Contrasting responses of soil fungal communities and soil respiration to the above- and below-ground plant C inputs in a subtropical forest

Article *in* European Journal of Soil Science · December 2018

DOI: 10.1111/ejss.12777

CITATION
1

READS
254

6 authors, including:

Lingling Shi
Georg-August-Universität Göttingen
25 PUBLICATIONS **136** CITATIONS
SEE PROFILE

Wenting Feng
Chinese Academy of Agricultural Sciences
23 PUBLICATIONS **265** CITATIONS
SEE PROFILE

Xin Jing
KU Leuven
36 PUBLICATIONS **396** CITATIONS
SEE PROFILE

Huadong Zang
China Agricultural University
33 PUBLICATIONS **129** CITATIONS
SEE PROFILE

Contrasting responses of soil fungal communities and soil respiration to the above- and below-ground plant C inputs in a subtropical forest

Fungi mediate soil respiration without plant C

L. L. SHI [a, b], W. T. FENG [c *], X. JING [d,e], H. D. ZANG [a], P. MORTIMER [b] & X. M. ZOU [f]

[a] Department of Agricultural Soil Science, University of Göettingen, 37077, Göettingen, Germany

[b] Key Laboratory for Plant Diversity and Biogeography of East Asia, Kunming Institute of Botany, Chinese Academy of Sciences, Heiongtan, 650201, Kunming, Yunnan, China

[c] National Engineering Laboratory for Improving Quality of Arable Land, Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, Beijing 100081, China

[d] Rubenstein School of Environment and Natural Resources, University of Vermont, Burlington, VT 05405, USA

[e] Gund Institute for Environment, University of Vermont, Burlington, VT05405, USA

[f] Department of Environmental Sciences, University of Puerto Rico, P.O. Box 70377, San Juan, PR 00931-8377, USA

Correspondence: W. T. FENG. E-mail: fengwenting@caas.cn

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/ejss.12777

Summary

The roles of soil fungal diversity and community composition in regulating soil respiration when above- and below-ground plant carbon (C) inputs are excluded remain unclear. In the present study, we aimed to examine: (i) how does the exclusion of above- and below-ground plant C inputs affect soil respiration and soil fungi singly and in combination? and (ii) are changes in soil fungal diversity aligned with changes in soil respiration? A field experiment with manipulation of plant C inputs was established in a subtropical forest in southwest China in 2004 with litter removal and tree stem-girdling to exclude inputs of the above- and below-ground plant C, respectively. In 2009, we measured the rates of soil respiration with an infrared gas analyser and soil fungal community structure using Illumina sequencing. We found that the rates of soil respiration were reduced significantly by litter removal and girdling, by similar magnitudes. However, they were not decreased further by the combination of these two treatments compared to either treatment alone. In contrast, litter removal increased the diversity of soil fungal communities, whereas girdling decreased the abundance of symbiotrophic fungi but increased the abundance of saptrotrophic and pathotrophic fungi. These changes in soil fungal community might initiate CO_2 emission from soil C decomposition, offsetting further decline in soil respiration when plant C inputs are excluded. These results revealed that the exclusion of the above- and below-ground plant C inputs led to contrasting soil fungal communities but similar soil function. Our findings suggest that both above- and below-ground plant C are important in regulating soil respiration in subtropical forests, by limiting substrates for soil fungal growth and altering the diversity and composition of soil fungal community.

Keywords: biodiversity, girdling, Illumina sequencing, litter removal, saprotroph, soil respiration, symbiotroph

Highlights

- Litter removal and girdling decreased soil respiration by similar magnitudes
- The combination of litter removal and girdling did not further decrease soil respiration
- Litter removal significantly increased species richness of soil fungal communities
- Girdling changed the abundance of functional guilds of soil fungal communities

Introduction

Plant carbon (C) is an important substrate for soil fungi, therefore, changes in the above- and below-ground plant C inputs can alter soil fungal community composition and functioning, such as soil C decomposition which is reflected by soil respiration (Hanson *et al.*, 2000; Högberg *et al.*, 2001; Schaefer *et al.*, 2009; Yarwood *et al.*, 2009). The quantity and quality of above- and below-ground plant C in forests are increasingly threatened by global changes in for example elevated carbon dioxide (CO_2) and nitrogen (N) deposition and management, such as harvesting of non-timber forest products (Jackson *et al.*, 2009; Li *et al.*, 2015). A greater understanding of how changes in plant C inputs regulate soil respiration and fungal communities will help to predict how soil C decomposition responds to global changes.

It remains unclear to what extent changes in above- and below-ground plant C inputs affect soil respiration, and whether this occurs by altering the soil fungal community or soil properties. Litter manipulation treatments, such as litter removal and girdling, not only change the supply of substrates for soil fungi but also alter the soil fungal community and soil properties (Högberg *et al.*, 2001; Yarwood *et al.*, 2009; Wang *et al.*, 2013). These changes in biotic and abiotic factors further influence soil respiration. Plant leaf litter and roots have different chemical compositions (Kögel-Knabner, 2002) and provide various substrates for soil fungi, resulting in different rates of decomposition of soil organic C. In general, belowground plant C is more important for soil C decomposition than that aboveground, because fresh photosynthates transported to roots stimulate rhizosphere microbes and enhance decomposition of complex C compounds (Yarwood *et al.*, 2009; Pausch & Kuzyakov, 2017). To compare the effects of the above- and below-ground plant C inputs, they must be assessed in the same ecosystem. However, a few studies only have examined

the effects of both above- and below-ground plant C exclusion simultaneously on the soil fungal community and soil respiration (Yarwood *et al.*, 2009; Wang *et al.*, 2013).

Changes in above- and below-ground plant C also alter the composition and diversity of soil fungi and further influence C decomposition. Increases in soil fungal diversity are assumed to enhance C decomposition because of niche differentiation and complementary substrate use of the soil fungal community (van der Wal *et al.*, 2013). Disturbances might increase soil fungal diversity according to the intermediate disturbance hypothesis (Connell, 1978), resulting in enhanced soil respiration which indicates increasing soil C decomposition. Litter manipulation treatments (e.g. litter removal and trenching) usually disturb the soil and lead to large fluctuations in soil microclimate (e.g. moisture and temperature) (Sayer, 2006; Schaefer *et al.*, 2009; Chen *et al.*, 2010). Under these circumstances, it is not clear whether increases in soil fungal diversity offset declines in soil respiration when above- and below-ground plant C inputs are excluded. Meanwhile, with the manipulation of plant litter, diversity of soil fungal community is complicated by changes in soil properties. Removal of plant litter reduces soil C availability and changes soil pH and soil organic matter composition (Sayer, 2006), whereas tree stem girdling and root trenching increase N availability and soil moisture (Brant *et al.*, 2006; Feng *et al.*, 2009; Chen *et al.*, 2012). To advance our understanding of the effects of plant C inputs on soil respiration, we need to investigate the changes in biotic and abiotic factors associated with the manipulations of litter.

In addition, the limitations of techniques also impede our understanding of how soil fungi mediate soil respiration with changes in plant C inputs. Most of plant C manipulation used the phospholipid-derived fatty acids (PLFA) method to assess soil microbial composition and diversity (Table 1). This method has shown divergent

responses to plant C manipulation. It determines rough categories of fungal community, but cannot identify the entire profile of the soil fungal community or the huge number of rare fungal species (Frostegård *et al.*, 2011). In contrast, high-throughput sequencing techniques (e.g. Illumina sequencing) can detect millions of fungal DNA sequences, and together with bioinformatics databases (i.e. NCBI (https://www.ncbi.nlm.nih.gov/) and UNITE (https://unite.ut.ee/)) can provide a more informative profile of soil fungal community composition and diversity (Zhou *et al.*, 2015). However, there is a lack of research that uses DNA sequencing methods to examine changes in the soil fungal community with manipulation of above- and below-ground plant C inputs.

A long-term experiment involving plant C manipulation has been carried out in a subtropical montane evergreen forest in southwest China since 2004. Litter removal, tree stem-girdling and root trenching treatments were used to exclude the above- and below-ground plant C inputs (Högberg *et al.*, 2001; Li *et al.*, 2004; Brant *et al.*, 2006). Taking advantage of this experiment, we measured simultaneously soil respiration, soil properties and the diversity and composition of the soil fungal community. We aimed to test: (i) How does soil respiration respond to the exclusion of above- and below-ground plant C inputs singly and in combination? Is soil respiration reduced further by the combination of above- and below-ground plant C exclusion compared to either treatment alone? (ii) Does soil fungal diversity increase with the exclusion of above- and below-ground plant C inputs? (iii) Do the responses of soil respiration to plant C manipulation treatments align with those of the soil fungal community? What are the potential links between them?

Materials and methods

Study site

The study site is located at the Ailao Field Station for Forest Ecosystem Studies (24°32′N, 101°01′E, 2476 m above sea level) in Yunnan Province, southwest China. It has a monsoon climate, with mean annual precipitation of 1840 mm and mean monthly temperature between 5.4 °C and 23.5 °C (Zhang *et al.*, 1983). The vegetation type is subtropical montane evergreen forest, dominated by the subtropical evergreen broad-leaved species *Lithocarpus jingdongensis* Y. C. Hsu et H. J. Qian, *Rhododendron leptothrium* Balf. f. & Forrest, *Vaccinium duclouxii* (Levl.) Hand. - Mazz., *Lithocarpus xylocarpus* (kurz) markg., *Castanopsis wattii* (King ex Hook. f.) A. Camus, *Schima noronhae* Reinw. ex Bl. Bijdr., *Hartia sinensis Dunn* in Hook. Ic. Pl. t. and *Manglietia insignis* (Wall.) Bl.. The annual plant litterfall varies from 5.4 to 8.2 t dry matter ha^{-1} $year^{-1}$ with the average of 7.1 t dry matter ha^{-1} $year^{-1}$ (Liu *et al.*, 2003). The soil is classified as an Alfisol (Chan *et al.*, 2006).

