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Contribution of above ground litterfall and roots to the soil CO_2 efflux of two sub-tropical *Cunninghamia lanceolata* and *Castanopsis carlesii* forests

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ABSTRACT

Soil respiration (R_S) is the largest terrestrial carbon (C) flux to the atmosphere, and it can be influenced by changing input of plant C from above- and/or below ground. Especially in tropical and sub-tropical ecosystems, the contributions of litter respiration (R_L), autotrophic respiration (R_A) and mineral soil respiration (R_M) are still poorly understood. In the present study, R_S was measured under untreated control (CT), root exclusion (NR), litterfall exclusion (NL), and combined litterfall and root exclusion (NRNL) in a subtropical *Cunninghamia lanceolata* plantation and a secondary *Castanopsis carlesii* forest for three years. In addition, litter input, litter and soil chemistry, and microbial biomass and community structure (PLFAs) were assessed. R_S was significantly higher in the *C. carlesii* forest than in the coniferous *C. lanceolata* forest. R_L and R_A were significantly higher in the *C. carlesii* forest than in the *C. lanceolata* forest, while there was no significant difference in R_M. R_M, R_A, and R_L contributed 55%, 29%, and 16% to R_S under *C. lanceolata*, and 39%, 32%, and 29% under *C. carlesii*, respectively. Above ground litter input and microbial biomass were lower in the coniferous *C. lanceolata* forest. Soil microbial biomass was significantly lower in NL, NR and NRNL in both forests. NL had most pronounced effects on the microbial community composition in the *C. carlesii* soil, whereas NR and NRNL affected the community composition in *C. lanceolata* soil. Overall, the unexpectedly small and only insignificant additive effects of litter exclusion and root exclusion in the combined treatment (NRNL) suggest that yet unresolved interactions had accelerated the decomposition of mineral soil organic matter and R_M under this lowest plant C-input scenario. Hence, in the case that above and below ground plant C inputs change simultaneously, effects on R_S and its components might be more complex than suggested by single-C-source manipulation studies.

1. Introduction

Globally, 55% of forest carbon (C) is stored in sub/tropical forests and they contain almost 30% of global soil organic C (SOC) and regulate a major exchange of C with the atmosphere through photosynthetic C uptake and respiration (Pan et al., 2011). Soil respiration (Rs) is one of the largest terrestrial C fluxes from soil to the atmosphere, and tropical and subtropical forests contribute more to global Rs than any other biome (Raich et al., 2002; Xu and Shang, 2016). The quantity and quality of plant C (i.e., above ground litter and root C) inputs to the soil are key drivers of heterotrophic soil respiration (R_H) by providing organic C for microbial decomposition. Furthermore, roots actively respire (root respiration) and exudate labile C, which is readily decomposed within the rhizosphere; both sources comprising the autotrophic soil CO_2 efflux (R_A). However, climatic change (i.e., warming, drought, and extreme weather events) could change the quality and quantity of plant litter inputs into the soil (Liu et al., 2016), as well as the allocation of C into the root and the rhizosphere, thereby affecting future soil C cycling and stocks (Bastos and Fleischer, 2021; Eastman et al., 2021; Gao et al., 2021). Therefore, we need to improve our

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understanding of how litter input and C allocation to roots influence Rs dynamic and soil C cycling in the yet understudied sub-tropical forest ecosystems (Crowther et al., 2016; Stephenson and Das, 2020; Sayer et al., 2011; Suseela and Tharayil, 2018).

Recent studies showed that variations in aboveground and belowground production of plant litter may increase or decrease Rs due to the different qualities and/or quantities of the litter (Yi et al., 2007; Wang et al., 2013; Fan et al., 2015; Wang et al., 2017; Chen and Chen, 2018). The contribution of R_A to Rs was found to vary considerably and to depend on the quantity of fine root biomass and forest types. R_A estimates in subtropical forests ranged between 24% in a Chinese fir plantation (Wang et al., 2017) to 35.4% in a monsoon evergreen broad-leaf forest (Yi et al., 2007) and averaged 34% globally (Chen and Chen, 2018). Comparisons between R_A and aboveground litter respiration (R_L) also gave very different pictures. A recent global meta-analysis suggested that the contributions of litter respiration (R_L, 23%) were lower than those of R_A (34%) (Chen and Chen, 2018). Other studies found that the contributions of R_L and R_A to Rs were rather equal in a subtropical coniferous forest (Wang et al., 2013, 2017). Thus, the relative contributions of R_L and R_A are likely site-specific and/or depend on plant traits and hence tree species composition. Plant traits such as litterfall and root chemical composition could affect their contribution to Rs because they affect decomposition rates by altering the microbial biomass, microbial activity, and microbial community composition (Wang et al., 2013, 2017). The chemical composition of roots and litterfall varies widely among tree species in highly diverse subtropical forests (Fan et al., 2015; Ni et al., 2021). Arbuscular mycorrhiza and ectomycorrhiza plants dominate in most natural and anthropogenic ecosystems, and they differ in belowground C allocation, the capacity of organic nutrient acquisition, and therefore play an important role in the forest C cycling and nutrient acquisition (Tedersoo and Bahram, 2019). For example, Yan et al., (2019) showed that ectomycorrhizal fungal respiration represented 41% of total rhizosphere respiration in *larch* plantations in Northern China. Therefore, spatial and temporal variation in quality and quantity of plant C inputs could differently affect the contribution of R_L and R_A to Rs in subtropical forests of various tree species compositions.

Soil microorganisms play a crucial role in regulating soil C and nutrient cycling. Tree species may directly influence soil microbial biomass and community structure through different quantities and quality of both above- and belowground C inputs (i.e., litter chemistry, root exudates) (Wang et al., 2013; Creamer et al., 2015; Wan et al., 2015; Sasse et al., 2018; Williams and de Vries, 2020). Especially between broadleaf and coniferous species differences in microbial community composition and soil C cycling can be expected (Templer et al., 2003; Weand et al., 2010). It has been shown that root exclusion significantly reduced the total microbial biomass and the fungal biomass in broadleaf as well as in coniferous subtropical forests of China (Wan et al., 2015; Wu et al., 2018; Liu 2019a). On the other hand, it was reported that litterfall exclusion significantly increased the ratio of gram-positive bacterial to gram-negative bacteria in a subtropical forest (Wang et al., 2013; Wu et al., 2018). Gram-positive bacteria were found to use more SOM-derived carbon sources while gram-negative bacteria rather rely on readily degradable plant C sources (Kramer et al., 2008). Other studies also demonstrated that litterfall exclusion enhanced fungal abundance towards species tending to utilize more stable C in the temperate forest (Nemergut et al., 2010; Pisani et al., 2016). Overall, the responses of soil microbial community composition and function to plant C-input is complex and tree species composition exerts a strong influence.

Detrital Input and Removal Treatment (DIRT) experiments were widely used to assess the contributions of plant litter (i.e., litterfall and root) to Rs (Fekete et al., 2014; Huang and Spohn, 2015; Wang et al., 2017). However, most of the experiments were conducted in boreal or temperate biomes. Forests in tropical and subtropical regions were less well studied (Wang et al., 2017; Liu et al., 2019a). To fill this knowledge gap, we established a combined litterfall- and root exclusion experiment in two subtropical broadleaf and coniferous forests.

We specifically addressed the following questions: 1) how do a broadleaved *Castanopsis. carlesii* and a coniferous *Cunninghamia lanceolata* subtropical forest vary in R_S and its components (R_L, R_A, and R_M) and 2) are the differences related to specific C input via above-ground litter, roots, and/or soil microbial biomass and/or community composition? We hypothesized 1) that Rs was higher in the *C.carlesii* forest, primarily as a matter of higher absolute and relative contribution of R_L. We further hypothesized 2) that in the simultaneous litter and root exclusion (NRNL) treatment effects were additive and therefore most pronouncedly reduced Rs. With regard to treatment effects on soil microbiology, we hypothesized 3) that litter and root exclusion reduced soil microbial biomass and affected the community structure in the mineral topsoil. Root exclusion was hypothesized to have most pronounced effects on soil microbial biomass and community structure in both forests since fine root-associated C has been identified as the primary C source for microorganisms in many forest soils (Liu et al., 2019a; Bahram et al., 2020).

2. Materials and methods

2.1. Site description and experimental design

The study site is located at the Sanming Forest Ecosystem and Global Change National Observation and Research Station, Fujian Province, China (26° 19′ N, 117° 36′ E). The site is exposed to a subtropical monsoonal climate with annual precipitation of 1670 mm and a mean annual temperature of 18.7 °C. A 3-year manipulation experiment was conducted in a *Cunninghamia lanceolata* (coniferous, symbiosis with arbuscular mycorrhizae (AM)) plantation (Liu et al., 2015) and a secondary *Castanopsis carlesii* (broadleaved, symbiosis with ectomycorrhiza, ECM) (Haug et al., 1994) forest (approximately 800 m apart). In 1975, part of natural *Castanopsis carlesii* forests was clear-cut, slashed, and burned. In 1976, the soil was prepared by digging holes, and then, 1-year-old seedlings of *C. lanceolate* were planted at 3000 trees per hectare. Thinning took place twice at stand ages of 10 and 15 years. *Diplospora dubia, Ilex pubescens*, and *Dicranopteris dichotoma* were the dominant species in the understory layer of the *C. lanceolata* plantation. The diameter of trees at breast height, total tree height, and stand density of the plantation in 2012 averaged 15.6 cm, 18.2 m, and 2858 trees ha^{-1}, respectively. The *C. carlesii* secondary forest originated in 1976 by natural regeneration after heavy selective logging. The diameter of trees at breast height, total tree height, stand density of the natural secondary *C. carlesii* forest in 2012 averaged 15.5 cm, 18.5 m, and 3788 trees ha^{-1}, respectively. Understory vegetation mainly included *Diplospora dubia, Ilex pubescens*, and *Ardisia punctate* in the *C. carlesii* forest. The aboveground biomasses of the two forests estimated based on DBH, tree height and tree density in 2020 were 134 ± 32 and 264 ± 83 t ha^{-1}, respectively (Ni et al., 2021). The soil types of two forests were classified as red soil (State soil Survey Service of China, 1998), equivalent to Ultisoils in the USDA Soil Taxonomy.

