded from litter removal plots with 1-mm nylon mesh suspended one meter above the ground. Double aboveground litter input was achieved by adding litter taken from litter removal plots and distributed with gentle raking (to avoid disturbance) biweekly since January 2013. To exclude roots, a narrow trench was excavated along the perimeter of the plot to approximately 0.6 m depth. Before backfilling, nylon mesh (45- μ m) sheets (Sefar,

Switzerland) were inserted to exclude root ingrowth but allow water and microbes to move freely. All above-ground plants within the plots was removed by hand to prevent root growth.

Soil sampling and measures

In March 2016, nine soil cores (diameter = 3.5 cm) from each plot were collected from the top layer of soil (0–10 cm) after removing the litter layer, and mixed to form a composite soil sample. Because Xu et al. (2013) showed with a meta-analysis that DIRT treatments strongly influence carbon concentration in the 0–5 cm mineral soil but not at greater depths, we conservatively decided to sample the top 10 cm of soil. Soil samples were kept at 4 °C during transport to laboratory, and then sieved to 2-mm and processed for soil chemical analyses. Subsamples were oven-dried for 48 h at 105 °C to calculate gravimetric moisture concentration. Soil pH was measured with a pH meter in a 1:2.5 soil/water suspension. Total soil C and N was analyzed in subsamples using a high-temperature combustion total CN analyzer (Elementar Vario MAX, Germany). All soil carbon was assumed to be organic; carbonate minerals do not persist in acidic soils such as those at our study site. Dissolved organic carbon (DOC) in the soils was extracted with 0.5 M K₂SO₄ in a ratio of 1:5 by shaking at 200 rpm for 1 h, then filtering through 0.45-mm Millipore filter paper (Jones and Willett 2006). Concentrations of DOC were determined with a Shimadzu TOC-TN analyzer (Shimadzu Corp., Kyoto, Japan). Microbial biomass C and N was determined on fresh soil samples using the chloroform fumigation extraction method with a 0.5 M K₂SO₄ solution (Vance et al. 1987; Carter and Gregorich 2006). MBC and

MBN were calculated as the difference in extractable C before and after fumigation using a conversion factor (kc) of 0.45 and 0.5 (Joergensen 1996), respectively.

PLFA analysis

Soil microbial community structure was determined using phospholipid fatty acids (PLFAs) as described by (Wan et al. 2015). The abundance of individual fatty acids was expressed as nanomole per gram of dry soil. Briefly, 8 g of soil was freeze-dried using a one phase solvent consisting of a 0.8:1:2 mixture of potassium phosphate buffers (pH 7.4), chloroform, and methanol. The lipids were split into neutral lipids, glycolipids and phospholipids by eluting with chloroform, acetone and methanol from a silica-filled solid phase extraction column. After mild alkaline methanolysis to form fatty acid methyl esters (FAMES), individual FAMES were analyzed by gas chromatography (Hewlett Packard 5890 GC, Agilent, USA). Peak areas and the resulting amount of PLFA were calculated relative to the internal standard PLFA 19:0. The peaks were identified on the basis of their retention times in comparison with a standard mixture using an identification software (MIDI Inc., Newark, DE). Gram-positive (GP) bacteria were identified by summing i14:0, i15:0, a15:0, i16:0, i17:0 and a17:0. Gram-negative (GN) bacteria were identified by summing 16:1 ω 7c, 16:1 ω 9c, cy17:0, 18:1 ω 7c, 18:1 ω 5c, and cy19:0 (Frostegård et al. 2011). The 18:1 ω 6 and 18:2 ω 6,9 PLFA was used as a marker for fungi (Frostegård et al. 2011). Actinobacteria were identified using the 10Me16:0, 10Me17:0, and 10Me18:0 (Frostegård et al. 2011). The biomarker used for arbuscular mycorrhizal fungi (AMF) was 16:1 ω 5 (McKinley et al. 2005). While the dominant tree species