Experimental design

This study used a split-plot design to exclude above- and below-ground plant C inputs (Figure 1). Tree stem-girdling and root trenching were designed to exclude below-ground plant C inputs to soil. Litter removal was designed to exclude the aboveground plant C inputs to soil. Four pairs of plots (each 20 m × 20 m) with 32 subplots (2 m × 3 m) were established in early February 2004. Specifically, four plots were randomly selected for the stem girdling (G) treatment and the other four plots had no girdling treatment. Litter removal (LR), root trenching (RT) and their combination were conducted in each of the eight main plots. These manipulations generated eight treatments: CCK (control), CNL (control, litter removal), CNR (control, root trenching), CNLR (control, litter removal + root trenching), GCK (girdling), GNL (girdling, litter removal), GNR (girdling, root trenching) and GNLR (girdling, litter removal + root trenching). All the subplots were selected to avoid woody stems inside

the 2 m × 3 m subplots. In the four girdled plots, a 5-cm wide band was peeled down to the xylem for each tree (diameter >2 cm) at breast height (Högberg *et al.*, 2001). The girdled plots and their perimeters were trenched down to 40-cm soil depth, and the trenches were lined with plastic sheets and refilled with soil (Liu & Zou, 2002). In the girdled plots, grasses and shrubs in the understory were kept intact. In the subplots of litter removal, wooden structures covered with a 1-mm nylon mesh screen were erected at a height of 1 m to intercept above-ground plant litter. The organic layer (~2 cm) above the mineral soil was removed, and grass and shrubs growing in the litter removal subplots were cleared. The perimeters of the root-trenching subplots were trenched as described above for the girdled plots. Although both trenching and girdling are designed to exclude the below-ground plant C inputs to the soil, they are based on different mechanisms. Root trenching causes a sudden cut-off of root C inputs, whereas stem girdling results in a gradual reduction of the below-ground C inputs and does not disturb the soil's physical structure substantially (Högberg *et al.*, 2001). The latter is more appropriate for examining the effects of photosynthates transported belowground on soil microbes, in particular the fungi that rely greatly on root exudates.

Field sampling and laboratory analyses

We collected 32 soil samples, one from each subplot, in December 2009. In each subplot (2 m × 3 m), three soil cores (2.5-cm diameter) were taken randomly from the mineral soil at 0–10-cm depth and then pooled as one composite sample. The samples were passed through a 2-mm sieve, and visible plant roots that passed through the sieve were removed with tweezers. Subsamples were immediately transported to the laboratory in Kunming, Yunnan province and stored at −20 °C for DNA extraction

and further sequencing analyses. The remaining samples were used for soil chemical analyses.

We measured soil respiration the day before soil sampling using a portable infrared gas analyser (LI-COR 820, Lincoln, NE, USA). At the same time, we measured soil temperature at five random locations around the respiration chambers (25-cm diameter × 30-cm height) at 5-cm depth, using a digital thermometer.

Soil microbial biomass C (MBC) was assessed using the fumigation incubation method, which is based on the difference in CO_2 released between fumigated and unfumigated samples during the first 10 days of laboratory incubation. Soil microbial biomass C was calculated using a correction coefficient (k_C) of 0.45, the value commonly used for forest soil (Jenkinson, 1976). Based on the MBC measurement described above, we determined soil labile organic C (LOC) using the sequential chloroform fumigation–incubation method (Zou *et al.*, 2005; Feng *et al.*, 2011). Soil pH was measured with a pH meter (PHS-3C; Shanghai Precision & Scientific Instrument, Shanghai, China) after homogenizing fresh soil samples in water to a saturated colloid (water: soil ratio =2.5). A subset of fresh soil (15–20 g) was oven dried at 105 °C for 24 hours to determine soil moisture. Soil total organic C content was analysed using the chromic acid wet oxidation method. Total N was detected using an Auto Kjeldahl Unit model K370 (BUCHI, Flawil, Switzerland). Hydrolysable N was converted to ammonium by reacting with iron (II) sulphate and sodium hydroxide using a diffusion procedure. Soil ammonium (NH_4^+-N) and nitrate (NO_3^--N) concentrations were determined using indophenol and cadmium reduction methods, respectively (Allen & Tildesley, 1989).

DNA amplicon and Illumina sequencing of fungal communities

Soil DNA was extracted using the extraction Kit (Bio Mio Lab Inc., Solana Beach, CA, USA) by following the instructions of manufacturer. We amplified the ITS1 region using the primers ITS1F (5´-GAACCWGCGGARGGATCA-3´) and ITS2 (5´GCTGCGTTCTTCATCGATGC-3´). The amplifications of PCR were done in a 20-µl reaction mixture including 50 ng of DNA, 4 µl of HOT MOL Pol Blend Master Mix (Molegene, Germany), and 0.5 mM each of the primers. The reaction conditions were 15 minutes at 95°C, followed by 35 cycles of 30 s at 95 °C, 30 s at 52 °C, 55 °C or 58 °C and 30 s at 72 °C. The final PCR products were purified with Agencourt AM Pure XP SPRI magnetic beads (Beckman Coulter, Fullerton, CA, USA). The Illumina sequencing was carried out on an Illumina MiSeq sequencer at the Research and Testing Laboratory (http://www.researchandtesting.com) (RTL, Lubbock, TX, USA).

Bioinformatics analysis: we used the standard microbial analysis pipeline of the Research and Testing Laboratory. The raw reads in the format of FASTQ were obtained using the PEAR Illumin paried-end read merger. De-noising was carried out for the entire region of the barcoding fragment prior to performing any other steps of the bioinformatics analysis. Reads were run through an internally developed quality trimming algorithm. We kept those sequences with a Phred score > 25. The trimmed reads were then sorted from the longest to the shortest. To remove duplicated sequences, dereplication based on the prefixes of reads was performed using the USEARCH algorithm. Clustering at 4% divergence was performed using the USEARCH clustering algorithm. Sequences less than 10 bps in length were not saved to the output file.

Selection of operational taxonomic units (OTUs) was performed with the UPARSE OTU selection algorithm to classify clusters into OTUs. De novo chimeric

detection was performed for each OUT with the UCHIME algorithm (Edgar *et al.*, 2011). We excluded OTUs with less than 10 reads following a recommendation in the manual of the program OUT pipe wrapper (http://drive5.com/usearch/manual/). There were 1311 OTUs used in further classification analysis. Taxonomic assignment of representative sequences for each OTU was done against the UNITE + INSD + environment database of unite (http://unite.ut.ee/index.php) using the USEARCH global alignment algorithm. Once taxonomic classification was achieved, fungal OTUs were classed by their life style (i.e. mycorrhizal, saprotrophic, pathogen, lichen or unknown) according to FUNGuild v1.0 (Nguyen *et al.*, 2015). The FUNGuild v1.0 is a database hosted by Github (https: //github.com /UMNFuN/FUNGuild). The database currently contains a total of 9476 entries, with 66% at the genus level and 34% at the species level. We have classified our entries into three broad functional groupings referred to as trophic modes: pathotroph, symbiotroph and saprotroph (Tedersoo *et al.*, 2014).

In total, 1,041,411 raw sequences were obtained from the Illumina sequencing, and 1,006,403 of these were retained for further analysis after quality filtering, de-noising, the removal of short and chimera sequences and the removal of non-fungal sequences. An average of 13,541 and a minimum of 10,000 sequences were obtained per sample. After rarefaction, 10000 sequences of each sample were set aside for further analysis. The raw data have been deposited in SRA (NCBI) database with accession numbers SAMN05904657 to SAMN05904688.

Statistical analyses

We performed the analysis of variance (ANOVA) with multiple error terms to examine the main effects and interactions of the experimental treatments (e.g. litter removal, girdling and root trenching) on soil properties (i.e. respiration, soil total organic C,

MBC, LOC, total N, NH_4^+, NO_3^-, pH, temperature and moisture). In brief, the ANOVA was done with the aov.ftest function (https://github.com/pascal-niklaus/pascal) to calculate the correct *F* ratio and *P* values. We fitted the appropriate ANOVA models using a hierarchical structure to specify error strata (i.e. plot pair + girdling/(litter removal + root trenching)), with litter removal (two levels) and root trenching (two levels) nested within the girdling treatment (two levels). We used graphical displays to show any significant main effects or interactions.