We randomly established three 20 m × 20 m blocks in April 2012 in each of the two forests. Within each block, we randomly assigned four subplots (1 m × 1 m) to one of four treatments: untreated control (CT), root exclusion (NR), litterfall exclusion (NL), and combined root and litterfall exclusion (NRNL). In the NL and NRNL plots, the litter layer was removed at the beginning of the study. A horizontal 1-mm nylon mesh screen 1 m above the ground was installed to exclude aboveground litterfall in NL and NRNL plots, the litterfall was removed biweekly from the screens. In the NR and NRNL plots, a trench was dug to a depth of 60–80 cm cutting all fine roots and c. 90% of larger roots (Lyu et al., 2019a). A 0.149-mm nylon mesh (Sefar, Switzerland) screen that allows water and nutrients to pass through freely was inserted as root barriers around the trenched plots. New vegetation was removed biweekly by hand from the NR and NRNL plots. We assumed that no roots grew into the trenched plots during the experiment.

2.2. Soil respiration and components

One 20 cm diameter polyvinyl chloride (PVC) collar (8 cm height) was inserted into the soil to a depth of 5 cm to anchor the Rs chamber on each plot. Each collar was placed in the center of a plot in June 2012. To minimize the effect of soil disturbance and decomposition of dead fine roots caused by trenching, Rs was measured at biweekly intervals from January 2013 (8-months post trenching) to December 2015 using a Li-Cor 8100 infrared gas analyzer (Li-Cor Inc., Lincon, NE, USA). Previous studies showed that most dead roots in trenched plots had decomposed within seven to twelve months and that confounding effects of dead root decomposition diminished thereafter (Subke et al., 2006; Sayer et al., 2011).

We distinguished between three components of soil respiration:

$$\mathrm{Litterrespiration}(R_L) = \mathrm{RespirationinCTplots} - \mathrm{RespirationinNLplots} \quad (1)$$

$$\mathrm{Autotrophicrespiration}(R_A) = \mathrm{RespirationinCTplots} - \mathrm{RespirationinNRplots} \quad (2)$$

$$\mathrm{Mineralsoilrespiration}(R_M) = \mathrm{RespirationinCTplots} - R_L - R_A \quad (3)$$

Mineral soil respiration (R_M) represents the CO_2 flux due to the decomposition of organic C in the mineral soil (heterotrophic), R_L representing the heterotrophic CO_2 flux from the decomposition of fresh surface litter, and R_A representing root respiration plus the CO_2 released during the decomposition of root exudates. We expected that effects of NL and NR were additive in the NRNL treatment and that:

$$\mathrm{RespirationinNRNLplots} \approx R_M \quad (4)$$

Annual CO_2 efflux was calculated as:

$$M = \sum R \times 3600 \times 24 \times (t_{i+1} - t_i) \times (12/44) \times 10^{-6} \quad (5)$$

where M is the annual CO_2 efflux (g CO_2-C m^{-2} yr^{-1}), R is the Rs rate (μmol m^{-2} s^{-1}), i is the sampling number, and t is the sampling time based on Julian day.

Soil temperature (0–5 cm,°C) and soil moisture (0–12 cm, %) were simultaneously monitored adjacent to each soil collar using a hand-held long-stem thermometer (Model SK-250WP, Sato Keiryoki Mfg. Co. Ltd., Tokyo, Japan) and time-domain reflectometry (TDR) (Model TDR300, Spectrum Technologies Inc., Plainfield, IL, USA), respectively (Liu et al., 2017).

2.3. Soil sampling and analysis

Five soil cores samples (0−10 cm depth) per plot were collected randomly with a 3.5-cm diameter corer and combined to gain one mixed sample per plot in May 2016. The samples were stored in airtight polypropylene bags and kept at 4 °C during transportation to the laboratory. Stones, roots, and large organic residues were manually removed before being sieved to 2-mm. Each sample was separated into two subsamples: one dried for analysis of SOC and total N (TN), the other one stored at 4 °C for analyses of phospholipid fatty acids (PLFAs), dissolved organic carbon (DOC), dissolved organic nitrogen (DON), and mineral nitrogen (NH_4^+-N and NO_3^--N). SOC and TN were determined using a CN elemental analyzer (Elementar Vario MAX, Hanau, Germany). Soil DOC and DON were extracted by deionized water at 20 °C; the mixture was filtered through a 0.45-μm filter membrane (Jones and Willett, 2006). The DOC concentration was determined using a Shimadzu TOC-VCPH/CPN analyzer (Tokyo, Japan). The mineral N was extracted from the soil using 2 M KCl and measured using a continuous flow analyzer (Skalar san++, Netherlands) (Carter and Gregorich, 2006).

2.4. Root biomass and litterfall

Roots were sampled with a soil auger of 4.5 cm in diameter, twenty soil cores were taken randomly in each site at 0-10 cm depth at beginning of April 2012. Roots were separated from the soil in the laboratory and then washed carefully. The roots were placed into an oven for 48 h at a temperature of 65 °C and weighted.

In the center of each block, five litterfall collectors of 0.2 mm nylon mesh with a surface area of 0.5 m^2 were randomly installed 50 cm above ground level. Litterfall was collected at biweekly intervals from January 2013 to December 2015. The litterfall was placed into an oven for 48 h at a temperature of 65 °C and weighted.

Dried roots and litterfall were ground to a powdered form using a mortar and pestle and passed through a 0.149 mm sieve before measuring the C, N, and P concentrations. C and N concentrations were measured using a CN auto-analyzer (Vario Max CN, Elementar, Langenselbold, Germany). P concentrations were measured by first digesting the samples with H_2SO_4 and $HClO_4$ ratio (4:1), passing them through a 0.45 μm glass fiber filter (Q/IEF J01-1997, Shanghai, China), and then using a continuous flow analyzer (Skalar san++, Netherlands) (Zhang et al., 2019). Fine root biomass C stocks were calculated by multiplying the fine root dry weight with root C contents. Litterfall C inputs were calculated by multiplying the litterfall dry weight with litterfall C contents.

2.5. Microbial biomass and community structure

The microbial biomass and community structure were determined using the phospholipid fatty acids (PLFAs) analysis as described by Bardgett et al. (1996). The concentration of each PLFA was calculated based on the 19:0 internal standard concentrations. The sum of lipids with chain lengths from C10 to C20 was calculated for total lipid abundance and microbial biomass (Li et al., 2020). PLFAs biomarkers of i14:0, i15:0, i16:0, i17:0, a15:0, and a17:0 represented gram-positive bacteria (GP) (Denef et al., 2009), and gram-negative bacteria (GN) were identified by summing 16:1 ω9c, 16:1 ω7c, 18:1 ω7c, 18:1 ω5c cy17:0 and cy19:0 (Frostegard et al., 2011; Ushio et al., 2013). The biomarkers of 18:2 ω6,9c and 18:1 ω9c were used as indicators of fungi, and the biomarker for arbuscular mycorrhizal fungi (AMF) was 16:1 ω5c (Swallow et al., 2009). The biomarkers used for actinomycetes (ACT) were 16:0 10-methyl, 17:0 10-methyl, and 18:0 10-methyl. The ratios of fungal to bacterial PLFAs (F:B), gram-positive bacteria to gram-negative bacteria (GP:GN), and total saturated fatty acids (14:0, 15:0, 16:0, 17:0, 18:0) to monounsaturated fatty acids (16:1 ω9c, 16:1 ω7c, 16:1 ω5c, 18:1 ω9c, 18:1 ω7c, 18:1 ω5c) (Sat:Mono) were used as indicators of microbial physiology and microbial community composition (Bardgett et al., 1996; Li et al., 2020).

2.6. Statistical analysis

The impacts of root exclusion, litterfall exclusion, and combined root and litterfall exclusion and their interaction on R_S and its components were assessed by repeated-measures analysis of variance (ANOVA). The differences between treatments in SOC, TN, DOC, DON, NH_4^+-N, NO_3^--N, PLFAs, and Rs components were analyzed using one-way analysis of variance (ANOVA) followed by least significant difference (LSD) tests. Two-way ANOVA with Tukey comparisons was used to test the differences in treatments for soil chemical parameters, DOC, and PLFAs. Statistically, significant differences were set at P values < 0.05, unless otherwise stated. Principle components analysis (PCA) was used to examine the differences in soil microbial community structure among treatments. The relationship between microbial community structure and soil characteristics was explored using redundancy analysis (RDA). The R package Corrplot (Wei, 2016) was used to calculate the Spearman correlation between the Rs and different variables as well as between each variable.

We used a structural equation modeling (SEM) approach to examine the relationships among Rs, fine root biomass C, litterfall biomass C input, soil chemical and microbial properties. The litterfall biomass C

input was calculated/modelled using the average annual production from 2013 to 2015 in each forest, and the fine root biomass C was from April 2012. All the data in the SEMs were scaling by one standard deviation (Lefcheck, 2016). We used composite variables to explain the collective effects of litterfall C input, root biomass C, soil N status (DON, NH_4^+-N, NO_3^--N, and mineral nitrogen), and microbial properties (GN bacteria and fungi) on Rs. Each of the composite variables was selected based on the multiple regression for mass loss rate and Akaike's Information Criterion (AIC). Model fit was assessed using Fisher's C statistic, where good-fitting models yield small C statistics and P values > 0.05 indicate that the data is well represented by the model. Piecewise SEM was based on linear mixed-effects models using the R package *piecewiseSEM* (Lefcheck, 2016). All the statistical analyses were performed in R v. 3.6.3 and with a significance level of 0.05 (R Core Team, 2019).