Table 1 Experimental treatments

Treatment	Method
Control (CT)	Normal litter inputs
No Litter (NL)	Aboveground inputs are excluded from plots. Leaf litter was totally removed by rake
Double Litter (DL)	Aboveground leaf inputs are doubled by adding litter removed from No Litter plots
No Roots (NR)	Roots are excluded with impenetrable barriers
Double Litter (DL)	Aboveground leaf inputs are doubled by adding litter removed from No Litter plots
No Roots + Double litter (NRDL)	No roots and double aboveground litter

C. carlesii is ectomycorrhizal, arbuscular plant species such as *Schima superba* are also abundant at the study site. Total bacterial PLFAs were calculated as the sum of GN and GP. The ratio of fungal to total bacterial PLFAs (F:B) was used to estimate the ratio of fungal to bacterial biomass in soils.

Data analysis

Data were checked for normality and homogeneity of variance prior to statistical analysis, and logarithmically transformed when appropriate. The differences between treatments in soil chemical parameters, microbial biomass C, microbial biomass N, DOC, GN, GP, Fungal, AMF, total PLFAs and F:B were analyzed using one-way analysis of variance (ANOVA) followed by Duncan multiple comparison tests. Two-way ANOVA was used to assess the effects of aboveground litter, belowground litter manipulations and their interactions on soil properties, MBC MBN, community of the five functional groups (GP, GN, AFM, actinobacteria and fungi), GP:GN and F: B. The significance threshold α was established at 0.05. All statistical analyses were conducted using R version 3.2.2 (R core team 2014), with the ‘agricolae’ package for Duncan multiple comparison tests. Data in tables and figures are reported as means \pm 1 standard error (SE).

Differences in soil microbial community structure among treatments were examined using principle components analysis (PCA) on the relative mole abundances of PLFAs in samples. A redundancy analysis (RDA) was used to explore the relationship between the microbial community structure and soil characteristics. Soil properties were tested for significant contributions to the variation in the PLFA data using the Monte Carlo permutation test ($P < 0.05$). The PCA and RDA were processed using Canoco software 5.0 (ter Braak and Smilauer 2012).

Results

Effects of above and below-ground litter manipulation on soil property and microbial biomass C, N

There were no significant differences in soil moisture, total N concentration, C:N or pH of the 0–10 cm soil among treatments (Table 2). Total C concentration was significantly lower in DL, NR NRDL and NI treatment

than in CT, but not different between NL and CT (Table 2); DOC concentration did not differ between the CT and NR treatments but was 15% higher in the DL treatment than the control, while it was significantly lower in NL, NRDL and NI treatments by 14%, 7% and 48%, respectively, relatively to the control (Table 2). MBC and MBN were significantly different among treatments (Table 2). Root manipulation had a stronger effect on MBC and MBN than did aboveground litter manipulation. MBC was lower by 17% in DL, 20% in NL, 37% in NR, 71% in NRDL and 55% in NI compared to the control treatment while MBN concentration was lower by 23.3%, 11.6%, 50%, 26.7% and 32.6% in DL, NL, NR, NRDL and NI treatment, respectively compared to the control (Table 2). The ratio of MBC to MBN did not differ between aboveground litter manipulation and control plots; compared to controls, this ratio increased in NR plots but decreased in NRDL and NI plots.

Microbial PLFAs

The aboveground litter manipulation and root exclusion treatments significantly reduced the PLFA concentrations (Table 3). Total PLFAs were lower in root-exclusion plots than in aboveground treatment plots, which in turn had lower total PLFAs than the control (Table 3). Compared to the control, the total PLFAs were 17% lower in DL, 31% in NL, 56% in NR, 53% in NRDL and 52% in NI. There were no significant differences in GP:GN ratio, F:B ratio, arbuscular mycorrhizal fungi and fungi were among treatments (Table 3). Biomass of GP was not different between CT and DL, but was significantly lower by 30%, 54%, 58% and 54% in NL, NR, NRDL and NI, respectively relative to the control. The GN showed similar patterns with GP. Actinobacteria were 56% lower in NR and 58% in NI relative to the control, but not significantly different among NL, DL, NRDL and CT (Table 3).

