Current Research in Environmental & Applied Mycology (Journal of Fungal Biology)
8(2): 224–237 (2018) ISSN 2229-2225

www.creamjournal.org **Article**

Doi 10.5943/cream/8/2/7

Short-term study of subalpine forest soils reveals that microbial communities are strongly influenced by the litter and organic layers

Luo X [1,2,4], Xu JC [1,3], Karunarathna SC [1], Hyde KD [1,4], Mortimer PE [1*]

[1]Key Laboratory of Economic Plants and Biotechnology, Kunming Institute of Botany, Chinese Academy of Sciences, 132 Lanhei Road, Kunming 650201, China
[2]School of Biology and Food Engineering, Chuzhou University, Chuzhou, 239000, China
[3]World Agroforestry Centre, East and Central Asia Office, 132 Lanhei Road, Kunming 650201, China
[4]Center of Excellence in Fungal Research, Mae Fah Luang University, Chiang Rai, 57100, Thailand

Luo X, Xu JC, Karunarathna SC, Hyde KD, Mortimer PE 2018 – Short-term study of subalpine forest soils reveals that microbial communities are strongly influenced by the litter and organic layers. Current Research in Environmental & Applied Mycology (Journal of Fungal Biology) 8(2), 224–237, Doi 10.5943/cream/8/2/7

Abstract

Given the wide range of factors influencing soil microbial communities, the aim of our study was to investigate how these communities respond to changes in plant inputs, litter, and removal of soil organic layers, in a subalpine forest. Our study site was located on the slopes of Yulong Snow Mountain, Yunnan, China. We analyzed the effect of litter, tree roots, and the organic horizon on soil microbial communities, using phospholipid fatty acid (PLFA) analysis and by monitoring any changes in soil properties, over two months. Our results showed that the gram-positive bacteria and gram-negative bacteria ratio (G^+/G^-), after the second month of all treatments, was significantly higher than after the first month of treatment. The biomass of the soil microbial community is sensitive to response to variation of the soil environment. Removal of the organic horizon and additional litter coverage significantly decreased the biomass of fungi, fungi/bacteria and total PLFA, and significantly increased the G^+/G^- ratio compared with the control and other treatments after two months. Organic horizon and litter layer removal significantly increased the G^+/G^- ratio. Litter removal significantly increased the biomass of arbuscular mycorrhizal fungi. Contrary to our expectations, root removal had no effect on the biomass of the soil microbial communities during two months' treatment.

Key words – litter – organic matter – phospholipid fatty acid – root cutting – soil properties

Introduction

Soil microbial communities are responsible for 80–90% of biotic soil processes, mediating soil organic matter decomposition and nutrient cycling in forest ecosystems (Barreiro et al. 2015, Yao et al. 2016). Soil microbes can break down plant material and regulate plant growth and community composition in forests by influencing soil moisture and the supply of soil nutrients. Soil microbial communities are affected by both biotic and abiotic factors (Lodge et al. 2014). Understanding the mutual relationships between microbial communities and these biotic and abiotic factors is critical to our understanding of soil ecological processes.

Biotic and abiotic factors, which include leaf litter type, plant species composition, soil organic matter, pH, temperature and moisture, all regulate soil microbial communities (Hirano et al.

Submitted 14 February 2018, Accepted 8 March 2018, Published 12 March 2018
Corresponding Author: Peter E. Mortimer – e-mail – peter@mail.kib.ac.cn

2017, Lodge et al. 2014). Urbanova et al. (2015) showed that the effect of tree species on the composition of microbial community was demonstrated to be stronger than other soil properties and to explain a large proportion of variation in community composition. Plant species and traits significantly influence soil microbial community abundance and structure in several ways, including the production of litter, rhizodeposition, direct interactions with root-symbiotic microorganisms, indirect biotic effects on soil microorganisms mediated by herbivores or soil fauna or though the alteration of the microclimate (Augusto et al. 2015, DeBellis et al. 2006, Pei et al. 2016, Prescott & Grayston 2013). Greater plant species diversity and richness increases rhizosphere carbon inputs into the soil environment, thus resulting in enhanced microbial activity and carbon storage (Lange et al. 2015, 2014). Tree species differ in the litter quality (e.g. C/N) and quantity and produce different root exudates (Pei et al. 2016). Root exudates provide easily degradable carbon sources for soil microbial community thus the impact of tree species, root exudates and leaf litter on microbial communities will vary (Legay et al. 2014). Furthermore, leaf litter provides a habitat for many microbes and can also form a protective layer over the soil surface that can regulate soil microbial climate conditions (Hobbie 2000, Sayer 2006).