3. Results

3.1. Soil respiration and its components

The annual soil CO_2 effluxes of CT, NR, NL and NRNL were 832 ± 59, 587 ± 68, 702 ± 63 and 511 ± 73 g CO_2-C m^{-2} yr^{-1} in the *C. lanceolata* forest, and all significantly lower than in the *C. carlesii* forest 1321 ± 61, 906 ± 58, 930 ± 48 and 893 ± 61 g CO_2-C m^{-2} yr^{-1}, respectively. Mean soil CO_2 effluxes in NR, NL, and NRNL were 33%, 17%, and 36% (Fig. 1b), and 31%, 29%, and 32% (Fig. 1d) lower than in the corresponding control (CT) plots in both forests, respectively. Mean annual estimates of R_L, R_A, and R_M were 131 ± 3, 245 ± 11, and 457 ± 71 g CO_2-C m^{-2} yr^{-1} in the *C. lanceolata* forest (Fig. 2b) and were 388 ± 16, 415 ± 27 and 520 ± 51 g CO_2-C m^{-2} yr^{-1} in the *C. carlesii* forest (Fig. 2d), respectively. R_L and R_A in the *C. lanceolata* forest were significantly lower than in the *C. carlesii* forest, but R_M was similar in the two forest types (Figs. 2b, d). Due to a gradual decrease in soil CO_2 efflux in NR plots throughout the study, the estimated contribution of R_M increased from 2013 to 2014 and 2015 in the *C. carlesii* forest (Figs. 2d). The average estimated contributions of R_M, R_A, and R_L to Rs over all three study years were 55%, 29%, and 16% in the *C. lanceolata*, and 39%, 32%, and 29% in *C. carlesii* forest, respectively (Table 1). Soil CO_2 efflux in NRNL (893 and 511 g CO_2-C m^{-2} yr^{-1}) was close to that in NR (906 and 587 g CO_2-C m^{-2} yr^{-1}) and did only partly reflect the expected additive effects of combined leaf litter and root exclusion (Eq. (4)).

3.2. Chemistry of above- and belowground litter, soil physical, and biochemical properties

The average annual litterfall C in the *C. lanceolata* and the *C. carlesii* forests were 209 and 295 g C m^{-2} yr^{-1}, respectively, during 2013-2015. Root biomass C was approximately 89 and 110 g C m^{-2} in 2012, respectively (Table S1). Annual litterfall C and root biomass C in the *C. lanceolata* forest were significantly lower than in the secondary *C. carlesii* forest (Table S1). The C:P and N:P ratio of leaf litter was significantly lower than those of roots, and C:N ratio of leaf litter was significantly higher than that of roots in the *C. lanceolata* forest. However, the C:N and C:P ratios of leaf litter were significantly lower than those of roots, whereas the N:P ratio of leaf litter was significantly higher than that of roots in the *C. carlesii* forest (Table S1).

Soil temperature showed typical seasonal and annual variations from 2013 to 2015 (Fig. S1a, c) and was unaffected by the treatments in both forests (Fig. S1). Root exclusion significantly increased soil moisture in both forests (Fig. S2). Litterfall exclusion significantly reduced soil moisture in the *C. carlesii* forest, but not in the *C. lanceolata* forest (Fig. S2). In the combined NRNL treatment, soil moisture was similarly enhanced as under NR in the *C. lanceolata* forest, while the increase was less pronounced in the *C. carlesii* forest (Fig. S2).

In the *C. lanceolata* forest, SOC, TN, DOC, and DON contents showed no significant difference between NR, NL, NRNL, and CT. The NH_4^+-N content in NL was significantly higher than in CT. The NO_3^--N content significantly increased in NR and NRNL but was lower in NL when compared to CT (Table 2). In the *C. carlesii* forests, NL significantly decreased SOC, TN, DOC, and NH_4^+-N contents (Table 2) and NR significantly reduced the DOC and NH_4^+-N contents but increased the NO_3^--N content compared to CT; The DOC content also showed a decline in NRNL when compared to CT (Table 2). There was a significant interaction effect between forest type and treatment on SOC, TN, DOC, DON, NH_4^+-N and NO_3^--N concentrations (Table 2).

3.3. Microbial biomass and community structure

The total PLFAs, bacterial, fungal, AMF, ACT biomass, and F:B ratio was significantly lower in the *C. lanceolata* than in the *C. carlesii* forest (Table 3). Compared to CT, the NR, NL, and NRNL treatments all significantly decreased the total, bacterial, fungal, AMF, and ACT PLFAs in both forests (Table 3). GN bacterial abundance was highest in CT

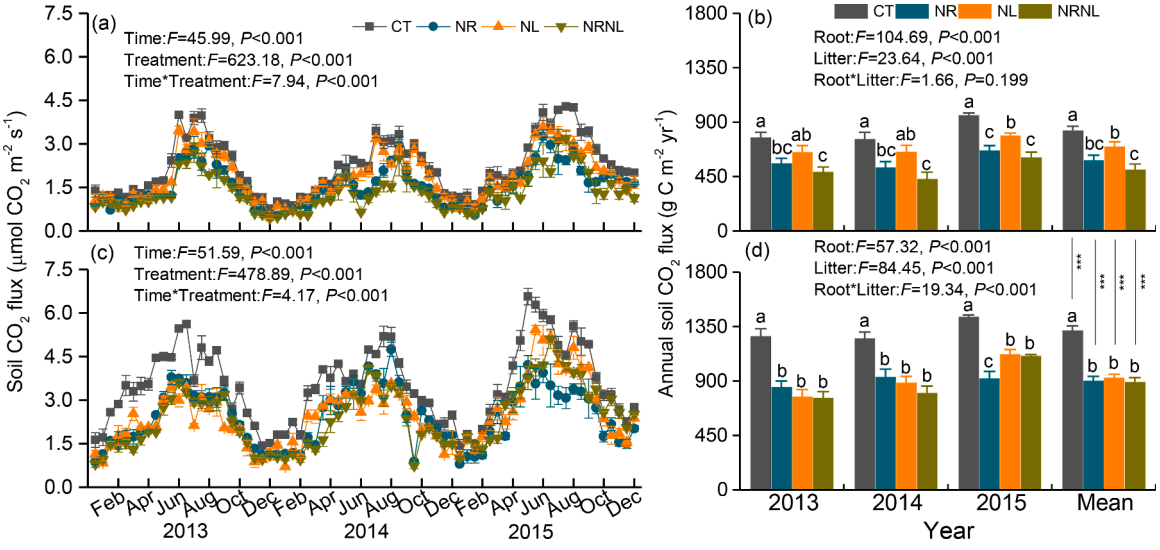

Fig. 1. Seasonal flux dynamics (a, c) and cumulative annual soil CO_2 effluxes (b, d) in the litterfall exclusion and trenching experiment from 2013 to 2015 in a *C. lanceolata* plantation (a, b) and a secondary *C. carlesii* forest (c, d) (mean ± 1 standard error). CT, control; NL, litterfall exclusion; NR, root exclusion; NRNL, litterfall and root exclusion; Different letters denote significance at the $P = 0.05$ level; Asterisks represent significant differences between the forests (*** $P < 0.001$).

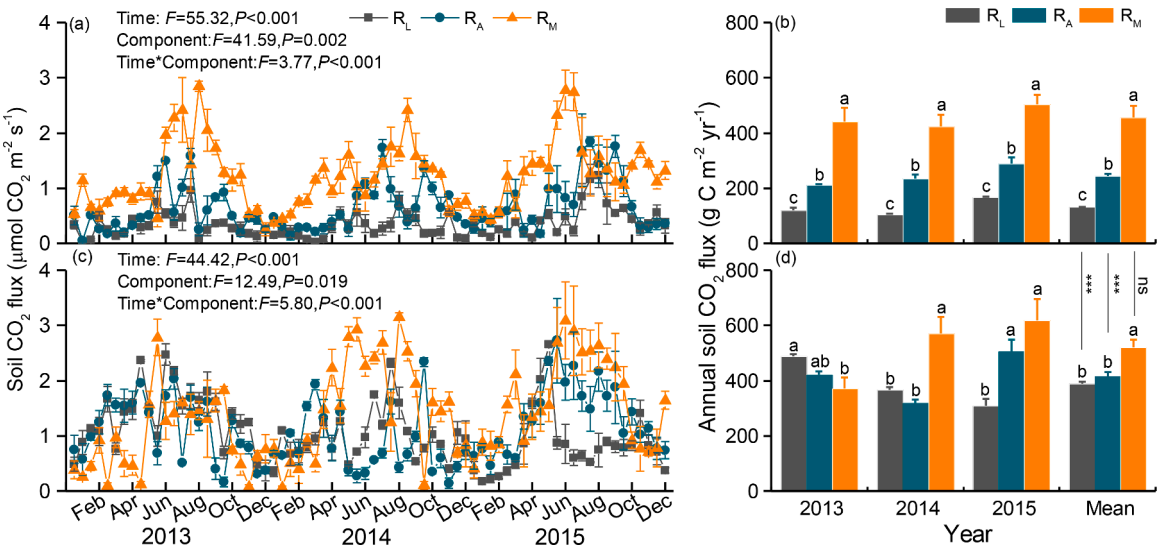

Fig. 2. Seasonal flux dynamics (a, c) and cumulative annual soil CO_2 effluxes (b, d) from leaf litter decomposition (R_L), autotrophic respiration (R_A), and mineral soil heterotrophic respiration (R_M) from 2013 to 2015 in a *C. lanceolata* plantation (a, b) and a secondary *C. carlesii* forest (c, d); Different letters denote significance at the $P = 0.05$ level; Asterisks represent significant differences between the forests (*** $P < 0.001$), "ns" indicates no statistically significant difference.

Table 1
Annual contributions of heterotrophic respiration from aboveground litter decomposition (R_L), belowground autotrophic respiration (R_A), and heterotrophic respiration from mineral soil organic matter decomposed (R_M) to total soil respiration (Rs) from 2013 to 2015; Different letters denote significance at $P = 0.05$ level.

Forest type	Time	R_L(%)	R_A(%)	R_M(%)
Cunninghamia lanceolata plantation	2013	15.7 ± 1.9 **ab**	27.5 ± 1.3 **a**	56.7 ± 3.2 **a**
	2014	13.9 ± 0.7 **b**	30.8 ± 1.7 **a**	55.2 ± 2.2 **a**
	2015	17.3 ± 0. **a**	30.2 ± 2.7 **a**	52.4 ± 2.8 **a**
	Mean	15.7 ± 0.8	29.5 ± 1.9	54.6 ± 2.7
secondary *Castanopsis Carlesii* forest	2013	38.2 ± 1.2 **a**	32.8 ± 0.5 **a**	28.8 ± 1.7 **b**
	2014	29.2 ± 1.4 **b**	25.5 ± 1.9 **b**	45.2 ± 3.1 **a**
	2015	21.5 ± 1.9 **c**	35.4 ± 3.2 **a**	42.9 ± 5.1 **a**
	Mean	29.3 ± 0.4	31.4 ± 1.2	39.2 ± 1.6

(10.5 nmol g^{-1} soil), followed by NL (6.5 nmol g^{-1} soil) and NRNL (6.2 nmol g^{-1} soil), and lowest in NR (5.6 nmol g^{-1} soil) in the *C. lanceolata* forest, while in the *C. carlesii* forests, GN bacterial abundance was highest in CT (13.1 nmol g^{-1} soil), followed by NR (10.2 nmol g^{-1} soil), and lowest in NL (6.2 nmol g^{-1} soil) and NRNL (7.4 nmol g^{-1} soil) (Table 3). In the *C. lanceolata* forest, NR significantly increased GP:GN and Sat:Mono ratios. In the *C. carlesii* forests, compared to CT, the GP:GN and Sat:Mono ratios were significantly decreased under NR, while the Sat:Mono ratio was significantly enhanced under NL (Table 3). There was a significant interaction effect between forest type and treatment on total PLFAs, bacterial, fungal, AMF biomass, GP:GN, and Sat:Mono ratios (Table 3).