To examine the main effects of litter removal, girdling, root trenching and their interactions on soil fungal community diversity and composition, we used the same statistical approach as described above (i.e. ANOVA with two error terms, main plot and split plot). First, we calculated biodiversity indices including species richness (the number of fungal operational taxonomic units, OTUs), effective number of species (exponent of Shannon–Wiener index) and Pielou's evenness (the ratio of Shannon–Wiener index to ln(species richness)). Species richness is the simplest measure of soil fungal diversity in this study, but it does not account for the relative abundance and the distribution of each species in a community. The effective number of species index avoids potential misinterpretations because of the nonlinearity between species richness and the Shannon–Wiener index (Jost, 2006). Pielou's evenness index is a measure of equability that does not take into account the number of species (i.e. species richness). Second, we fitted the Poisson log-normal abundance distribution to examine whether girdling, litter removal and root trenching influenced the patterns of composition of the soil fungal community. We fitted a generalized linear model with logarithmic link function using the rad.lognormal function of the vegan package in R (Oksanen, 2008). We estimated two parameters ($\ln \mu$ and $\ln \sigma$) of the Poisson log-normal distribution as follows:

$$a_i = \exp(\ln(\mu) + \ln(\sigma) \times N),$$

where a_i is the expected abundance of a species at rank i, μ and σ are the mean and standard deviation of the logarithm, respectively, and N is the normal deviate. Finally, we used ANOVA with multiple error terms to test whether girdling, litter removal, root trenching and their interactions significantly influenced soil fungal community diversity and composition (species richness, effective number of species, Pielou's evenness, lnμ, and lnσ). We used graphical displays to show any significant main effects or interactions. In addition, we performed nonmetric multidimensional scaling (NMDS) with the Bray–Curtis dissimilarity index using the metaDMS function to visualize changes in soil fungal community composition for each treatment (i.e. girdling, litter removal and root trenching). As described above, we used ANOVA with multiple error terms to examine the effects of girdling, litter removal, root trenching and their interactions on the abundances of soil fungal guilds (i.e. symbiotroph, pathotroph and saprotroph). We reported only the girdling effect on those three fungal guilds because the effects of litter removal and root trenching were not significant.

Pearson correlation analysis was used to examine the bivariate relations between soil properties (i.e. respiration, soil total organic C, MBC, LOC, total N, NH_4^+ and NO_3^-, pH, temperature and moisture) and soil fungal community diversity and composition (i.e. species richness, effective number of species, Pielou's evenness, lnμ and lnσ). Soil total organic C, NH_4^+ and NO_3^- were transformed to natural logarithms, and LOC was transformed to square roots to satisfy the assumptions of ANOVA and Person's correlation analysis. All statistical analyses were performed in R 3.3.2 (R Core Team 2016, http://www.R-project.org).

Results

Soil respiration was smaller for the girdling ($F_{1,3}$ = 20.4; P = 0.021) and litter removal ($F_{1,18}$ = 7.22; P = 0.016) treatments than the control (Figures 2 and S2 (Supporting Information); Table 2). Specifically, girdling reduced soil respiration from 1.26 ± 0.09 to 0.92 ± 0.05 g CO_2 m^{-2} $hour^{-1}$ (mean ± 1SE hereafter) and litter removal reduced soil respiration from 1.20 ± 0.10 to 0.97 ± 0.06 g CO_2 m^{-2} $hour^{-1}$. There was no statistically significant difference between declines in soil respiration induced by girdling compared to litter removal (Figures 2 and S2). Briefly, the interaction between girdling and litter removal was significant (Figure S2); Table 2). Compared to the control (1.49 ± 0.11 g CO_2 m^{-2} $hour^{-1}$), soil respiration was not reduced further by the combination of girdling and litter removal (0.92 ± 0.09 g CO_2 m^{-2} $hour^{-1}$), compared to the individual treatments of litter removal (1.03 ± 0.09 g CO_2 m^{-2} $hour^{-1}$) or girdling (0.91 ± 0.07 g CO_2 m^{-2} $hour^{-1}$) (Figures 2 and S2). We did not find any effect of root trenching on soil respiration (Table 2).

Girdling, litter removal and their interactions had no significant effects on soil LOC, MBC, total N, NH_4^+ and temperature, but significantly affected other soil properties such as soil total organic C (Table 2). Soil total organic C declined with girdling from 74.8 ± 3.9 to 67.8 ± 3.2 g kg^{-1} ($F_{1,3}$ = 25.96; P = 0.015) (Figure 3a; Table 2). Soil moisture increased with girdling treatment from 22.4 ± 1.2 to 29.1 ± 1.6 % ($F_{1,3}$ = 11.05; P = 0.045) (Figure 3b; Table 2). Soil NO_3^--N increased significantly with girdling from 1.4 ± 0.4 to 6.0 ± 0.9 mg kg^{-1} ($F_{1,3}$ = 15.18; P = 0.030) (Figure 3b; Table 2), and the girdling effect on soil NO_3^--N varied with litter removal ($F_{1,18}$ = 5.51; P = 0.031 for girdling • litter removal interaction) (Figure 3c; Table 2). Soil pH increased with litter removal from 4.01 ± 0.04 to 4.15 ± 0.04 ($F_{1,18}$ = 8.86; P = 0.009) (Figure 3c; Table 2). The effect of litter removal varied with girdling ($F_{1,18}$ = 7.64; P = 0.013 for girdling • litter removal interaction) (Figure 3d; Table 2). Soil temperature

decreased with litter removal from 11.59 ± 0.08 to 11.42 ± 0.09 ^{o}C ($F_{1,18}$ = 5.27; P = 0.034) (Figure 3e; Table 2). We did not find any significant effects of root trenching on those soil properties (Table 2).

Girdling, litter removal and their interactions had no significant effects on species abundance distribution (mean and standard deviation of the Poisson log-normal distribution) of the soil fungal community (P > 0.10; Table 3). The mean of the Poisson log-normal distribution was negatively correlated with soil microbial biomass C (Table 4). In addition, litter removal significantly increased species richness by 51% (from 146 ± 19 to 220 ± 21 number of OTUs; $F_{1,18}$ = 6.17; P = 0.024) and the effective number of species by 47% (from 2.92 ± 0.25 to 4.29 ± 0.38; $F_{1,18}$ = 10.40; P = 0.054) (Figure 4a,b; Table 3). Girdling and root trenching had an interactive effect on the effective number of species (Figure 4c; Table 3). Litter removal increased community evenness from 0.41 ± 0.03 to 0.52 ± 0.03 (from 2.92 ± 0.25 to 4.29 ± 0.38; $F_{1,18}$ = 5.06; P = 0.038) (Figure 4d; Table 3). In addition, species richness of the soil fungal community was negatively correlated with soil total organic C (Pearson correlation coefficient: r = −0.38), whereas it was positively correlated with soil NO_3^--N (r = 0.60) (Table 4).

In contrast, girdling and root trenching had no main effects on species richness and the effective number of species of soil fungal communities (Table 3). However, compared with controls, composition of the soil fungal community in girdling plots shifted to the left of the first axis of NMDS (Figure S1 (Supporting Information); $F_{1,3}$ = 25.8; P = 0.015). Furthermore, girdling reduced the abundance of symbiotrophic fungi by 75%, whereas it increased the abundance of both pathographic fungi (from 137 ± 47 to 610 ± 284 individuals) and saprotrophic fungi (from 891 ± 200 to 4456 ± 894 individuals) (Figure 5).

Discussion

Effects of plant C exclusions on soil respiration

We predicted that girdling and root trenching would decrease the rate of soil respiration more than litter removal, and that the combined treatments (litter removal + girdling) would further reduce soil respiration because of the removal of all plant C inputs. In contrast to our prediction, litter removal reduced soil respiration to the same extent as girdling, and root trenching did not affect soil respiration (Figure 2; Table 2). The decrease in soil respiration observed with litter removal in the present study was similar to the effects of litter removal reported for other studies. Without litter cover, soil respiration decreased by 40–63% in the subtropical plantations (Mao *et al.*, 1992). In tropical forests, litter removal showed a decrease in soil respiration of 28–54% (Li *et al.*, 2004; Vasconcelos *et al.*, 2004). Our study shows that litter removal affected soil respiration by the same magnitude as girdling. Although many studies have examined the effects of girdling and litter removal on soil respiration (Högberg *et al.*, 2001; Binkley *et al.*, 2006, Scott-Denton *et al.*, 2006; Chen *et al.*, 2010), few compared the effects of above- and below-ground plant C inputs in the same ecosystem (Sayer, 2010; Wang *et al.*, 2013). Our results provide quantitative evidence of the relative importance of above- compared with below-ground plant C in regulating soil C decomposition. It suggests that aboveground plant C is as important as belowground plant C in regulating soil C decomposition.

Root trenching and tree girdling had different effects on soil respiration because they reduce belowground plant C inputs based on different mechanisms. Roots died immediately after trenching and the decomposition of root residue was in the late stage when we sampled, which was five years after the treatment resulting in a small rate of decomposition of roots. Thus, root trenching had small effects on soil

respiration at our sampling time. Moreover, the lack of effects of root trenching on soil respiration are because the autotrophic soil respiration, which determines the difference in soil respiration between the control and root trenching, was very small at the time of sampling, i.e. December. Consecutive measures of soil respiration from March 2004 to March of 2007 showed a consistent pattern of a small rate of autotrophic soil respiration in December (Schaefer *et al.*, 2009).

Tree girdling still affected soil respiration when we sampled. Root decomposition started much later in girdled plots than in trenching plots because trees in the girdled plots began to die and shed leaves in the summer of 2007 (Schaefer *et al.*, 2007). However, trees in this forest have the ability to sprout and grow twigs on the girdled tree trunks, which might supply photosynthates for soil microbes. This could explain why we can detect the effects of root decomposition on soil respiration in girdled plots five years after treatment. The effects of girdling on soil respiration differ in different ecosystems, depending on the types of ecosystem and tree species and the length of time of treatment. Different effects of girdling might be explained by differences in the ability to resprout and in the availability of carbohydrates stored below the girdled stem among tree species (Binkley *et al.*, 2006; Chen *et al.*, 2010). Girdling reduced soil respiration by 53–65% in boreal spruce and pine forests (Högberg *et al.*, 2001; Bhupinderpal-Singh *et al.*, 2003), and by 18–37% in subtropical and tropical plantations (Binkley *et al.*, 2006; Chen *et al.*, 2010), but it increased soil respiration in a subalpine pine forest (Scott-Denton *et al.*, 2006). The different effects of girdling and root trenching on soil respiration we observed suggest that it is important to partition autotrophic and heterotrophic soil respiration to understand the responses of soil respiration to global changes in forest. We should

consider the methodological differences when estimating heterotrophic soil respiration.