Abiotic properties are linked not only to plant traits but also to soil microbial communities. Variation in soil microbial communities can be explained by abiotic factors like climate, pH and soil properties (Vries et al. 2012). Furthermore, climatic variances, such as fluctuations in precipitation and temperature could affect soil microbial communities indirectly by altering soil microbial growth and activity (Sayer et al. 2017). Furthermore, soil microbial community structure can mediate strong indirect effect on processes of soil nutrient cycling such as soil nutrient status, and soil organic matter quality and quantity (Lange et al. 2014). Variation in soil microbial communities change soil fertility and above ground plant biomass (Ostertag & Hobbie 1999, Sayer & Tanner 2010).

Fatty acid chains within intact phospholipid molecules in microbial membranes can rapidly degrade upon cell death and increase the predictive power of ecosystem models. Therefore, Phospholipid fatty acid (PLFA) analysis is an effective and reliable biomarker method to determine soil microbial structure (Frostegård et al. 2011, Schnecker et al. 2012, Sun et al. 2015). PLFA analysis can give a quantitative outline of microbial community composition, including fungal and bacterial biomass, and the response of these groups to physiological stress (Drenovsky et al. 2004).

Although past studies, such as the work of Urbanova et al. (2015), have shown that tree species is the dominant factor influencing soil microbial community composition, especially for fungi, we aimed to investigate if this is true for soil microbial communities in a subalpine forest, and what other factors may also influence these communities. Thus, in the present study we established plots in a subalpine forest, consisting of uniform vegetation and slope, in order to test the short-term response of soil microbial communities to changes in key biotic and abiotic factors. The objectives of this study were: 1) to evaluate the effect of plant root, litter, and organic matter on soil microbial communities; 2) to evaluate the effect of treatment time on soil microbial communities; and 3) to determine whether changes to soil microbial community composition are correlated with soil organic matter.

Materials & Methods

Description of sampling sites

Our study site was located on the slopes of the Yulong Snow Mountain (26°59′–27°17′N, 100°04′–100°15′E, approximately 3300 masl) (Fig. 1), situated on the southeastern side of the Tibetan Plateau, forming part of the southernmost Hengduan Mountains, in southwestern China. Our site (27°85′22"N, 100°13′24"E) was established in a coniferous forest (more than 300 years old). The climate consists of a summer monsoon wet season, lasting from May to October. The dry season is dominated by the Qinghai-Tibetan Plateau circulation and westerly winds, which last from November to April (Zeng et al. 2016). Mean annual temperature is about 10.3 °C, while the mean annual precipitation is 786.5 mm. The vegetation type in the region is classified as subalpine

coniferous forest and is dominated by coniferous species such as *Picea likiangensis*, *Abies forrestii*, *Pinus yunnanensis*, *Pinus armandii*, and mixed with some broad leaf shrubs and small trees. The site has about 60–70% canopy cover. The slope of the site ranged between 24–31 degrees.