Redundancy analysis showed that the microbial communities in NR and NRNL were separated from those in CT and NL, indicating that trenching significantly changed the structure of the soil microbial community under *C. lanceolata* (Fig. 3). The NL treatment was separated from CT, NR, and NRNL, indicating that litter exclusion significantly changed the soil microbial community under *C. carlesii* and that N availability (TN content) was an important factor (Fig. 3). All of the abiotic factors explained 50.8% and 57.1% of the variance in the microbial community composition in *C. lanceolata* and *C. carlesii* forest, respectively (Fig. 3).

The ratios of GP:GN and Sat:Mono were significantly higher in the *C. lanceolate* forest (Table 3) and were significantly negatively correlated with the contents of DON and mineral N (Fig. 4).

3.4. Relationships between Rs and biochemical properties

Across both forests from 2013 to 2015, field Rs was significantly positively correlated with soil temperature (Fig. S3). The annual Rs rates

Table 2
Surface mineral soil (0–10 cm) biochemical properties in May 2016. CT, control; NL, litter exclusion; NR, root exclusion; NRNL, litter and root exclusion; Different letters denote significance at $P = 0.05$ level.

Forest type	Treatment	SOC (g kg^{-1})	TN (g kg^{-1})	DOC (mg kg^{-1})	DON (mg kg^{-1})	NH_4^+-N (mg kg^{-1})	NO_3^--N (mg kg^{-1})
Cunninghamia lanceolata plantation	CT	17.2 ± 0.3 **a**	1.1 ± 0.1 **ab**	43.9 ± 1.3 **ab**	2.0 ± 0.1 **a**	7.3 ± 0.4 **b**	0.28 ± 0.02 **c**
	NR	15.7 ± 1.2 **a**	1.0 ± 0.1 **b**	35.3 ± 4.8 **b**	3.2 ± 0.4 **a**	7.5 ± 0.2 **b**	0.59 ± 0.06 **a**
	NL	17.3 ± 1.1 **a**	1.3 ± 0.1 **ab**	54.5 ± 0.3 **a**	2.6 ± 0.2 **a**	9.5 ± 0.4 **a**	0.12 ± 0.02 **d**
	NRNL	17.6 ± 2.1 **a**	1.3 ± 0.2 **a**	49.9 ± 4.3 **a**	3.8 ± 1.1 **a**	8.3 ± 0.1 **b**	0.42 ± 0.04 **b**
Secondary *Castanopsis carlesii* forest	CT	20.9 ± 1.5 **a**	1.4 ± 0.1 **a**	44.8 ± 2.1 **a**	1.8 ± 0.2 **a**	8.2 ± 0.3 **a**	0.24 ± 0.02 **b**
	NR	18.8 ± 1.4 **a**	1.3 ± 0.1 **a**	38.6 ± 2.2 **b**	1.8 ± 0.1 **a**	5.3 ± 0.2 **c**	0.42 ± 0.06 **a**
	NL	12.4 ± 1.0 **b**	0.9 ± 0.1 **b**	35.2 ± 0.7 **b**	1.5 ± 0.3 **a**	6.7 ± 0.3 **b**	0.28 ± 0.04 **b**
	NRNL	17.2 ± 1.2 **a**	1.2 ± 0.1 **a**	20.5 ± 1.6 **c**	0.7 ± 0.1 **b**	6.2 ± 0.4 **bc**	0.31 ± 0.01 **ab**
Two-way ANOVA							
Forest type (F)		0.17	0.80	**34.90** ***	**18.64** **	**42.22** ***	2.08
Treatment (T)		**3.87** *	3.06	**7.13** **	0.65	**8.98** **	**23.03** ***
F*T		**5.13** *	**9.42** **	**18.15** ***	**3.26** *	**12.37** ***	**6.55** **

Table 3
Concentrations of microbial PLFAs (nmol g^{-1} soil) in the different treatments in May 2016. CT, control; NL, litter exclusion; NR, root exclusion; NRNL, litter and root exclusion; AMF, arbuscular mycorrhizal fungi; GP, gram-positive bacteria; GN, gram-negative bacteria; F:B, fungi to bacteria ratio; GP:GN, gram-positive bacteria to gram-negative bacteria; Sat:Mono, total saturated fatty acids to monounsaturated fatty acids. Different letters denote significance at $P = 0.05$ level.

Biomarker	Cunninghamia. lanceolata plantation				secondary Castanopsis carlesii forest				Two-way ANOVA		
	CT	NR	NL	NRNL	CT	NR	NL	NRNL	Forest type (F)	Treatment (T)	F*T
Total PLFAs	44.1 ± 1.5 **a**	26.2 ± 0.8 **b**	26.7 ± 0.3 **b**	25.9 ± 1.4 **b**	54.5 ± 1.8 **a**	38.9 ± 1.9 **b**	26.4 ± 0.5 **c**	29.9 ± 1.3 **c**	**51.9** ***	**124.3** **	**10.2** **
Bacteria	20.2 ± 0.7 **a**	12.0 ± 0.3 **b**	12.3 ± 0.3 **b**	12.3 ± 0.7 **b**	24.2 ± 0.8 **a**	17.6 ± 0.8 **b**	11.5 ± 0.1 **c**	13.3 ± 0.6 **c**	**34.5** ***	**122.6** ***	**11.6** ***
Fungi	4.5 ± 0.3 **a**	2.3 ± 0.2 **b**	2.6 ± 0.1 **b**	2.3 ± 0.1 **b**	7.6 ± 0.2 **a**	5.6 ± 0.5 **b**	3.5 ± 0.1 **c**	4.3 ± 0.3 **c**	**177.2** ***	**63.7** ***	**10.8** ***
AMF	1.5 ± 0.1 **a**	0.8 ± 0.1 **b**	0.9 ± 0.1 **b**	0.9 ± 0.1 **b**	1.8 ± 0.1 **a**	1.5 ± 0.2 **b**	0.9 ± 0.1 **c**	1.1 ± 0.1 **c**	**27.9** ***	**32.0** ***	**4.8** *
Actinomycetes	4.7 ± 0.4 **a**	2.7 ± 0.1 **b**	2.7 ± 0.1 **b**	2.5 ± 0.2 **b**	5.1 ± 0.1 **a**	3.6 ± 0.3 **b**	2.8 ± 0.1 **c**	2.7 ± 0.1 **c**	**7.9** *	**58.3** ***	1.9
GP	9.7 ± 0.2 **a**	6.5 ± 0.1 **b**	5.8 ± 0.3 **b**	6.1 ± 0.4 **b**	11.1 ± 0.1 **a**	7.4 ± 0.5 **b**	5.3 ± 0.1 **c**	5.9 ± 0.4 **c**	**5.2** *	**124.6** ***	**5.3** *
GN	10.5 ± 0.5 **a**	5.6 ± 0.1 **c**	6.5 ± 0.1 **b**	6.2 ± 0.3 **bc**	13.1 ± 0.7 **a**	10.2 ± 0.4 **b**	6.2 ± 0.1 **c**	7.4 ± 0.3 **c**	**56.4** ***	**83.9** ***	**15.0** ***
F:B	0.22 ± 0.02 **a**	0.19 ± 0.01 **a**	0.22 ± 0.02 **a**	0.19 ± 0.01 **a**	0.32 ± 0.01 **a**	0.32 ± 0.02 **a**	0.30 ± 0.01 **a**	0.32 ± 0.01 **a**	**155.1** ***	0.6	2.0
GP:GN	0.93 ± 0.02 **b**	1.16 ± 0.01 **a**	0.90 ± 0.04 **b**	0.96 ± 0.01 **b**	0.82 ± 0.02 **a**	0.73 ± 0.02 **b**	0.86 ± 0.01 **a**	0.80 ± 0.05 **ab**	**74.5** ***	2.3	**19.1** ***
Sat:Mono	0.94 ± 0.03 **b**	1.09 ± 0.01 **a**	0.97 ± 0.07 **ab**	0.99 ± 0.02 **ab**	0.84 ± 0.01 **b**	0.73 ± 0.03 **c**	0.86 ± 0.01 **a**	0.79 ± 0.03 **bc**	**69.8** ***	0.3	**6.5** **

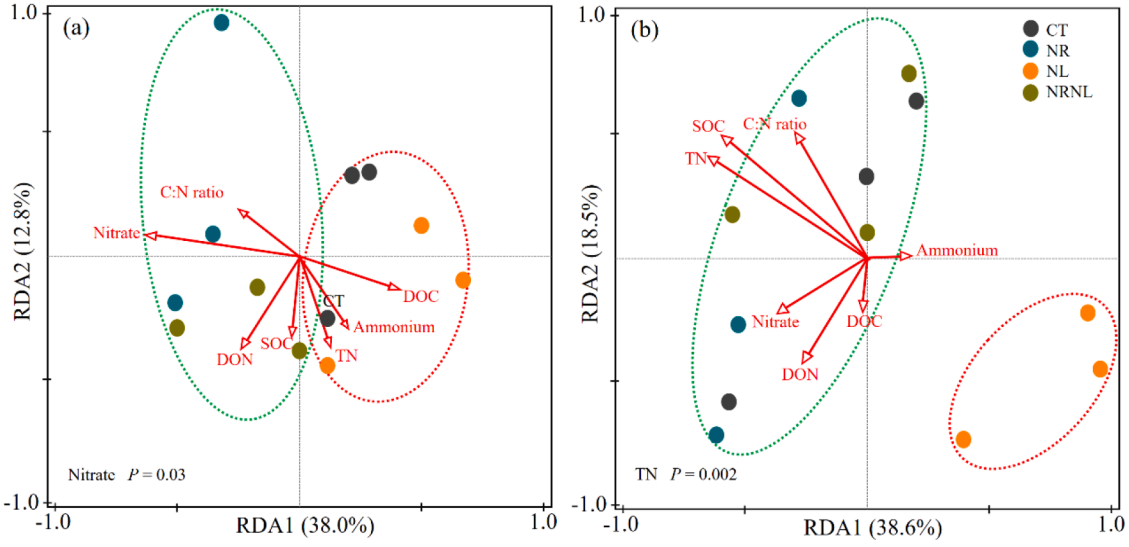

Fig. 3. Redundancy analysis of the relationships between abiotic factors and soil microbial PLFAs (mol%) under *C. lanceolata* (a) and *C. carlesii* (b). Percentages indicate the proportion of variance explained by each axis. DOC, dissolved organic carbon; DON, dissolved organic nitrogen. Red arrows reflect the abiotic factors. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

were negatively correlated with the contents of moisture and DON, and the ratios of GP:GN and Sat:Mono (Fig. 4). However, annual Rs rates were positively correlated with the concentration of total PLFAs, bacteria, fungi, GP, GN, actinobacteria, arbuscular mycorrhizal fungi, and the F:B ratios (Fig. 4). The SEM with litterfall, roots, soil N status, and microbial biomass could explain 89% of the total variance in Rs ($C = 5.43$, $df2$, $P = 0.7$, AIC = 56.32, BIC = 76.34, Fig. 5). Roots, soil N status, and microbial biomass were the most important variables exerting a directly positive effect on Rs. Roots and litterfall had an indirect effect on Rs by their positive effects on microbial biomass (Fig. 5).