The controlling factors of soil microbial community structure

Approximately 98% of the variation of PLFA in soil samples can be explained by the first principal component (PC1 94.7%) and second principal component (PC2 3.5%) (Fig. 1). It was possible to distinguish two different groups in the PCA biplot, one group (upper left), was made up of samples from root exclusion root

Table 2 Soil properties (means \pm 1 SE) under different litter (aboveground) and root treatments in a natural *Castanopsis* forest

Parameter	Treatment						Treatment effects		
	CT	DL	NL	NR	NRDL	NI	Litter	Root	Litter*Root
Moisture (%)	28.8 \pm 0.1a	29.9 \pm 0.1a	28.2 \pm 0.1a	28.5 \pm 0.1a	31.6 \pm 0.3a	27.6 \pm 0.1a	1.91	0.01	0.51
Total C concentration (g·kg ⁻¹)	28.5 \pm 0.9a	22.6 \pm 1.5d	27.3 \pm 2.2ab	26.0 \pm 1.2bc	25.3 \pm 2.6c	22.5 \pm 1.8d	1.81	1.08	2.28
Total N concentration (g·kg ⁻¹)	1.7 \pm 0.0a	1.4 \pm 0.1a	1.6 \pm 0.2a	1.5 \pm 0.1a	1.5 \pm 0.1a	1.4 \pm 0.1a	1.68	2.57	1.55
C/N	17.7 \pm 1.0a	16.3 \pm 0.3a	16.7 \pm 0.3a	17.4 \pm 0.3a	16.8 \pm 0.7a	16.7 \pm 0.4a	1.73	0.03	0.23
DOC (mg·kg ⁻¹)	55 \pm 1.2b	63 \pm 2.1a	48 \pm 0.9d	57 \pm 6.8b	51 \pm 2.6c	29 \pm 2.6e	20.65***	13.06**	4.82*
pH	4.9 \pm 0.1a	4.7 \pm 0.1a	4.7 \pm 0.1a	4.6 \pm 0.1a	4.7 \pm 0.1a	4.4 \pm 0.1a	4.16*	13.07**	3.77*
MBC (mg·kg ⁻¹)	435 \pm 54a	363 \pm 4b	349 \pm 47c	274 \pm 20d	127 \pm 10f	197 \pm 24e	6.33*	49.01***	1.05
MBN (mg·kg ⁻¹)	28.7 \pm 3.8a	22.0 \pm 1.0c	25.3 \pm 3.8b	14.3 \pm 3.0e	21.0 \pm 2.6 cd	19.3 \pm 2.2d	0.05	8.91*	2.66
MBC:MBN	15.3 \pm 1.7bc	16.7 \pm 0.6b	13.6 \pm 0.6c	20.7 \pm 4.8a	6.3 \pm 1.1e	10 \pm 0.1d	5.69*	2.66	6.57*

The numbers in the columns under “treatment effects” are F-values of two-factor (aboveground litter and root treatments) ANOVA. Different letters indicate significant differences between treatments at $p = 0.05$. Bold font indicates a significant effect ($p < 0.05$). *: $p < 0.05$, **: $p < 0.01$ and ***: $p < 0.001$

plots and the other group consisted of samples from plots with root presence. Aboveground treatments did not cluster cleanly in the biplot.

The RDA ordination biplot showed that soil properties explained 58.8% of the variance, with axis 1 explaining 58.2% of the variance and axis 2 explaining another 0.6% (Fig. 2). Soil microbial community structure was significantly related to soil the concentration s of MBC (Fig. 2, $F = 16.4$, $P = 0.002$).