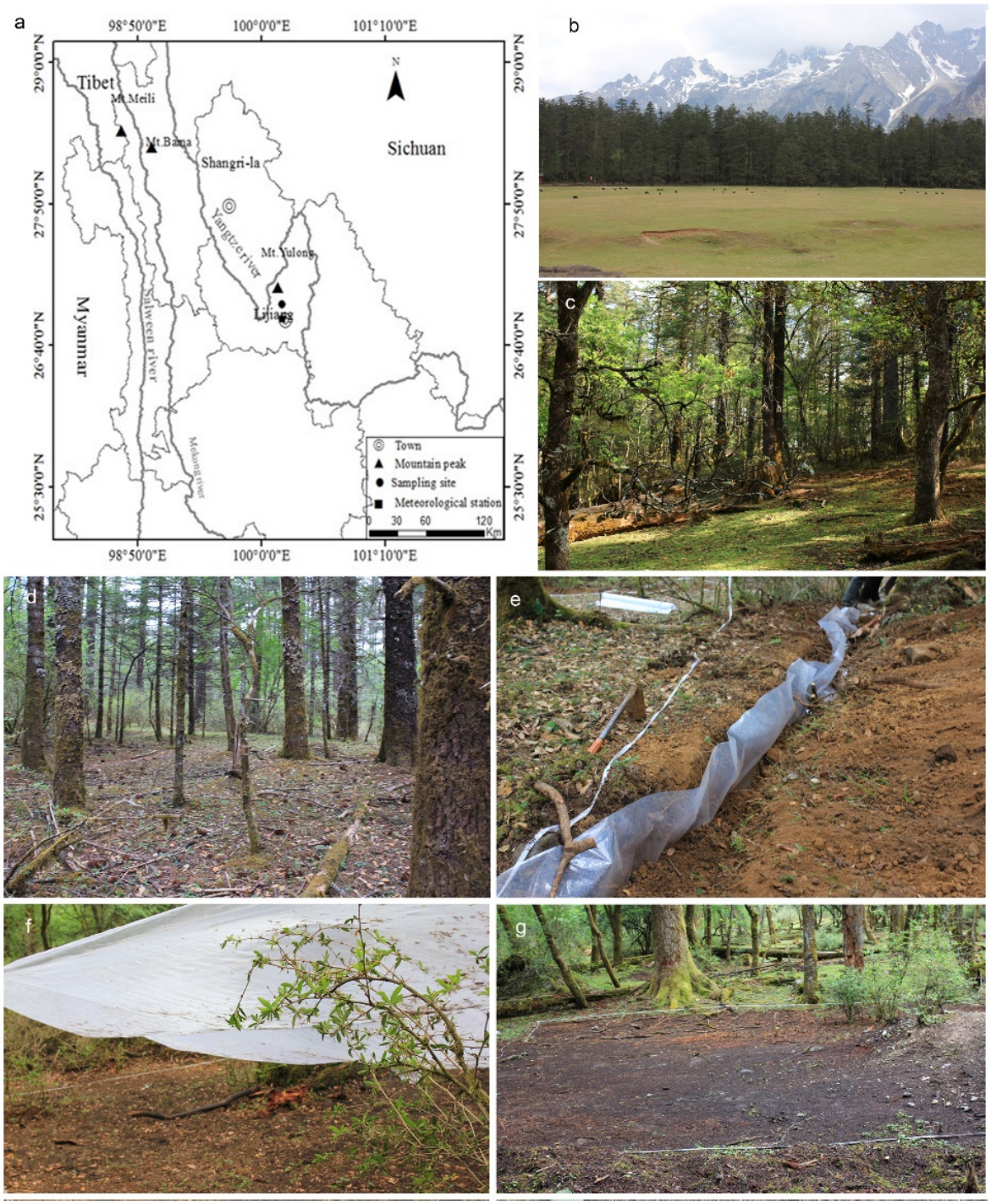

Fig. 1 – Location (a) and appearance of the forest understory (b) from our study. Dominant tree species included *Picea likiangensis*, *Abies forrestii*. The four treatments are also shown: (c) control (CK), (d) organic horizon removal and then covered with litter (O^-L^+), (e) cutting of external root connections (NR), (f) litter removal (NL), and (g) organic horizon removal (NO).

Field plot design

Twenty-five plots (10 m × 10 m) including four treatments and a control (CK) were established in Yulong Snow Mountain Nature Reserve (Fig. 1). All plots were established within 3,200 to 3,400 m elevation, with five replicates for each treatment and control. Every plot was

separated from other neighboring plots by at least 50 m. Five plots were randomly chosen as CK plots. The four treatments were: cutting external root connections (NR), litter removal (NL), organic horizon removal, which included the removal of both the organic horizon and the litter layer (NO), and removal of the organic horizon followed by the replacement of the litter layer (O^-L^+). For NR, there were no trees inside the plots and canals were dug along plot boundaries (40 cm depth, 10 cm width), severing all roots going into the plots, followed by the insertion of a thin plastic sheet to prevent the growth of external roots into the plots (Feng et al. 2009). In NL plots, the litter and moss layers were removed and the O horizon was retained. In NO plots, the litter and moss layers, along with the O horizon were removed. The depth of the litter layer, moss layer, and O horizon was about 5–10 cm. In NL and NO plots, to eliminate the effect of freshly fallen litter of the stand forest during the investigating period, a sloping, clear mesh was fixed (mesh size: 2 mm × 2 mm) 1.8 m above the plot. The mesh allowed for moisture exchange, light penetration, and air circulation. Scheduled cleaning was carried out weekly to avoid a buildup of litter on the net and thus allowing sunlight to reach the soil surface. In O^-L^+ treatment, the litter and O horizon layers were removed and then the plots were later covered with the original litter that had been removed from that plot. Controls were set up which had no treatments applied. All experimental treatments were positioned within a 0.5 m buffer zone, with boundary around the plots. We tried to minimize disturbance. All the treatments were established in the first half of June 2015.