Changes in soil fungal community composition and diversity on soil respiration

Girdling and litter removal changed substrates, soil microclimate and soil properties for soil fungal growth and created niches for new fungal species to grow (Sayer *et al.*, 2006; Feng *et al.*, 20009; Yarwood *et al.*, 2009). In the present study, girdling shifted the composition of soil fungal communities, with decreasing symbiotrophic fungi and increasing saprotrophic and pathogenic fungi (Figure 5). This is consistent with the findings of previous studies, which showed that discontinuing plant root C inputs led to the replacement of mycorrhizal fungi by fungal opportunists (Leake *et al.*, 2002; Yarwood *et al.*, 2009; Lindahl *et al.*, 2010). Saprotrophic fungi turn over faster and have more ability to decompose soil C than mycorrhizal fungi (Cooke *et al.*, 1984; Hobbie *et al.*, 1999), which is supposed to increase soil respiration. However, soil respiration decreased with increasing saprotrophic fungi in the girdling treatment. This is probably because a decrease in soil mycorrhizal fungi might reduce autotrophic soil respiration, which is a component of soil respiration. It could also be that pathogenic and other opportunistic fungi replaced soil mycorrhizal fungi, but they did not always have more ability to decompose complex soil C and increase soil CO_2 efflux. It is worth noting the observed increase in pathogenic fungi (Figure 5), which could cause negative effects on forest health. For example, the soil fungus *Caloscypha fulgens* is a pathogen for plant seeds and roots (Sutherland, 1979) and has the potential to threaten plant growth and vegetation succession.

The absence of effects of root trenching on soil fungal community is probably because soil fungi had already adapted to changes in substrates and soil microclimate

induced by this treatment prior to when we sampled. In contrast to girdling, root trenching immediately terminates belowground plant C inputs and leads to increased root debris. Labile substrates from the decomposition of root debris that affect soil microbial community decrease with time (Sun *et al.*, 2013). Five years after the initiation of root trenching the decomposition of root debris would have stabilized. This could explain why it is difficult to observe the influences of root trenching on the soil fungal community.

Litter removal increased the species richness, effective number of species and evenness of soil fungi (Figure 4). The reason could be that the litter removal treatment removed both the plant litter layer and the organic soil layer. The former is accumulated plant material and the latter is the decomposed organic layer with depth of ca. 1–5 cm. This treatment removed not only substrates for soil fungi but also altered the mycorrhizal fungi which was the dominant species in these two layers (Tedersoo *et al.*, 2003). Disturbing soil mycorrhizal fungi by the litter removal treatment alleviated the competition between mycorrhizal fungi and the other fungal species (e.g. saprotrophs and pathotrophs), and retained energy, nutrients and space for them to growth (Lindahl *et al.*, 1999; Leake *et al.*, 2002; Lindahl *et al.*, 2010). These changes in soil fungal community structure might increase their adaptation to greater fluctuations of soil microclimate and enhance CO_2 emission from soil C decomposition, according to the positive correlation between litter or wood decomposition and soil fungal diversity suggested in some studies (Setälä & Mclean, 2004; Deacon *et al.*, 2006; LeBauer *et al.*, 2010). However, soil respiration did not increase with litter removal (Figure 2). Without the aboveground inputs of plant C, soil substrates for soil fungal growth probably decreased considerably, and the effects of changes in soil fungal diversity on soil respiration were indirect. Because soil

fungal diversity was not significantly correlated with soil respiration in this study but was negatively correlated with soil total organic C (Table 4), suggested that increasing soil fungal diversity enhanced soil C decomposition and resulted in reducing soil C content. Soil respiration in the litter removal treatment combined with girdling did not decrease further when compared with girdling and litter removal (Figures 2 and S2). The reason could be that increasing soil fungal diversity in this treatment led to more CO_2 emission from soil C decomposition, offsetting further decline in soil respiration but not to the extent to increase soil respiration. The result also suggests that the exclusion of above- and below-ground plant C inputs altered the soil fungal community in a synergistic way in decomposing soil C. Litter removal increased soil fungal diversity, whereas girdling attenuated root competition with fungi for water and nutrients by cutting off plant uptake. Soil moisture and NO_3^- concentrations increased with girdling in this study (Figure 3). Without water and nutrient limitation, soil fungal communities may shift to those species able to utilize the remaining more complex organic C.

Confounding effects of soil microclimate and properties on soil respiration

We suggest two reasons why soil respiration was not reduced further by girdling combined with litter removal in this subtropical forest. First, effects of girdling and litter removal on soil respiration might result from changes in soil temperature, moisture and pH induced by these two treatments (Sayer, 2006; Feng *et al.*, 2009; Chen *et al.*, 2010). The soil organic layer functions as a physical protection for soil microbes (Sayer, 2006). The removal of litter not only reduces substrates and nutrients for soil microbes but also exposes soil microbes to greater environmental fluctuations, which impede CO_2 emission from soil C decomposition. Second,

although girdling limited the photosynthates transported below ground, carbohydrates stored in roots and stems below breast height would still provide substrates for soil fungal growth and increase fungal ability to decompose soil C with resulting CO_2 emission.

Conclusions

This study showed that litter removal decreased soil respiration to the same extent as girdling five years after initiation of the treatments, suggesting that aboveground plant C is as important as belowground plant C in regulating soil respiration in subtropical forests. In contrast to our prediction, the combination of litter removal and girdling did not reduce soil respiration further compared to the individual treatments. This could be attributed to increasing species richness of the soil fungal community caused by litter removal and shifts in soil fungal composition with girdling. These changes might stimulate soil fungi to use complex soil C and initiate soil C decomposition, thereby counteracting any further decrease in soil respiration.

Supporting Information

Figure S1 The NMDS analysis shows the main effects of girdling, litter removal and root trenching on soil fungal community composition.

Figure S2 Effects of girdling, litter removal and their interactions on soil respiration. G, girdling. LR, litter removal. Bars show mean ± 1SE (n = 8).

References listed in Table 1

Acknowledgements

We thank Xinliang Xu, Penlin Kayaalp, Deli Zai and Peter Allen for critical reviews of this manuscript. We also thank the staff of Ailaoshan Station for Forest Ecosystem Studies, Chinese Academy of Sciences and the Ailaoshan Nature Reserve for their support in the field. This study was supported by the National Natural Science Foundation of China (31600428 and 31700455) and the 973 key project of the National Natural Science Foundation of China (2014CB954101).

References

Allen M.P. & Tildesley D.J. (1989) Computer Simulation of Liquids. Oxford University Press, New York, NY.

Bhupinderpal-Singh, Nordgren, A., Lofvenius, M.O., Högberg, M.N., Mellander, P.E. & Högberg, P. (2003) Tree root and soil heterotrophic respiration as revealed by girdling of boreal Scots pine forest: extending observations beyond the first year. *Plant Cell and Environment*, **26**, 1287–1296.

Binkley, D., Stape, J.L., Takahashi, E.N. & Ryan, M.G. (2006) Tree-girdling to separate root and heterotrophic respiration in two Eucalyptus stands in Brazil. *Oecologia*, **148**, 447–54.

Brant, J.B., Myrold, D.D. & Sulzman, E.W. (2006) Root controls on soil microbial community structure in forest soils. *Oecologia*, **148**, 650–659.

Chan, O.C., Yang, X., Fu, Y., Feng, Z., Sha, L., Casper, P. & Zou, X. (2006) 16S rRNA gene analyses of bacterial community structures in the soils of evergreen broad-leaved forests in south-west China. *FEMS Microbiology Ecology*, **58**, 247–259.

Chen, D.M., Zhang, Y., Lin, Y.B., Zhu, W.X. & Fu, S.L. (2010) Changes in belowground carbon in *Acacia crassicarpa* and *Eucalyptus urophylla* plantations after tree girdling. *Plant and Soil*, **326**, 123–135.

Chen, D.M., Zhou, L.X., Wu, J.P., Hsu, J.N., Lin, Y.B. & Fu, S.L. (2012) Tree girdling affects the soil microbial community by modifying resource availability in two subtropical plantations. *Applied Soil Ecology*, **53**, 108–115.

Connell, J.H. (1978) Diversity in tropical rain forests and coral reefs. *Science*, **199**, 1302–1310.

Cooke, R.C. & Rayner, D.M. (1984) *Ecology of Saprotrophic Fungi*. Longman. London, UK.

Deacon, L.J., Prycemiller, E.J., Frankland, J.C., Bainbridge, B.W., Moore, P.D. & Robinson, C.H. (2006) Diversity and function of decomposer fungi from a grassland soil. *Soil Biology & Biochemistry*, **38**, 7–20.

Edgar, R.C., Haas, B.J., Clemente, J.C., Quince, C. & Knight, R. (2011) UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics*, **27**, 2194–2200.

Feng, W., Schaefer, D.A., Zou, X. & Zhang, M. (2011) Shifting sources of soil labile organic carbon after termination of plant carbon inputs in a subtropical moist forest of southwest China. *Ecological Research*, **26**, 437–444.

Feng, W., Zou, X. & Schaefer, D. (2009) Above- and belowground carbon inputs affect seasonal variations of soil microbial biomass in a subtropical monsoon forest of southwest China. *Soil Biology and Biochemistry*, **41**, 978–983.