4. Discussion

4.1. Plant C input and soil CO_2 fluxes

As hypothesized, R_S was higher in the *C. carlesii* forest than in the *C. lanceolata* plantation and the C input manipulations indicated that higher root, and especially higher litter C inputs were the primary causes. Since annual litterfall C input was about 25% higher in the *C. carlesii* forest, a larger contribution of R_L could be anticipated; but litter input among forest types did not only differ in quantity; it differed in quality as well. Above ground litter in the secondary *C. carlesii* forest had a lower C:N and C:P ratio and higher N:P ratio, indicating that the quality of above ground litter was higher than that of *C. lanceolata*. In a detailed litter characterization study at the same sites, Ni et al. (2021) made similar observations and further found that *C. carlesii* litter decomposed twice as fast as *C. lanceolata* needles and released significantly more N during its decomposition. Such higher litter inputs, as well as turnover rates, can reasonably explain the higher contribution (absolute and relative) of R_L to R_S in the broadleaved secondary *C. carlesii* forest.

Interestingly, our annual estimates of R_L did not match the site

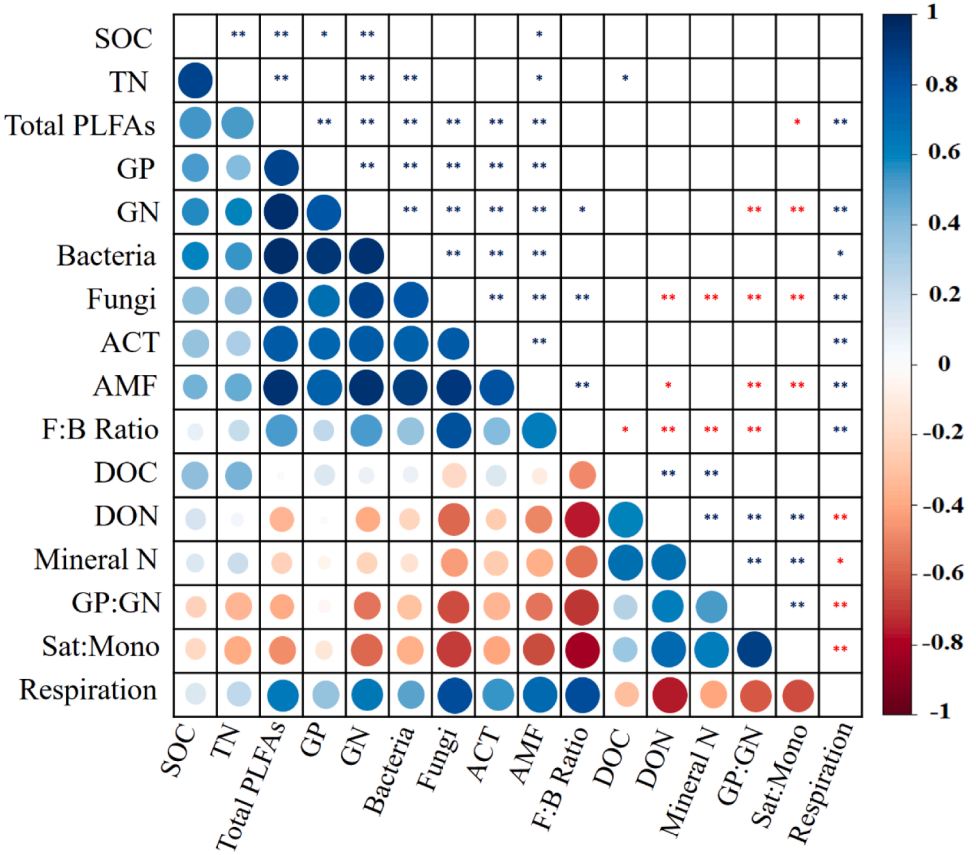

Fig. 4. Correlation matrix (Spearman ranks) of environmental, soil chemical and microbial variables. SOC, soil organic carbon; TN, soil total nitrogen; GP, gram-positive bacteria; GN, gram-negative bacteria; ACT, actinomycetes; AMF, arbuscular mycorrhizal fungi; DOC, dissolved organic carbon; DON, dissolved organic nitrogen; F:B, fungi to bacteria ratio; GP:GN, gram-positive bacteria to gram-negative bacteria; Sat:Mono, total saturated fatty acids to monounsaturated fatty acids. Circles with blue and red colors indicate positive and negative relationships, respectively. Circle size indicates the coefficient, * indicates $P < 0.05$, ** indicates $P < 0.01$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

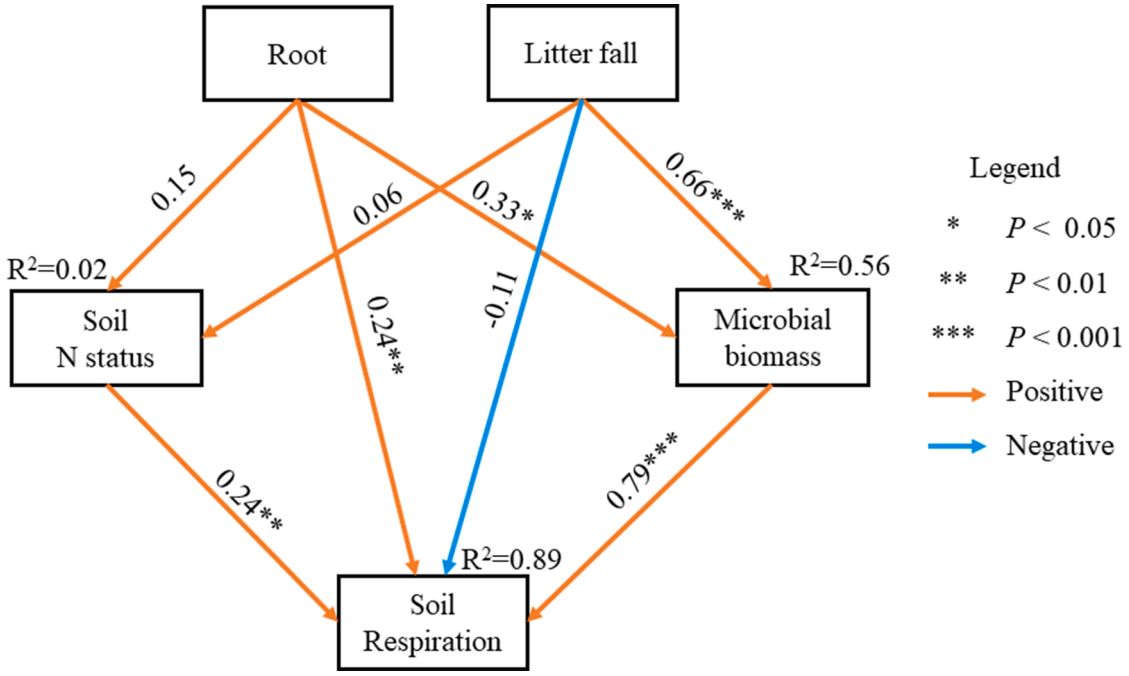

Fig. 5. Structural equation modeling (SEM) analysis of the effect of root and litter fall exclusion on the annual soil respiration rate. Soil N status (DON, NH_4^+-N, NO_3^--N and mineral N); Microbial biomass (gram-negative bacterial and fungal PLFAs). The orange and light blue arrows represent significant positive and negative pathways, respectively. Numbers at arrows are standardized path coefficients and arrow width is proportional to the strength of the relationship. R^2 values on top of response variables indicate the proportion of variation explained by relationships with other variables. The final results of model fitting were: $C = 5.43$, $df = 2$, $P = 0.7$, AIC = 56.32, BIC=76.34.

specific annual litterfall C inputs in both forest types. In the *C. carlesii* forest, R_L (~x223C 390 g CO_2-C m^{-2} yr^{-1}) was higher than annual litter C input (~x223C 290 g C m^{-2} yr^{-1}). This was surprising since R_L and litter input were expected to be in a similar range, considering that the soil C pool of this natural forest can be assumed near a steady state (Giardina and Ryan, 2002). There are two complementary explanations for the mismatch between R_L and litter C input in the *C. carlesii* forest. The first one is that litterfall was methodologically underestimated by losing a fraction of litter through wind blow-out from litter traps or non-accounting of ground-level lateral litter input beneath the litter traps. However, though this cannot be totally ruled out, it is rather unlikely that the litter estimates are biased, especially since Ni et al. 2021 found very similar C input rates throughout a 10-year intensive litter collection study in the same forest stands. The second, and more likely explanation for the mismatch, is that litter removal not only excluded respiration from decaying litter, but as well negatively affected R_H in the *C. carlesii* forest soil. Labile C input from decaying leaf litter could have exerted a positive effect ("priming") on the decomposition of SOM (Liu et al., 2017; Lyu et al., 2019b; Fanin et al., 2020). The observed decrease in microbial biomass in the mineral topsoil of the NL treatment supports this explanation, as does the decrease in mineral soil DOC concentrations. DOC concentrations are indicators of easily available C for soil microbes (Barea et al., 2005; Kuzyakov and Cheng, 2001) and are often positively related to R_S, such as it was the case in our present experiment (Liu et al., 2017). Accordingly, R_L in the *C. carlesii* forest likely not only represented the pure CO_2 efflux from decomposing litter, but a fraction of (primed) R_M in addition.