Discussion

Soil carbon concentration

The reduced soil C concentration in the double-litter input treatment relative to the control (Table 2) stands in contrast to the results of many DIRT experiments in temperate and tropical forests, which generally showed either no significant differences (Sulzman et al. 2005;

Table 3 Microbial biomass (nmol g⁻¹, means \pm 1 SE) of soil microbial groups in different litter treatments

Parameter	Treatment						Treatment effects		
	CT	DL	NL	NR	NRDL	NI	Litter	Root	Litter*Root
GP	9.2 \pm 1.8a	7.8 \pm 1.6ab	6.4 \pm 0.5b	4.2 \pm 0.4c	3.8 \pm 0.2c	4.2 \pm 0.8c	0.82	17.33**	0.85
GN	8.2 \pm 1.1a	6.7 \pm 1.6ab	5.8 \pm 0.6bc	3.5 \pm 0.4d	3.8 \pm 0.3d	4.1 \pm 1.1 cd	0.45	15.2**	1.21
AMF	0.6 \pm 0.1a	0.5 \pm 0.1a	0.4 \pm 0.1a	0.2 \pm 0.1a	0.4 \pm 0.1a	0.4 \pm 0.1a	0.12	5.8**	1.88
Actinobacteria	3.7 \pm 0.6a	3.1 \pm 0.8ab	2.6 \pm 0.3ab	1.6 \pm 0.2b	2.0 \pm 0.3b	1.5 \pm 0.3b	0.75	13.2**	0.68
Fungi	3.1 \pm 0.4a	2.5 \pm 0.6a	1.9 \pm 0.2a	1.3 \pm 0.2a	1.7 \pm 0.3a	1.7 \pm 0.7a	0.38	6.57**	1.89
Total	24.7 \pm 3.9a	20.6 \pm 4.7b	17.1 \pm 1.7c	10.8 \pm 1.3d	11.7 \pm 0.7d	11.9 \pm 3.1d	0.59	14.88**	1.11
GP:GN	1.1 \pm 0.1a	1.2 \pm 0.1a	1.1 \pm 0.1a	1.2 \pm 0.1a	1.0 \pm 0.1a	1.1 \pm 0.1a	0.34	0.41	1.43
F:B	0.18 \pm 0.01a	0.17 \pm 0.01a	0.15 \pm 0.01a	0.16 \pm 0.01a	0.22 \pm 0.03a	0.20 \pm 0.03a	1.17	2.39	1.93

The numbers in the columns under “treatment effects” mean F-values generated by two-factor (aboveground litter and root treatments) ANOVA. Different letters indicate significant differences between treatments at $p = 0.05$. Bold font indicates a significant effect ($p < 0.05$). *: $p < 0.05$, **: $p < 0.01$ and ***: $p < 0.001$

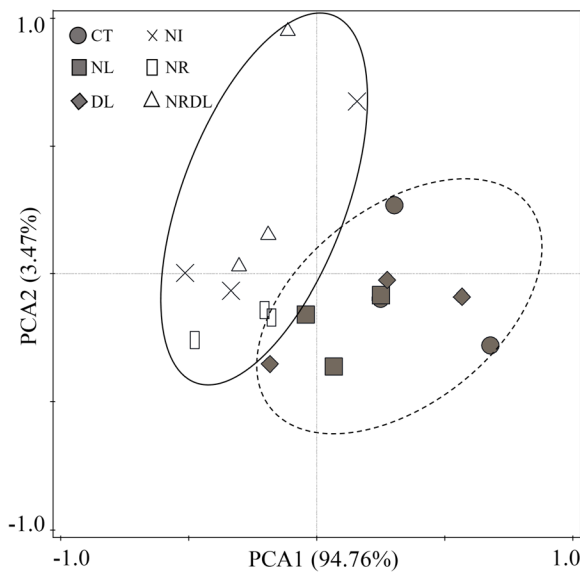


Fig. 1 Scatter plot of the PLFA mol% data of 0–10 soils in different plots showing their scores or correlations to the first and second principal components. Percentages represent the amount of variability explained by the principal component

Bowden et al. 2014; Lajtha et al. 2018) or increased soil C concentration (Leff et al. 2012; Fekete et al. 2014; Lajtha et al. 2014b) in response to aboveground litter