Frostegård, Å., Tunlid, A. & Bååth, E. (2011) Use and misuse of PLFA measurements in soils. *Soil Biology & Biochemistry*, **43**, 1621–1625.

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 methods and observations. *Biogeochemistry*, **48**, 115–146.

Hobbie, E. A., Macko, S. A., & Shugart, H. H. (1999). Insights into nitrogen and carbon dynamics of ectomycorrhizal and saprotrophic fungi from isotopic evidence. *Oecologia*, **118**, 353–360.

Högberg, P., Nordgren, A., Buchmann, N., Taylor, A.F.S., Ekblad, A., Högberg, M.N. *et al.* (2001) Large-scale forest girdling shows that current photosynthesis drives soil respiration. *Nature*, **411**, 789–792.

Jackson, R.B., Cook, C.W., Pippen, J.S. & Palmer, S.M. (2009) Increased belowground biomass and soil CO_2 fluxes after a decade of carbon dioxide enrichment in a warm-temperate forest. *Ecology*, **90**, 3352–3366.

Jenkinson, D.S. (1976) The effects of biocidal treatments on metabolism in soil—IV. The decomposition of fumigated organisms in soil. Soil Biology and Biochemistry, 8, 203–208.

Jost, L. (2006) Entropy and diversity. *Oikos*, **113**, 363–375.

Kögel-Knabner, I. (2002) The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter. *Soil Biology & Biochemistry*, **34**, 139–162.

Leake, J.R., Donnelly, D.P., & Boddy, L. (2003) Interactions between ectomycorrhizal and saprotrophic fungi. In: Mycorrhizal Ecology (eds van der Heijden, M.G.A. & Sanders, I.R.), pp. 345–372. Springer, Berlin–Heidelberg.

Lebauer, D.S. (2010) Litter degradation rate and β-glucosidase activity increase with fungal diversity. *Canadian Journal of Forest Research*, **40**, 1076–1085.

Lindahl, B.D., De Boer, W., & Finlay, R.D. (2010). Disruption of root carbon transport into forest humus stimulates fungal opportunists at the expense of mycorrhizal fungi. *The ISME Journal*, **4**, 860–872.

Lindahl, B., Stenlid, J., Olsson, S. & Finlay, R. (1999) Translocation of 32 P between interacting mycelia of a wood-decomposing fungus and ectomycorrhizal fungi in microcosm systems. *New Phytologist*, **144**, 183–193.

Li, W., Jin, C., Guan, D., Wang, Q., Wang, A., Yuan, F. & Wu, J. (2015) The effects of simulated nitrogen deposition on plant root traits: A meta-analysis. *Soil Biology & Biochemistry*, **82**, 112–118.

Li, Y., Xu, M., Sun, O.J. & Cui, W. (2004) Effects of root and litter exclusion on soil CO_2 efflux and microbial biomass in wet tropical forests. *Soil Biology & Biochemistry*, **36**, 2111–2114.

Liu, W.Y., Fox, J.E.D. & Xu, Z.F. (2003) Litterfall and nutrient dynamics in a montane moist evergreen broad-leaved forest in Ailao Mountains, SW China. *Plant Ecology*, **164**, 157–170.

Liu, Z.G. & Zou, X.M. (2002) Exotic earthworms accelerate plant litter decomposition in a Puerto Rican pasture and a wet forest. *Ecological Applications*, **12**, 1406–1417.

Mao, D.M., Min, Y.W., Yu, L.L., Martens, R. & Insam, H. (1992) Effect of afforestation on microbial biomass and activity in soils of tropical China. *Soil Biology & Biochemistry*, **24**, 865–872.

Nguyen, N.H., Song, Z., Bates, S.T., Branco, S., Tedersoo, L., Menke, J. *et al.* (2016) FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild. *Fungal Ecology*, **20**, 241–248.

Oksanen, J., Kindt, R., Legendre, P., O'Hara, B., Simpson, G.L., Solymos, P. *et al.* (2008) vegan: Community Ecology Package. R package version 1.13-0 R Project for Statistical Computing, Vienna, Austria.

Pausch, J. & Kuzyakov, Y. (2017) Carbon input by roots into the soil: Quantification of rhizodeposition from root to ecosystem scale. *Global Change Biology*, **24**, 1–12.

Sayer, E.J. & Tanner, E.V.J. (2010) A new approach to trenching experiments for measuring root-rhizosphere respiration in a lowland tropical forest. *Soil Biology & Biochemistry*, **42**, 347–352.

Sayer, E.J. (2006) Using experimental manipulation to assess the roles of leaf litter in the functioning of forest ecosystems. *Biological Reviews*, **81**, 1–31.

Schaefer, D.A., Feng, W. & Zou, X. (2009) Plant carbon inputs and environmental factors strongly affect soil respiration in a subtropical forest of southwestern China. *Soil Biology & Biochemistry*, **41**, 1000–1007.

Scott-Denton, L.E., Rosenstiel, T.N. & Monson, R.K. (2006) Differential controls by climate and substrate over the heterotrophic and rhizospheric components of soil respiration. *Global Change Biology*, **12**, 205–216.

Setälä, H. & McLean, M.A. (2004) Decomposition rate of organic substrates in relation to the species diversity of soil saprophytic fungi. *Oecologia*, **139**, 98–107.

Sun, T., Mao, Z. & Han, Y. (2013) Slow decomposition of very fine roots and some factors controlling the process: a 4-year experiment in four temperate tree species. *Plant and Soil*, **372**, 445–458.

Sutherland, J.R. (1979) The pathogenic fungus Caloscyphafulgens in stored conifer seeds in British Columbia and relation of its incidence to ground and squirrel-cache collected cones. *Canadian Journal of Forest Research*, **9**, 129–132.

Tedersoo, L., Bahram, M., Põlme, S., Kõljalg, U., Yorou, N.S., Wijesundera, R. *et al.* (2014) Global diversity and geography of soil fungi. *Science*, **346**, 1–11.

Tedersoo, L., Kõljalg, U., Hallenberg, N. & Larsson, K.H. (2003) Fine scale distribution of ectomycorrhizal fungi and roots across substrate layers including coarse woody debris in a mixed forest. *New Phytologist*, **159**, 153–165.

van der Wal, A., Geydan, T.D., Kuyper, T.W. & de Boer, W. (2013) A thready affair: linking fungal diversity and community dynamics to terrestrial decomposition processes. *FEMS Microbiology Reviews*, **37**, 477–494.

Vasconcelos, S.S., Zarin, D.J., Capanu, M., Littell, R., Davidson, E.A., Ishida, F.Y. *et al.* (2004) Moisture and substrate availability constrain soil trace gas fluxes in an eastern Amazonian regrowth forest. *Global Biogeochemical Cycles*, **18,** 193–204.

Wang, Q.K., He, T.X., Wang, S.L. & Liu, L. (2013) Carbon input manipulation affects soil respiration and microbial community composition in a subtropical coniferous forest. *Agricultural and Forest Meteorology*, **178**, 152–160.

Werner, A., Zadworny, M. & Idzikowska, K. (2002) Interaction between Laccaria laccata and Trichoderma virens in co-culture and in the rhizosphere of Pinus sylvestris grown in vitro. *Mycorrhiza*, **12**, 139–145.

Yarwood, S.A., Myrold, D.D. & Hogberg, M.N. (2009) Termination of belowground C allocation by trees alters soil fungal and bacterial communities in a boreal forest. *FEMS Microbiology Ecology*, **70**, 151–162.

Zhang, K., 1983. The characteristics of mountain climate in the north of Ailao Mts. research of forest ecosystem on Ailao Mountains, Yunnan. *Yunnan Science and Technology Press*, Kunming, China.

Zhou, J., He, Z., Yang, Y., Deng, Y., Tringe, S.G. & Alvarez-Cohen, L. (2015) High-throughput metagenomic technologies for complex microbial community analysis: open and closed formats. *MBIO*, **6**. DOI: 10.1128/mBio.02288-14

Zou, X.M., Ruan, H.H., Fu, Y., Yang, X.D. & Sha, L.Q. (2005) Estimating soil labile organic carbon and potential turnover rates using a sequential fumigation-incubation procedure. *Soil Biology & Biochemistry*, **37**, 1923–1928.

FIGURE CAPTIONS

Figure 1 Schematic graph to show the experimental design of the present study.