Inversely, R_L (~x223C 130 g CO_2-C m^{-2} yr^{-1}) was lower than the annual litter C input (~x223C 210 g C m^{-2} yr^{-1}) in the *C. lanceolata* plantation - suggesting that above ground litter C accumulated in this forest. An accumulation of leaf litter in the *C. lanceolata* plantation can be anticipated, since the coniferous *C. lanceolata* trees were planted on former *C. carlesii* forest soil. Accumulation of needle litter and surface humus is a typical initial pattern in coniferous plantations after their establishment on agricultural soil or after conversion from broadleaved to coniferous forest (Mayer et al., 2020). There was no negative effect of NL on mineral topsoil DOC concentrations (Table 2), suggesting that labile C influx from the litter layer did not play a significant role in promoting SOM decomposition in the *C. lanceolata* soil. Microbial biomass was similarly decreased in the mineral NL topsoil, which might as well have resulted in a reduction of R_M. This, however, remains speculative and warrants further investigation. The different nutrient ratios of above ground and fine roots may serve as indicators for the role of above ground and root litter C input. Compared to roots, litterfall had a higher C:N ratio and lower C:P and N:P ratios in the *C. lanceolata* plantation, which means that the quality of litterfall was lower than that of fine root biomass (Creamer et al., 2015; Wang et al., 2017); pointing at the potential importance of root litter C inputs into this specific forest soil.

Root exclusion similarly reduced soil respiration rates by about 30% in both forest types. R_A contribution of around 30% lies well within the range of autotrophic forest soil respiration estimates globally (Subke et al., 2006; Chen and Chen, 2018) and in subtropical forests in particular (Wang et al., 2017; Liu et al., 2019b). Root exclusion had more pronounced negative effects on microbial biomass in the *C. lanceolata* forest (40% reduction, compared to ~x223C 30% reduction under *C. carlesii*). SEM results suggested that root exclusion had direct effects on Rs (exclusion of root respiration), and indirectly regulated Rs by decreased soil microbial biomass (Fig 5, Table 3). The negative effects on microbial biomass reflect the important role of root C inputs for soil microbial populations, particularly in the *C. lanceolata* forest soil. In this forest type, C inputs from roots seem to clearly dominate inputs from aboveground litterfall, whereas in the broadleaved *C. carlesii* forest, our results suggest that aboveground litterfall and root C inputs affected R_S in a similar magnitude. It has to be noted that the trenching method, which was used for root exclusion, is a rather rough approach and is associated with potential biases. Soil moisture tends to be higher in trenched plots, as there is no water uptake by the cut-off tree roots (Díaz-Pinés et al., 2010; Fekete et al., 2016). This can be particularly problematic during dry periods, during which R_S is suppressed on control plots, but not in trenched plots (Schindlbacher et al., 2009). In the current study, soil moisture was indeed higher in trenched plots, but the differences were comparably small throughout the study period (Fig. S2). With this regard, the insertion of a mesh instead of a foil to restrict root ingrowth could have had positive implications by allowing for some lateral water outflow from the trenched plots. On the other hand, the 0.149-mm nylon mesh likely was too wide to restrict any ingrowth of mycorrhizal mycelia, or even of very fine roots into the trenched plots (Heinemeyer et al., 2007; Yan et al., 2019). It has been shown that ectomycorrhizal fungal respiration can contribute significantly to R_A. Heinemeyer et al. (2007) found that ectomycorrhizal fungal respiration contributed 25% to the total forest soil CO_2 efflux in a temperate *Pinus contorta* forest and Yan et al. (2019) estimated that 41% of the rhizosphere respiration in larch plantations was mycorrhizal respiration. Hence an ingrowth of fine roots and/or mycorrhizal hyphen throughout the three years study would have caused an underestimation of R_A. However, soil samples in 2016 were free of fine roots, indicating that the mesh was too fine for root ingrowth and the relatively stable annual contributions of R_A throughout the three study years also suggest that mycorrhizal hyphae ingrowth did not play a significant role (in the case of significant ingrowth and respiration, R_A estimates would have gradually declined over time). Another source of bias is respiration associated with the decomposition of cut-off roots (Hanson et al., 2000; Tang et al., 2016). If cut-off roots were still present and decomposing during the study, R_A would have been underestimated as well. The lower R_A contribution during the first study year, as well as the gradually increasing R_M in the *C. lanceolata* forest soil (Table 1) might be an indication for an - at least initial - contribution of dead root respiration. However, the annual differences were small and only insignificant; indicating that dead root respiration did not cause significant bias, and that most of the (at least labile) root C was already decomposed during the eight months equilibration period prior to the experiment.

The mineral soil associated heterotrophic respiration (R_H) rates of 39 and 55% respectively, lay well within those from other subtropical forest ecosystems (Yi et al., 2007; Wang et al., 2017; Liu et al., 2019b). The similarly high R_H fluxes (457 and 520 g C m^{-2} yr^{-1}) among the two forests suggest a similar C loss by mineral SOM decomposition. However, litter C input was significantly lower in the *C. lanceolata* forest as were fine root biomass and R_A. Accordingly, the conversion of the secondary *C. carlesii* forest into a *C. lanceolata* plantation might lead to a net loss of mineral soil carbon. Such a C loss was supported by the ~x223C 30% reduction in C concentrations of the mineral topsoil C at the same sites, which were reported by Ni et al. (2021). However, in the current study, the SOC contents were only insignificantly lower in the *C. lanceolata* mineral soil (Table 2).

Contrary to our hypothesis, the R_M estimates from the combined NRNL treatment (Eq. 4) did not correspond with the R_M estimates that were calculated by adding the individual NL and NR treatment effects (Eq. 3). In the *C. carlesii* forest, the soil CO_2 effluxes in NRNL were nearly identical to those in NR and NL, and in the *C. lanceolata* plantation NRNL fluxes were as well only insignificantly lower than NR fluxes. We can only speculate about the reasons why the combined plant C input exclusion treatment showed much weaker responses than anticipated. In the *C. carlesii* forest, microbial biomass in NRNL was close to that of the NL treatment (but significantly lower than in CT and NR). In the *C. lanceolata* plantation, the microbial biomass in NRNL was almost identical with the microbial biomass in NL and NR (Table 3). It, therefore, seems that soil microbes in the NRNL plots of both forests sustained their biomass through a higher utilization of mineral SOM. This would also explain the correspondingly high soil CO_2 fluxes, as it was collaborated by the SEM, which clearly suggested microbial biomass as the main predictor of respiration rates.

4.2. Plant C input and soil microbial community

Soil microbial biomass was significantly lower in the *C. lanceolata* as well as the F:B ratio, which serves as an important functional indicator with regard to soil C cycling (Malik et al., 2016). Whereas the reason for the lower microbial biomass likely is the lower labile C input (see above), the reasons for the lower F:B ratio in the soil of the coniferous plantation remain open. Similar to our observations Lin et al. (2019), Wan et al. (2015) and Stefanowicz et al. (2021), as well found that fungi dominated over bacteria in soil of a broadleaved forest of *Castanopsis eyrei*, whereas the differences between fungal and bacterial biomasses were less pronounced in *C. lanceolata* soil. A potential reason for the variations in F:B ratios among the forest types could be the tree species specific mycorrhizal associations (AM with *C. lanceolata* and EM with *C. carlesii*) (Stefanowicz et al., 2021). Furthermore, in the *C. lanceolata* forest, a significant proportion of the fungal biomass could have been located in the litter and organic layer, which were not reflected in our mineral soil analyses.

As hypothesized, litterfall and root exclusion significantly reduced the microbial biomass and changed the microbial community composition in both forests. The results are consistent with most studies indicating that both, litterfall and root C-input (i.e., exudates), are import C sources for soil microbes (Kuzyakov and Cheng, 2001; Jones et al., 2004; Feng and Simpson, 2009). However, the microbial community composition in NR and NRNL differed from that in CT and NL in the *C. lanceolata* plantation, while the microbial community composition of NL was different from those in CT, NRNL, and NR in the secondary *C. carlesii* forest (Fig. 3). This implies that the changes of microbial community composition were more pronouncedly influenced by root exclusion in the *C. lanceolata* plantation, and most pronouncedly affected by litter exclusion in the secondary *C. carlesii* forest (Table 3 and Fig. 3 PCA results).

In the *C. lanceolata* plantation, NR significantly increased the GP:GN bacteria ratio, while the GP:GN ratio was significantly decreased in the secondary *C. carlesii* forests. Previous studies showed that GP bacteria form oligotrophic communities preferentially use recalcitrant C fractions under low nutrient availability, while GN bacteria are favored in soils with high nutrient content (Zechmerster-Boltenstern et al., 2015; Zhou et al., 2017). However, with this regard, the increased GP:GN ratio contradict the higher availability of N (i.e., DON, NO_3^--N) in NR in the *C. lanceolata* plantation, and the decreased GP:GN ratios in the secondary *C. carlesii* forest contradict the reduced N availability in the NR plots of this forest. A possible explanation is that soil microbes were co-limited by other elements rather than N. However, DOC contents did not show any significant relationship with GP:GN ratios in our study, though they were significantly negatively correlated with respiration rates (Fig. 3). A decrease in DOC contents typically intensifies the competition among microbes for available C (Blagodatskaya et al., 2014). Kieft et al. (1994) reported an increase in the Sat:Mono ratio when gram-negative bacteria faced C starvation. Therefore, this ratio has frequently been used as microbial stress indicator (Bardgett et al., 1996; Li et al., 2020). However, though the Sat:Mono ratios were increased in the NR treatment of the *C. lanceolata* forest, there was no general relationship between DOC and Sat:Mono ratios in the present study (Fig. 3). Actually, the Sat:Mono ratios could have been expected highest in the lowest plant C input treatment, but there was no significant difference between NRNL and CT in both forests, suggesting that the microbial population was not exposed to physiological stress.