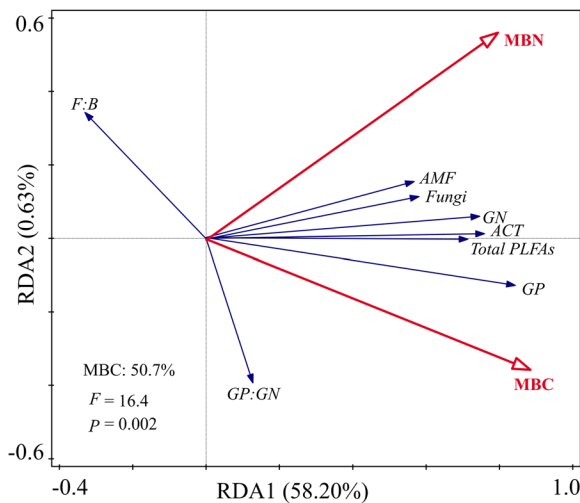


Fig. 2 Ordination biplot based on redundancy analysis (RDA) of PLFA in the soil samples. The dependent variables include biotic variable (solid arrow), gram-positive bacterial PLFAs (GP); gram negative bacterial PLFAs (GN); fungal PLFAs (Fungi); actinobacteria PLFAs (ACT); arbuscular mycorrhizal fungi PLFAs (AMF), total PLFAs (Total PLFAs) and abiotic variables (dashed arrow), microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN)

addition. However, many other studies have shown that adding fresh litter may result in priming effects (Sulzman et al. 2005; Lajtha et al. 2014b, 2018), the mineralization of older soil organic matter (Kuzyakov et al. 2000), while the net effect on soil C concentration depends on the magnitude of the priming effects and the amount of aboveground litter added. It has also been suggested that greater inputs of new organic C may compensate for the release of older soil organic C by priming following litter addition (Xu et al. 2013). In our study site, increased fresh litter addition resulted in a strong priming effect as indicated by elevated enzyme activities and soil respiration (Liu et al. 2017). This priming is likely an important factor leading to reduced soil C in litter addition plots found in the current experiment.

In contrast to aboveground litter addition, we found that aboveground litter removal did not significantly affect soil C concentration (Table 2). Most studies in temperate and tropical forests showed that litter removal resulted in decreased soil total C due to the lack of the fresh C input (Leff et al. 2012; Xu et al. 2013; Fekete et al. 2014). Although our study found decreased soil DOC concentration and MBC concentration in response to aboveground litter removal (Table 1), DOC and MBC together contribute only approximately 1% of total soil C in forest soil (Whalen et al. 2000). Thus, decreases in soil DOC and MBC had very limited effects on soil total C concentration. A possible explanation for the lack of changes in soil C in response to aboveground litter removal could be the short experiment period (3 years), relative to the residence time of soil organic matter. Most studies have shown that aboveground litter removal reduced soil C concentration 8–20 years or longer after the treatment (Bowden et al. 2014; Lajtha et al. 2014b). However, Leff et al. (2012) found that aboveground litter removal from the forest floor drove a 26% reduction after only 2 years of treatment in the wet tropical forest in Costa Rica. The difference between our study and the study of Leff et al. (2012) may be partially explained by the warmer and wetter climate in the tropical forest in Costa Rica (~25 °C and 4430 mm) than at our study site (19.5 °C and 1630 mm). Temperature and rainfall are important factors affecting litter decomposition and the leaching of fresh DOC from litter into soils (Cleveland and Townsend 2006). The high temperature and rainfall in Costa Rica likely contributed to rapid decomposition and leaching of DOC. The removal of aboveground litter cut off the supply of C from

litter to the soils and therefore reduced soil C concentration. The differences in soil C response to aboveground litter removal among forests with different climate and length of experimental treatments may illustrate the importance of climate and treatment time on the response of soil C to litter manipulation. In addition, our results suggest that soil C concentration is more sensitive to aboveground litter addition than to litter removal over short timescales (Table 2). However, it is important to note that changes in litter input due to climate change are not as drastic as is the manipulations in this and other DIRT experiments, some caution is needed when inferring the sensitivity of soil C to climate change from results of such studies.