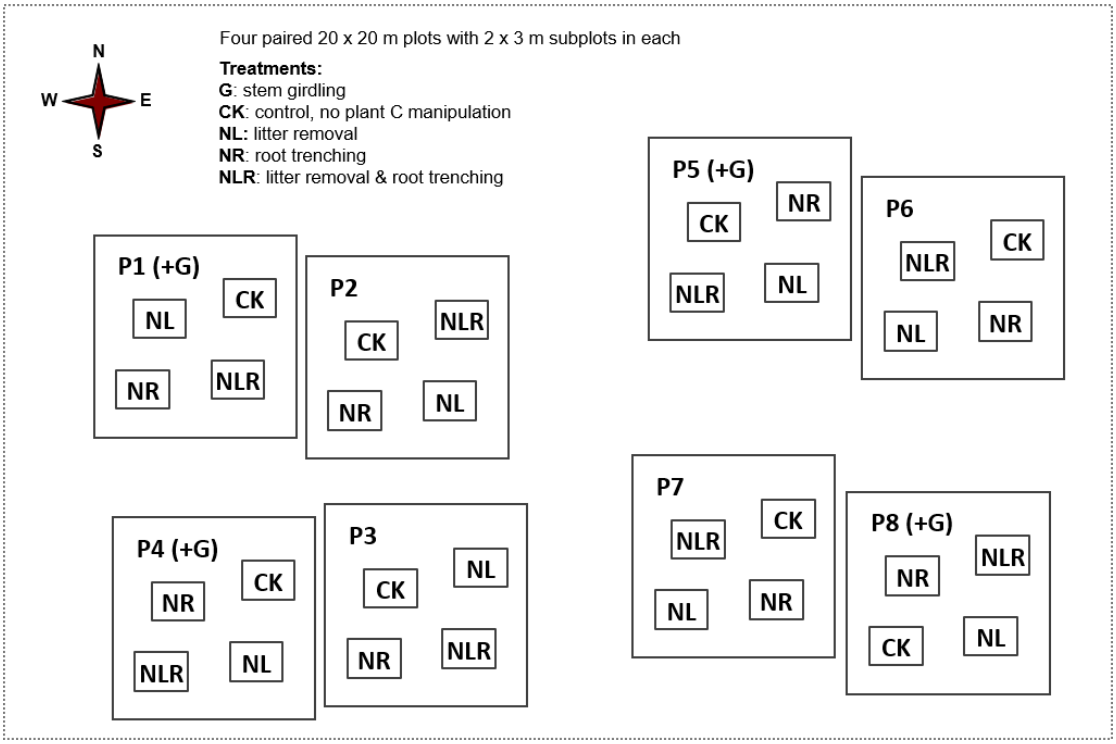


Figure 2 Changes in soil respiration and soil fungal species richness in the treatments of litter removal, girdling and their combination. Bars show mean ± 1SE (n = 8).

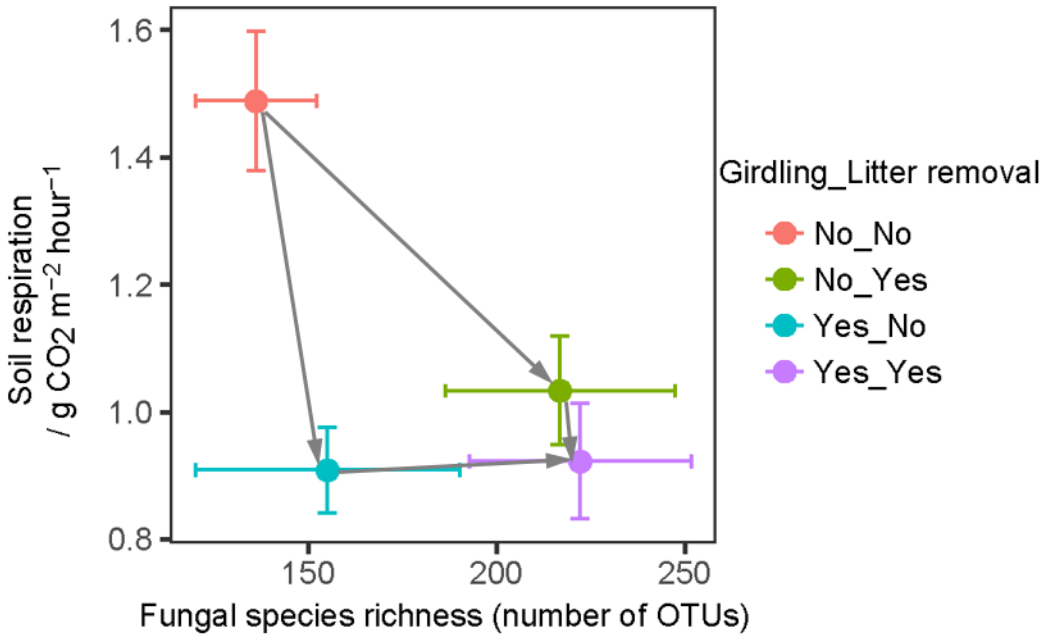


Figure 3 Effects of girdling, litter removal and their interactions on soil characteristics: (a) total organic carbon, (b) soil moisture, (c) nitrate, (d) pH and (d) temperature. G, girdling; LR, litter removal. We reported only the girdling effect in panels (a) and (b) because there were no significant effects of litter removal and trenching and their interactions. Bars show mean ± 1SE (n = 16 for a, b and n = 8 for b – d).

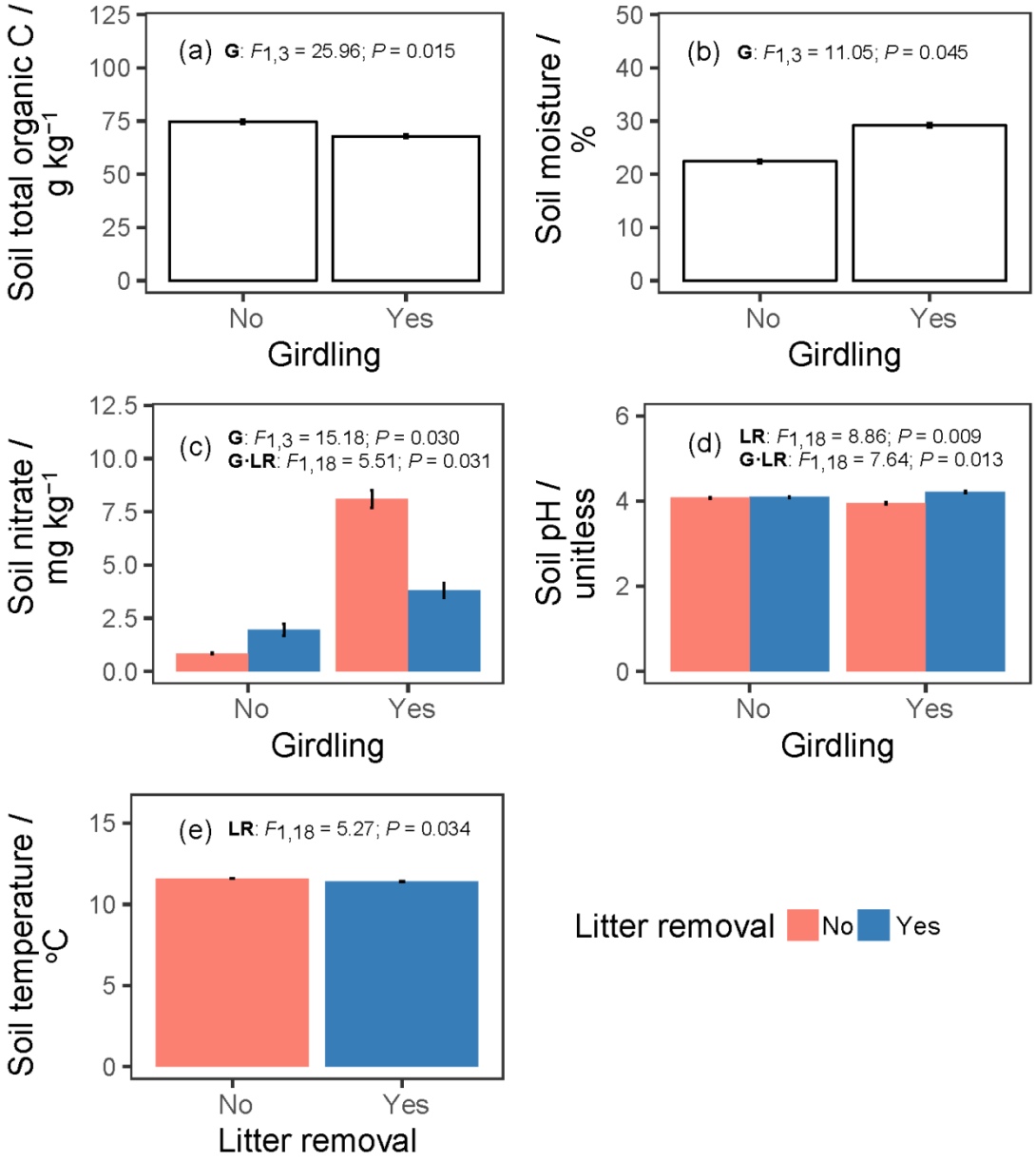


Figure 4 Effect of above- and below-ground plant C inputs on soil fungal diversity. (a) Species richness, (b) and (c) effective number of species, and (d) evenness. Bars show mean ± 1SE (n = 16 for a, b and d and n=8 for c).