It has to be noted that the microbial community and microbial biomass analyses were a point in time assessment three years after starting the plant C input manipulations. It has been extensively shown that microbial biomass as well as community composition can change significantly throughout seasons (Schindlbacher et al., 2011; Mellado-Vázquez et al., 2019; Fu et al., 2020) and that these changes can even depend on the tree species present in a specific forest stand (Thoms et al., 2013). Therefore, our microbial analyses rather represent a starting point and further research is required to better understand the interactions of plant C inputs and soil microbial functioning in the studied forests ecosystems.

5. Conclusion

The conversions of a secondary *C. carlesii* forest into a *C. lanceolata* plantation changed above and below ground plant C inputs. These changes affected the different soil respiration components (R_L, R_A, and R_M). Three years of intensive measurements of soil CO_2 fluxes from litter and root exclusion plots suggest contributions of R_M, R_A, and R_L in the range of 55%, 29%, and 16% under *C. lanceolata*, and of 39%, 32%, and 29% under *C. carlesii*, respectively. Lower R_L than above ground C litter input, and comparable high R_M rates indicate that the *C. lanceolata* plantation accumulated C in the litter layer or surface humus, but potentially lost C in the mineral soil. The strong reductions of plant C inputs in the combined litter and root exclusion treatment (NRNL) were not reflected in the soil respiration rates, which remained at levels, similar to those of single root exclusion (NR). This suggests that the microbial community had increased the decomposition of mineral soil C under the combined and most severe plant C input reduction. As a conclusion, accelerated soil C loss can be expected if C input declines sharply, e.g. under stand-replacing disturbance events in both forest types, but particularly under *C. lanceolata*.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.agrformet.2021.108671.

References

Bahram, M., Netherway, T., Hildebrand, F., Pritsch, K., Drenkhan, R., Loit, K., Anslan, S., Bork, P., Tedersoo, 2020. Plant nutrient-acquisition strategies drive topsoil microbiome structure and function. New Phytol. 227 (4), 1189–1199.

Bardgett, R.D., Hobbs, P.J., Frostegard, A., 1996. Changes in soil fungal:bacterial biomass ratios following reductions in the intensity of management of an upland grassland. Biol. Fert. Soils. 22 (3), 261–264.

Barea, J.M., Pozo, M., Azcón, R., Azcon-Aguilar, C., 2005. Microbial co-operation in the rhizosphere. J. Exp. Bot. 56 (417), 1761–1778.

Bastos, A., Fleischer, K., 2021. Effects of rising CO_2 levels on carbon sequestration are coordinated above and below ground. Nature 591, 532–534.

Blagodatskaya, E., Khomyakov, N., Myachina, O., Bogomolova, I., Blagodatsky, S., Kuzyakov, Y., 2014. Microbial interactions affect sources of priming induced by cellulose. Soil Biol. Biochem. 74, 39–49.

Carter, M.R., Gregorich, E.G., 2006. Soil sampling and methods of analysis. Can. J. Soil Sci., Chap. 19, 207–214.

Chen, X., Chen, H.Y.H., 2018. Global effects of plant litter alterations on soil CO_2 to the atmosphere. Glob. Change Biol. 24 (8), 3462–3471.

Creamer, C.A., Menezes, A.B.D., Krull, E.S., Sanderman, J., Farrell, M., 2015. Microbial community structure mediates response of soil C decomposition to litter addition and warming. Soil Biol. Biochem. 80, 175–188.

Crowther, T.W., Todd-Brown, K.E.O., Rowe, C.W., Wieder, W.R., Carey, J.C., Machmuller, M.B., Snoek, B.L., Fang, S., Zhou, G., Allison, S.D., et al., 2016. Quantifying global soil carbon losses in response to warming. Nature 540 (7631), 104–108.