Regardless of the treatment type (addition or removal) of aboveground litter treatment, soil C concentration was much lower in root removal treatments than in treatments with root presence (Table 2). Compared to the control, DOC was 7% lower in the NRDL treatment and 48% lower in the NI treatment. MBC was reduced by 37%, 71% and 55% in the NR, NRDL and NI plots, respectively. These results indicate that changes in belowground C supply plays a more important role on soil C than alteration in the quantity of aboveground litter. Several studies have suggested that roots may have greater contribution to the stable C pools than does aboveground litter (Brant et al. 2006a; Wang et al. 2013; Sokol and Bradford 2019). In contrast, the DIRT experiment at a Pennsylvania temperate deciduous forest found that aboveground litter was more important in maintaining soil C than was roots (Bowden et al. 2014). It appears that aboveground litter and belowground litter may exert different controls on soil C in different ecosystems.

Root exclusion is intended to prevent the flow of C fixed via photosynthesis from plant canopies to the soils, which occurs via multiple pathways: root turnover, allocation to mycorrhizae, and exudates (Epron et al. 2012; Gorka et al. 2019). The lack of difference in soil C concentration between NRDL and NR plots suggests that aboveground litter addition in the short term (i.e., three years) probably did not cause priming when live roots were excluded. That priming responses to aboveground litter manipulations are dependent on belowground C inputs is a key finding suggesting complex interactions between these functionally very different C inputs to the soil, which may be important to understand if the goal is to predict global change effects on soil C (Rasse et al. 2005). However, it is also important to note

that short-term and long-term responses could be different. Given the lower C concentration in NRDL than NL, it is possible that continual DL inputs to the NR plots may induce observable decreases of soil C with additional priming.

Effects of C input manipulation on the microbial community

The PLFA analysis showed that plot-scale manipulation of the aboveground litter and belowground litter (roots) significantly changed the microbial community biomass and community structures (Table 3, Fig. 1), which is consistent with previous studies (Brant et al. 2006b; Nemergut et al. 2010; Wang et al. 2013). However, our finding that belowground C inputs exerted greater influence on the soil microbial community than aboveground C inputs has only been reported by DIRT experiments conducted by Brant et al. (2006a) in temperate forests, and by Wang et al. (2013) in a subtropical plantation. The greater effects of belowground than aboveground C input on soil microbial community is likely associated with greater reduction in soil labile organic C in the root exclusion treatments than in the aboveground litter treatments. The lower bacterial biomass (GP and GN) in the root exclusion and litter removal treatments than in the controls suggests that litter removal may have decreased labile C inputs to soil and thus decreased the GN bacteria biomass, because the growth of GN is highly dependent on labile C (Waldrop and Firestone 2004).

The smaller quantities of actinobacteria biomarkers in the root exclusion treatments (Table 3) was not consistent with studies in temperate forests and subtropical Chinese fir plantations that reported increased actinobacteria biomass in response to root exclusion (Brant et al. 2006a; Wang et al. 2013; Pisani et al. 2016). Actinobacteria are filamentous heterotrophic bacteria that degrade recalcitrant C compounds (McCarthy and Williams 1992; Khamna et al. 2009). Studies show that root exclusion increases soil water concentration (due to lack of root uptake) which has a negative effect on actinobacteria biomass (Williams et al. 1972; Brant et al. 2006a) because actinobacteria has been reported to increase in relative abundance under low water availabilities (Griffiths et al. 1998; Šnajdr et al. 2008). However, the lack of difference among treatments in our study (Table 3) does not support the negative effects of root exclusion on

actinobacteria biomass via reducing water stress (Table 2). The important role of root C input on actinobacteria is in agreement with the significant effect of root exclusion not litter manipulation on the biomass of actinobacteria (Table 3).

Conclusions

This study demonstrates that the soil C concentration is more sensitive to changes in belowground than aboveground litter input in a subtropical forest. Most notably, aboveground litter addition and root exclusion each significantly reduced soil C concentrations and microbial biomass in the 0–10 cm soil layer. These results suggest that root-derived C inputs exert a stronger control on soil C concentration and microbial community structures than aboveground litter in the studied subtropical natural forest. In light of this study, it is essential that plant-soil feedbacks should be taken into account in predictions of the C sequestration potential of subtropical natural forests under global change.

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