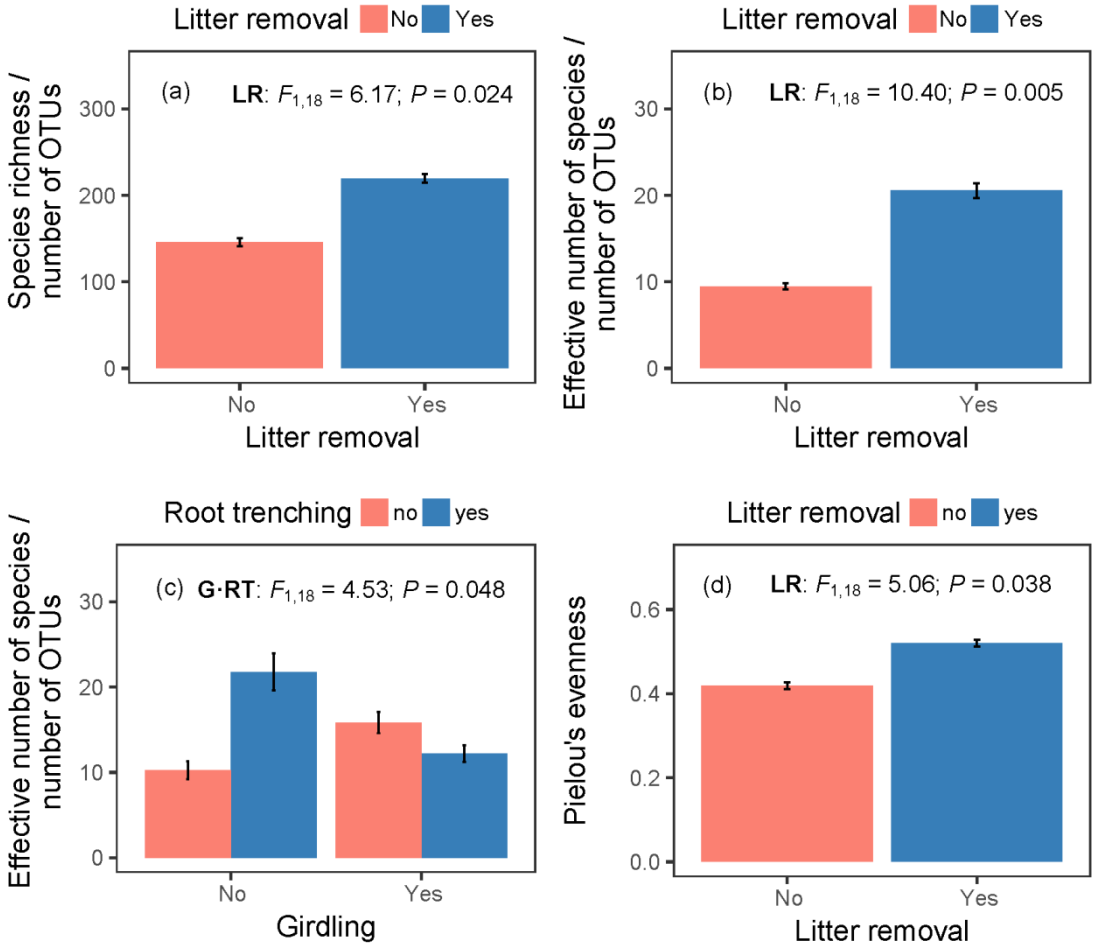
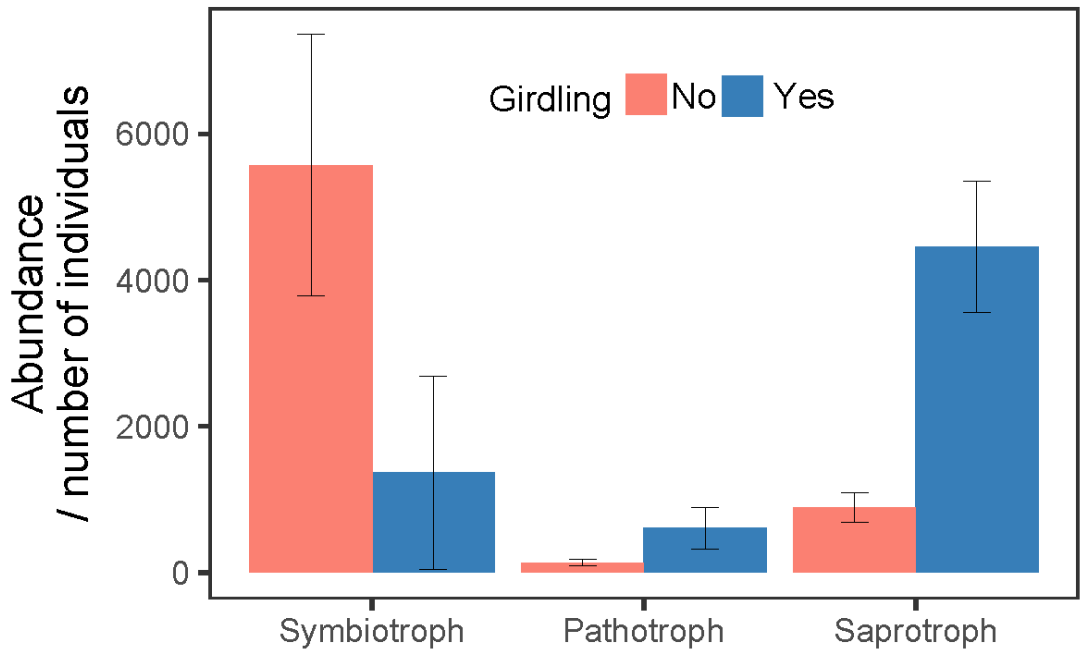


Figure 5 Girdling effect on functional guilds of soil fungi. Bars show mean ± 1 SE (*n* = 16).



TABLES

Table 1

Summary to show changes in soil microbial communities with the treatments of girdling, root trenching and litter removal examined in this study.

Treatments	Diversity	Microbial community composition	Fungal guilds		Method	Resources
			Symbio-troph	Sapro-troph		
Girdling	─	↓Fungi, bacteria (ns), total (ns)	─	─	PLFA	Siira-Pietikainen *et al.* (2001)
	Shannon index (ns)	─	ECM colonization (ns)	─	Sequencing	Druebert *et al.* (2009)
	─	↓Bacteria	↓ECM abundance;	↑abundance	LH-PCR	Yarwood *et al.* (2009)
	ECM: ↓Shannon index; evenness (ns)	─	↓ECM richness	─	Sequencing	Pena *et al.* (2010)
	─	↓Total PLFA	↓ECM biomass	─	PLFA	Kaiser *et al.* (2010)
	─	Total (ns), fungi (ns), bacteria (ns)	─	─	PLFA	Wu *et al.* (2011)
	─	↓Fungi, ↑bacteria, total (ns)	─	─	PLFA	Chen *et al.* (2012)
	─	─	↓Hyphal abundance	─	Real time PCR	Bergemann *et al.* (2013)
	─	↑Bacteria	↓ECM; ↓AFM	↑abundance	PLFA	Murugan *et al.* (2014)
Trenching	↓Simpson evenness	─	↓ECM infection rate	─	Microscopic identification	Simard *et al.* (1997)
	─	Total (↑/ns), bacteria (↑/ns), fungi (↓/ns)	─	─	PLFA	Siira-Pietikäinen *et al.* (2003)
	─	↓Fungi, ↑Actinomycete, ↓G^-	─	─	PLFA	Brant *et al.* (2006)
	─	↑Bacteria, ↑fungi, ↑Actinomycetes	─	─	PLFA	Wang *et al.* (2013)
Litter removal	─	↑Acidobacteria	─	─	qPCR, pyrosequencing	Nemergut *et al.* (2010)
	─	Total PLFA (ns)	─	─	PLFA	Elgersma *et al.* (2011)
	─	Total PLFA (ns)	─	─	PLFA	Leitner *et al.* (2016)
	─	Total PLFA (ns)	─	─	PLFA	Zhao *et al.* (2017)

PLFA, phospholipid fatty acids analysis; ns, no statistical difference; ─, no data available; ↓, decrease; ↑, increase; ECM, ectomycorrhizal fungi; AMF, arbuscular mycorrhizal fungi. Citations in the table are listed in the Supporting Information.

Table 2

Effects of girdling, litter removal, root trenching and their interactions on soil properties.

Variable	Source	df	ddf	SS	MS	*F*	*P*
Soil respiration	Plot pair	3	3	0.41	0.14	2.89	0.204
	Girdling (G)	1	3	0.96	0.96	**20.39**	**0.021**
	Main plot error	3		0.14	0.05		
	Litter removal (LR)	1	18	0.39	0.39	**7.22**	**0.016**
	Root trenching (RT)	1	18	0.11	0.11	1.98	0.177
	LR • RT	1	18	0.05	0.05	0.96	0.340
	G • LR	1	18	0.44	0.44	**8.15**	**0.011**
	G • RT	1	18	0.12	0.12	2.16	0.160
	G • LR • RT	1	18	0.00	0.00	0.02	0.887
	Residuals	18		0.97	0.05		
TOC	Plot pair	3	3	0.10	0.03	**11.13**	**0.040**
	Girdling (G)	1	3	0.08	0.08	**25.96**	**0.015**
	Main plot error	3		0.01	0.00		
	Litter removal (LR)	1	18	0.04	0.04	0.73	0.406
	Root trenching (RT)	1	18	0.01	0.01	0.13	0.720
	LR • RT	1	18	0.00	0.00	0.00	0.952
	G • LR	1	18	0.04	0.04	0.77	0.392
	G • RT	1	18	0.01	0.01	0.10	0.754
	G • LR • RT	1	18	0.00	0.00	0.01	0.915
	Residuals	18		0.99	0.06		
LOC	Plot pair	3	3	1.00	0.33	1.04	0.488
	Girdling (G)	1	3	0.47	0.47	1.46	0.314
	Main plot error	3		0.96	0.32		
	Litter removal (LR)	1	18	0.16	0.16	0.84	0.371
	Root trenching (RT)	1	18	0.27	0.27	1.45	0.245
	LR • RT	1	18	0.36	0.36	1.89	0.187
	G • LR	1	18	0.02	0.02	0.10	0.752
	G • RT	1	18	0.25	0.25	1.31	0.268
	G • LR • RT	1	18	0.15	0.15	0.80	0.384
	Residuals	18		3.41	0.19		
MBC	Plot pair	3	3	1.15	0.38	0.78	0.580
	Girdling (G)	1	3	1.98	1.98	4.01	0.140
	Main plot error	3		1.48	0.49		
	Litter removal (LR)	1	18	0.02	0.02	0.07	0.801