Denef, K., Roobroeck, D., Wadu, M.C.W.M., Lootens, P., Boeckx, P., 2009. Microbial community composition and rhizodeposit-carbon assimilation in differently managed temperate grassland soils. Soil Biol. Biochem. 41 (1), 144–153.
Díaz-Pinés, E., Schindlbacher, A., Pfeffer, M., Jandl, R., Zechmeister-Boltenstern, S., Rubio, A., 2010. Root trenching: a useful tool to estimate autotrophic soil respiration? A case study in an Austrian mountain forest. Eur. J. For. Res. 129 (1), 101–109.
Eastman, B.A., Adams, M.B., Brzostek, E.R., Burnham, M.B., Carrara, J.E., Kelly, C., McNeil, B.E., Walter, C.A., Peterjohn, W.T., 2021. Altered plant carbon partitioning enhanced forest ecosystem carbon storage after 25 years of nitrogen additions. New Phytol. 230 (4), 1435–1448.
Fan, J., Wang, J.S., Zhao, L.H., Wu, C.Y., Zhang, X.H., 2015. Effects of manipulated above- and belowground organic matter input on soil respiration in a Chinese Pine plantation. PLoS One 10 (5), e0126337.
Fanin, N., Alavoine, G., Bertrand, I., 2020. Temporal dynamics of litter quality, soil properties and microbial strategies as main drivers of the priming effect. Geoderma 337, 114576.
Fekete, I., Kotroczó, Z., Varga, C., Nagy, P.T., Várbíró, G., Bowden, R.D., Tóth, J.A., Lajtha, K., 2014. Alterations in forest detritus inputs influence soil carbon concentration and soil respiration in a Central-European deciduous forest. Soil Biol. Biochem. 74, 106–114.
Fekete, I., Varga, C., Biró, B., Tóth, J.A., Várbíró, G., Lajtha, K., Szabó, G., Kotroczó, Z., 2016. The effects of litter production and litter depth on soil microclimate in a central european deciduous forest. Plant Soil 398 (1-2), 291–300.
Feng, X., Simpson, M.J., 2009. Temperature and substrate controls on microbial phospholipid fatty acid composition during incubation of grassland soils contrasting in organic matter quality. Soil Biol. Biochem. 41 (4), 804–812.
Frostegard, A., Tunlid, A., Baath, E., 2011. Use and misuse of PLFA measurements in soils. Soil Biol. Biochem. 43 (8), 1621–1625.
Fu, D., Wu, X., Qiu, Q., Duan, C., Jones, D.L., 2020. Seasonal variations in soil microbial communities under different land restoration types in a subtropical mountains region. Southwest China Appl. Soil Ecol. 153, 103634.
Gao, D., Joseph, J., Werner, R.A., Brunner, I., Zürcher, A., Hug, C., Wang, A., Zhao, C., Bai, E., Meusburger, K., Gessler, A., Hagedorn, F., 2021. Drought alters the carbon footprint of trees in soils-tracking the spatio-temporal fate of ^{13}C-labelled assimilates in the soil of an old-growth pine forest. Glob. Change Biol. 27 (11), 2491–2506.
Giardina, C.P., Ryan, M., 2002. Total belowground carbon allocation in a fast-growing eucalyptus plantation estimated using a carbon balance approach. Ecosystems 5 (5), 487–499.
Hanson, P.J., Edwards, N.T., Garten, C.T., Andrews, J.A., 2000. Separating root and soil microbial contributions to soil respiration: a review of mrthods and observations. Biogeochemistry 48 (1), 115–146.
Haug, I., Weber, R., Oberwinkler, F., Tschen, J., 1994. The mycorrhizal status of Taiwanese trees and the description of some ectomycorrhizal types. Trees 8 (5), 237–253.
Huang, W., Spohn, M., 2015. Effects of long-term litter manipulation on soil carbon, nitrogen, and phosphorus in a temperate deciduous forest. Soil Biol. Biochem. 83, 12–18.
Jones, D.L., Hodge, A., Kuzyakov, Y., 2004. Plant and mycorrhizal regulation of rhizodeposition. New Phytol. 163 (3), 459–480.
Jones, D.L., Willett, V.B., 2006. Experimental evaluation of methods to quantify dissolved organic nitrogen (DON) and dissolved organic carbon (DOC) in soil. Soil Biol. Biochem. 38 (5), 991–999.
Kieft, T.L., Ringelberg, D.B., White, D.C., 1994. Changes in ester-linked phospholipid fatty acid profiles of subsurface bacteria during starvation and desiccation in a porous medium. Appl. Environ. Microb. 60 (9), 3292–3299.
Kramer, C., Gleixner, G., 2008. Soil organic matter in soil depth profiles: Distinct carbon preferences of microbial groups during carbon transformation. Soil Biol. Biochem. 40 (2), 425–433.
Kuzyakov, Y., Cheng, W., 2001. Photosynthesis controls of rhizosphere respiration and organic matter decomposition. Soil Biol. Biochem. 33 (14), 1915–1925.
Lefcheck, J.S., 2016. piecewiseSEM: Piecewise structural equation modelling in r for ecology, evolution, and systematics. Methods Ecol. Evol. 7 (5), 573–579.
Li, XJ., Xie, JS., Zhang, QF., Lyu, MK., Xiong, XL., Liu, XF., Lin, T., Yang, YS., 2020. Substrate availability and soil microbes drive temperature sensitivity of soil organic carbon mineralization to warming along an elevation gradient in subtropical Asia. Geoderma 164, 114198.
Lin, D., Pang, M., Fanin, N., Wang, H., Qian, S., Zhao, L., Yang, Y., Mi, X., Ma, K., 2019. Fungi participate in driving home-field advantage of litter decomposition in a subtropical forest. Plant Soil 434 (1), 467–480.
Liu, BT., Li, HB., Zhu, B., Koide, RT., Eissenstat, DM., Guo, DL., 2015. Complementarity in nutrient foraging strategies of absorptive fine roots and arbuscular mycorrhizal fungi across 14 coexisting subtropical tree species. New Phytol. 208 (1), 125–136.
Liu, J., Wu, N., Wang, H., Sun, J., Peng, B., Jiang, P., Bai, E., 2016. Nitrogen addition affects chemical compositions of plant tissues, litter and soil organic matter. Ecology 97 (7), 1796–1806.
Liu, X.F., Lin, T.C., Yang, Z.J., Vadeboncoeur, M.A., Lin, C.F., Xiong, D.C., Lin, W.S., Chen, G.S., Xie, J.S., Li, Y.Q., Yang, Y.S., 2017. Increased litter in subtropical forests boosts soil respiration in natural forests but not plantations of Castanopsis carlesii. Plant Soil 418 (1), 141–151.
Liu, X.F., Liu, T.C., Vadeboncoeur, M.A., Yang, Z.J., Chen, S.D., Xiong, D.C., Xu, C., Li, Y. Q., Yang, Y.S., 2019a. Root litter inputs exert greater influence over soil C than does aboveground litter in a subtropical natural forest. Plant Soil 444 (1), 489–499.
Liu, X.F., Chen, S.D., Yang, Z.J., Lin, C.F., Xiong, D.C., Lin, W.S., Xu, C., Chen, G.S., Xie, J.S., Li, Y.Q., Yang, Y.S., 2019b. Will heterotrophic soil respiration be more sensitive to warmingthan autotrophic respiration in subtropical forests? Eur. J. Soil. Sci. 70 (3), 655–663.
Lyu, M.K., Li, X.J., Xie, J.S., Homyak, P.M., Ukonmaanaho, K., Yang, Z.J., Liu, X.F., Ruan, C.Y., Yang, Y.S., 2019a. Root-microbial interaction accelerates soil nitrogen depletion but not soil carbon after increasing litter inputs to a coniferous forest. Plant Soil 444 (1), 153–164.
Lyu, M.K., Nie, Y.Y., Giardina, C.P., Vadeboncoeur, M.A., Ren, Y.B., Fu, Z.Q., Wang, M. H., Jin, C.S., Liu, X.M., Xie, J.S., 2019b. Litter quality and site characteristics interact to affect the response of priming effect to temperature in subtropical forests. Funct. Ecol. 33 (11), 2226–2238.
Mayer, M., Prescott, C.E., Abaker, W.E., Augusto, L., Cécillon, L., Ferreira, G.W., James, J., Jandl, R., Katzensteiner, K., Laclau, J.P., Laganière, J., Nouvellon, Y., Paré, D., Stanturf, J.A., Vanguelova, E.I., Vesterdal, L., 2020. Influence of forest management activities on soil organic carbon stocks: a knowledge synthesis. For. Ecol. Manage. 466, 118127.
Malik, A.A., Chowdhury, S., Schlager, V., Oliver, A., Puissant, J., Vazquez, P.G.M., Jehmlich, N., von Bergen, M., Griffiths, R.I., Gleixner, G., 2016. Soil Fungal:Bacterial Ratios Are Linked to Altered Carbon Cycling. Front. Microbiol. 7, 1247.
Mellado-Vázquez, P.G., Lange, M., Gleixner, G., 2019. Soil microbial communities and their carbon assimilation are affected by soil properties and season but not by plants differing in their photosynthetic pathways (C3 vs. C4). Biogeochemistry 142 (2), 175–187.
Ni, X.Y., Lin, C.F., Chen, G.S., Xie, J.S., Yang, Z.J., Liu, X.F., Xiong, D.C., Xu, C., Yue, K., Wu, F.Z., Yang, Y.S., 2021. Decline in nutrient inputs from litterfall following forest plantation in subtropical China. For. Ecol. Manage. 496, 119445.
Nemergut, D.R., Cleveland, C.C., Wieder, W.R., Washenberger, C.L., Townsend, A.R., 2010. Plot-scale manipulations of organic matter inputs to soils correlate with shifts in microbial community composition in a lowland tropical rain forest. Soil Biol. Biochem. 42 (12), 2153–2160.
Pan, Y., Birdsey, R.A., Fang, J.Y., Houghton, R., Kauppi, P.E., Kuraz, W.A., Phillips, O.L., Shvidenko, A., Lewis, S.L., Canadell, J.P., Ciais, P., Jackson, R.B., Pacala, S.W., McGuire, A.D., Piao, S.L., Rautiainen, A., Sitch, S., Hayes, D., 2011. A large and persistent carbon sink in the world's forests. Science 333 (6045), 988–993.
Pisani, O., Lin, L.H., Lun, O.O.Y., Lajtha, K., Nadelhoffer, K.J., Simpson, A.J., Simpson, M.J., 2016. Long-term doubling of litter inputs accelerates soil organic matter degradation and reduces soil carbon stocks. Biogeochemistry 127 (1), 1–14.
R Core Team, 2019. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Retrieved from. https://www.R-project.org/.
Raich, J.W., Potter, C.S., Bhagawati, D., 2002. Interannual variability in global soil respiration, 1980–94. Glob. Change Biol. 8 (8), 800–812.
Sasse, J., Martinoia, E., Northen, T., 2018. Feed your friends: do plant exudates shape the root microbiome? Trends Plant Sci. 23 (1), 25–41.
Sayer, E.J., Heard, M.S., Grant, H., Marthews, T.R., Tanner, E.V.J., 2011. Soil carbon release enhanced by increased tropical forest litterfall. Nat. Clim. Change 1 (6), 304–307.
Stefanowicz, A.M., Rożek, K., Stanek, M., Rola, K., Zubek, S., 2021. Moderate effects of tree species identity on soil microbial communities and soil chemical properties in a common garden experiment. For. Ecol. Manage. 482, 118799.
Schindlbacher, A., Zechmeister-boltenstern, S., Jandl, R., 2009. Carbon losses due to soil warming: do autotrophic and heterotrophic soil respiration respond equally? Glob. Change Biol. 15 (4), 901–913.
Schindlbacher, A., Rodler, A., Kuffner, M., Kitzler, B., Sessitsch, A., Zechmeister-Boltenstern, S., 2011. Experimental warming effects on the microbial community of a temperate mountain forest soil. Soil Biol. Biochem. 43 (7), 1417–1425.
Stephenson, N.L., Das, A.J., 2020. Height-related changes in forest composition explain increasing tree mortality with height during an extreme drought. Nat. Commun. 11 (1), 1–4.
Subke, J.A., Inglima, I., Francesca Cotrufo, M., 2006. Trends and methodological impacts in soil CO_2 efflux partitioning: a metaanalytical review. Glob. Change Biol. 12 (6), 921–943.
Suseela, V., Tharayil, N., 2018. Decoupling the direct and indirect effects of climate on plant litter decomposition: Accounting for stress-induced modifications in plant chemistry. Glob. Chang Biol. 24 (4), 1428–1451.
Swallow, M.J.B., Quideau, S.A., Mackenzie, M.D., Kishchuk, B.E., 2009. Microbial community structure and function: the effect of silvicultural burning and topographic variability in northern Alberta. Soil Biol. Biochem. 41 (4), 770–777.
Tang, X., Fan, S., Qi, L., Guan, F., Du, M., Zhang, H., 2016. Soil respiration and net ecosytem production in relation to intensive management in Moso bamboo forests. Catena 137, 219–228.
Tedersoo, L., Bahram, M., 2019. Mycorrhizal types differ in ecophysiology and alter plant nutrition and soil processes. Biol. Rev. 94 (5), 1857–1880.
Templer, P.H., Findlay, S., Lovett, G.M., 2003. Soil microbial biomass and nitrogen transformations among five tree species of the Catskill Mountains, New York, USA. Soil Biol. Biochem. 35 (4), 607–613.
Thoms, C., Gleixner, G., 2013. Seasonal differences in tree species' influence on soil microbial communities. Soil Biol. Biochem. 66, 239–248.
Ushio, M., Balser, T.C., Kitayama, K., 2013. Effects of condensed tannins in conifer leaves on the composition and activity of the soil microbial community in a tropical montane forest. Plant Soil 365 (1), 157–170.
Wan, X.H., Huang, Z.Q., He, Z.M., Yu, Z.P., Wang, M.H., Davis, M.R., Yang, Y.S., 2015. Soil C:N ratio is the major determinant of soil microbial community structure in subtropical coniferous and broadleaf forest plantations. Plant Soil 387 (1), 103–116.
Wang, Q., He, T., Wang, S., Liu, L., 2013. Carbon input manipulation affects soil respiration and microbial community composition in a subtropical coniferous forest. Agr. For. Meteorol. 178, 152–160.

Wang, Q., Yu, Y., He, T., Wang, Y., 2017. Aboveground and belowground litter have equal contributions to soil CO_2 emission: an evidence from a 4-year measurement in a subtropical forest. Plant Soil 421 (1), 7–17.
Weand, M.P., Arthur, M.A., Lovett, G.M., Mcculley, R.L., Weathers, K.C., 2010. Effects of tree species and N additions on forest floor microbial communities and extracellular enzyme activities. Soil Biol. Biochem. 42 (12), 2161–2173.
Wei, T., 2016. Package 'corrplot'. Statistician 56, 316–324. https://github.com/taiyun/corrplot.
Williams, A, De Vries, F.T., 2020. Plant root exudation under drought: implications for ecosystem functioning. New Phytol. 225 (5), 1899–1905.
Wu, J.J., Zhang, D.D., Chen, Q., Feng, J., Li, Q., Fan, Y., Zhang, Q., Cheng, X.L., 2018. Shifts in soil organic carbon dynamics under detritus input manipulations in a coniferous forest ecosystem in subtropical China. Soil Biol. Biochem. 126, 1–10.
Xu, M., Shang, H., 2016. Contribution of soil respiration to the global carbon equation. J. Plant Physiol. 203, 16–28.
Yan, T., Qiu, T,T., Song, H,H., Sun, Z.Z., Zeng, H., Peng, S,S., 2019. Ectomycorrhizal fungi respiration quantification and drivers in three differently-aged larch plantations. Agric. For. Meteorol. 265, 245–251.
Yi, Z., Fu, S., Yi, W., Zhou, G., Mo, J., Zhang, D., Ding, M., Wang, X., Zhou, L., 2007. Partitioning soil respiration of subtropical forests with different successional stages in south China. For. Ecol. Manage. 243 (2-3), 178–186.
Zechmerster-Boltenstern, S., Keiblinger, K.M., Mooshammer, M., Penuelas, J., Richter, A., Sardans, J., Wanek, W., 2015. The application of ecological stoichiometry to plantemicrobialesoil organic matter transformations. Ecol. Monogr. 85 (2), 133–155.
Zhang, Q.F., Zhou, J.C., Li, X.J., Yang, Z.J., Zheng, Y., Wang, J., Lin, W.S., Xie, J.S., Chen, Y.M., Yang, Y.S., 2019. Are the combined effects of warming and drought on foliar C:N:P:K stoichiometry in a subtropical forest greater than their individual effects? For. Ecol. Manage 448, 256–266.
Zhou, Z.H., Wang, C.K., Jiang, L.F., Luo, Y.Q., 2017. Trends in soil microbial communities during secondary succession. Soil Biol. Biochem. 115, 92–99.