	Root trenching (RT)	1	18	0.26	0.26	1.10	0.310
	LR • RT	1	18	0.74	0.74	3.16	0.093
	G • LR	1	18	0.02	0.02	0.10	0.752
	G • RT	1	18	0.23	0.23	0.99	0.333
	G • LR • RT	1	18	0.00	0.00	0.01	0.943
	Residuals	18		4.20	0.23		
Total N	Plot pair	3	3	7.28	2.43	2.16	0.273
	Girdling (G)	1	3	3.10	3.10	2.76	0.196
	Main plot error	3		3.37	1.12		
	Litter removal (LR)	1	18	0.84	0.84	0.20	0.658
	Root trenching (RT)	1	18	2.01	2.01	0.48	0.496
	LR • RT	1	18	0.01	0.01	0.00	0.958
	G • LR	1	18	3.35	3.35	0.81	0.381
	G • RT	1	18	0.21	0.21	0.05	0.826
	G • LR • RT	1	18	1.30	1.30	0.31	0.582
	Residuals	18		74.61	4.15		
NH_4^+-N	Plot pair	3	3	0.74	0.25	0.41	0.761
	Girdling (G)	1	3	0.48	0.48	0.80	0.439
	Main plot error	3		1.83	0.61		
	Litter removal (LR)	1	18	0.04	0.04	0.32	0.581
	Root trenching (RT)	1	18	0.01	0.01	0.09	0.764
	LR • RT	1	18	0.02	0.02	0.15	0.704
	G • LR	1	18	0.05	0.05	0.42	0.525
	G • RT	1	18	0.44	0.44	3.63	0.073
	G • LR • RT	1	18	0.18	0.18	1.45	0.245
	Residuals	18		2.20	0.12		
NO_3^--N	Plot pair	3	3	5.15	1.72	1.34	0.408
	Girdling (G)	1	3	19.44	19.44	**15.18**	**0.030**
	Main plot error	3		3.84	1.28		
	Litter removal (LR)	1	18	0.95	0.95	1.26	0.276
	Root trenching (RT)	1	18	0.14	0.14	0.18	0.677
	LR • RT	1	18	1.51	1.51	2.01	0.174
	G • LR	1	18	4.14	4.14	**5.51**	**0.031**
	G • RT	1	18	0.00	0.00	0.00	0.978
	G • LR • RT	1	18	0.41	0.41	0.55	0.469
	Residuals	18		13.53	0.75		
pH	Plot pair	3	3	0.36	0.12	**12.24**	**0.035**
	Girdling (G)	1	3	0.00	0.00	0.02	0.909
	Main plot error	3		0.03	0.01		
	Litter removal (LR)	1	18	0.16	0.16	**8.86**	**0.009**

	Root trenching (RT)	1	18	0.03	0.03	1.61	0.221
	LR • RT	1	18	0.01	0.01	0.36	0.556
	G • LR	1	18	0.13	0.13	**7.64**	**0.013**
	G • RT	1	18	0.00	0.00	0.05	0.824
	G • LR • RT	1	18	0.01	0.01	0.75	0.397
	Residuals	18		0.32	0.02		
Soil moisture	Plot pair	3	3	220.5	73.51	2.23	0.264
	Girdling (G)	1	3	364.8	364. 87	**11.05**	**0.045**
	Main plot error	3		99.01	33.00		
	Litter removal (LR)	1	18	23.53	23.53	1.01	0.330
	Root trenching (RT)	1	18	20.67	20.67	0.88	0.360
	LR • RT	1	18	33.05	33.05	1.41	0.251
	G • LR	1	18	100.7	100.7	4.30	0.053
	G • RT	1	18	23.53	23.53	1.01	0.330
	G • LR • RT	1	18	5.58	5.58	0.24	0.632
	Residuals	18		421.1	23.40		
Soil temperature	Plot pair	3	3	1.73	0.58	3.91	0.147
	Girdling (G)	1	3	0.06	0.06	0.42	0.565
	Main plot error	3		0.44	0.15		
	Litter removal (LR)	1	18	0.24	0.24	**5.27**	**0.034**
	Root trenching (RT)	1	18	0.08	0.08	1.72	0.207
	LR • RT	1	18	0.01	0.01	0.24	0.629
	G • LR	1	18	0.08	0.08	1.72	0.207
	G • RT	1	18	0.01	0.01	0.11	0.747
	G • LR • RT	1	18	0.03	0.03	0.67	0.424
	Residuals	18		0.84	0.05		

TOC, total organic C; LOC, labile organic C; MBC, microbial biomass C. df, degree of freedom; ddf, denominate degree of freedom; SS, sum of squares; MS, mean sum of squares. *F* and *P* values in bold show significant treatment effect.

Table 3

Effects of girdling, litter removal, root trenching and their interactions on soil fungal diversity and community composition.

Variable	Source	df	ddf	SS	MS	*F*	*P*
SR	Plot pair	3	3	28 078.1	9359.4	1.81	0.320
	Girdling (G)	1	3	1188.3	1188.3	0.23	0.665
	Main plot error	3		15 521.1	5173.7		
	Litter removal (LR)	1	18	43 734.0	43 734.0	**6.17**	**0.024**
	Root trenching (RT)	1	18	10 260.3	10 260.3	1.45	0.245
	LR • RT	1	18	1164.0	1164.0	0.16	0.691
	G • LR	1	18	371.3	371.3	0.05	0.822
	G • RT	1	18	38.3	38.3	0.01	0.943
	G • LR • RT	1	18	2261.3	2261.3	0.32	0.580
	Residuals	18		12 7605.1	7089.2		
ENS	Plot pair	3	3	11.4	3.8	6.97	0.073
	Girdling (G)	1	3	0.1	0.1	0.20	0.687
	Main plot error	3		1.6	0.6		
	Litter removal (LR)	1	18	14.9	14.9	**10.40**	**0.005**
	Root trenching (RT)	1	18	1.9	1.9	1.35	0.261
	LR • RT	1	18	0.2	0.2	0.14	0.713
	G • LR	1	18	1.7	1.7	1.18	0.293
	G • RT	1	18	6.5	6.5	**4.53**	**0.048**
	G • LR • RT	1	18	0.1	0.1	0.04	0.852
	Residuals	18		25.9	1.4		
Evenness	Plot pair	3	3	0.1	0.0	8.58	0.056
	Girdling (G)	1	3	0.0	0.0	0.24	0.661
	Main plot error	3		0.0	0.0		
	Litter removal (LR)	1	18	0.1	0.1	**5.06**	**0.038**
	Root trenching (RT)	1	18	0.0	0.0	1.65	0.216
	LR • RT	1	18	0.0	0.0	0.33	0.574
	G • LR	1	18	0.0	0.0	1.56	0.228
	G • RT	1	18	0.1	0.1	3.06	0.098
	G • LR • RT	1	18	0.0	0.0	0.23	0.639
	Residuals	18		0.3	0.0		
Ln μ	Plot pair	3	3	5.0	1.7	1.00	0.499
	Girdling (G)	1	3	1.1	1.1	0.69	0.469
	Main plot error	3		5.0	1.7		
	Litter removal (LR)	1	18	0.4	0.4	0.14	0.714

	Root trenching (RT)	1	18	5.6	5.6	1.85	0.191
	LR • RT	1	18	4.3	4.3	1.43	0.248
	G • LR	1	18	8.9	8.9	2.94	0.104
	G • RT	1	18	0.0	0.0	0.00	0.954
	G • LR • RT	1	18	0.0	0.0	0.01	0.935
	Residuals	18		54.3	3.0		
Ln σ	Plot pair	3	3	1.9	0.6	5.50	0.098
	Girdling (G)	1	3	0.1	0.1	0.80	0.438
	Main plot error	3		0.3	0.1		
	Litter removal (LR)	1	18	1.2	1.2	2.52	0.130
	Root trenching (RT)	1	18	1.1	1.1	2.28	0.149
	LR • RT	1	18	0.5	0.5	1.07	0.315
	G • LR	1	18	0.9	0.9	1.79	0.198
	G • RT	1	18	0.7	0.7	1.38	0.255
	G • LR • RT	1	18	0.0	0.0	0.03	0.860
	Residuals	18		8.8	0.5		

SR, species richness; ENS, effective number of species (exponential of Shannon–Wiener index); Evenness, Pielou's evenness (Shannon–Wiener index/ln(species richness)); log μ and log σ are the two parameters (mean and standard deviation) from the Poisson log-normal distribution of the abundance of soil fungal species. df, degree of freedom; ddf, denominator degrees of freedom; SS, sum of squares; MS, mean sum of squares. *F* and *P* values in bold show significant treatment effect.

Table 4

Pearson's correlations coefficients between soil properties and diversity and abundance distribution of the soil fungal community.

	Species richness	Effective number of species	Pielou's evenness	ln μ	ln σ
Soil respiration	−0.34	−0.26	−0.24	−0.14	0.22
Total organic C	**−0.38**	−0.29	−0.12	0.01	0.03
Labile organic C	−0.25	0.01	0.12	0.32	−0.25
Microbial biomass C	−0.22	−0.16	−0.19	**−0.40**	0.26
Total N	−0.22	−0.16	−0.02	0.09	−0.01
NH_4^+	−0.07	−0.01	0.07	0.20	−0.09
NO_3^-	**0.60**	0.24	0.13	−0.15	−0.02
pH	0.17	0.10	0.20	0.15	−0.20
Moisture	0.33	−0.03	−0.04	−0.16	0.10

Significant values are in bold.