Soil sample collection and analyses

Five soil samples from each plot were collected from the A horizon (0—10 cm depth) using a cylindrical soil borer. The soil samples were extracted along a diagonal line across every plot. Samples were extracted after one month (end of July) and two months (end of August 2015) from when the treatments were initiated. Five soil samples from each plot were mixed and divided between analyses. Fresh soil was sieved through a 2 mm mesh, and the remaining roots and stones were removed by hand. The soil samplings were divided into two parts. One part was air dried and sieved through a 0.3 mm mesh to analyze soil properties. The other parts of fresh samples underwent vacuum freeze-drying and were later used for PLFA analysis.

Soil properties were analyzed at Yunnan Agriculture Academy, Yunnan Province, China. Soil pH was determined in a soil: H_2O (1:2.5) solution using a pH meter (FE-20, Five Easy PlusTM, Mettler-Toledo, Germany). Soil available phosphorus (AP) was analyzed using Bray method (Bray & Kurtz 1945). Soil mechanical composition was determined according to the methods proposed by Liu & Huang (2005). The high-temperature catalytic combustion method was used to determine total carbon (TC), total nitrogen (TN), total hydrogen (TH) and total sulfur (TS) content of soil samples using elemental micro-analysis in the Elemental Analyzer Vario MICRO-cube according to the Pregl-Dumas method (Sugimura & Suzuki 1988). Soil C/N ratio values were calculated as the ratio of total carbon to total nitrogen.

Microbial community analyses

The soil microbial community was characterized using PLFA analysis that was performed in the laboratory of the South China Botanical Gardens, Chinese Academy of Sciences, based on the method of Bossio et al. (1998). The MIDI Sherlock^{TM} Microbial Identification System (MIDI, Newark, DET) was used to analyze each specific signature derived from the GC-MS for each of the individual PLFA.

Different PLFA were representative of different groups of soil microbial organisms from each soil sample. Twenty-nine soil microbial communities were identified as a specific microbial group through the use of their biomarkers (Table 1) according to references (Gui et al. 2017, Willers et al. 2015). Total microbial biomass was expressed as the sum of all the extracted PLFA. Biomass of every microbial group was estimated based on the 19:00 internal standard concentrations (Bossio et al. 1998).

Table 1 Phospholipid fatty acids used in the analysis and classification of the soil microbial communities in our study.

Soil microbial groups and ratios	Diagnostic fatty acids
Gram-positive bacteria (G^+)	i14:0, i15:0, a15:0, a16:0, i16:0, i17:0, a17:0, i18:0
Gram-negative bacteria (G^-)	16:1w7c, 16:1w11c, 17:1w8c, 18:1w5c, 18:1w7c, cy17:0, cy19:0, 15:0 3OH, 20:1w9c
Bacteria (B)	G^+, G^-, non-specific bacteria
Fungi (F)	18:1w9c, 18:3 w6c (6,9,12)
Saprotrophic fungi (Sap)	18:2w6c, 18:2w9c, 18:3w6c
Arbuscular mycorrhizal fungi (AMF)	16:1 w5c
Ratio of F to B (F/B)	F/B
Actinomycetes (A)	10-methyl branched
non-specific bacteria (OB)	14:0, 15:0, 16:0, 17:0, 18:0

The formula for calculating microbial biomass is as follows:

PLFA (ng/g dry soil) = (Response of unknown PLFA/ Response of 19:00 internal standard) × concentration of 19:00 internal standard (5 μg/ml) × (volume of sample (200 μl)) /mass of soil (8 g).

Climatic factors

Data on climatic factors during the investigating period were downloaded from National Science & Technology infrastructure (http://data.cma.cn/), including mean precipitation (MP) (mm), mean lowest temperature (MLT) (℃), mean highest temperature (MHT) (℃), mean temperature (MT) (℃), and mean relative humidity (MRH) (%) (Table 3).

Statistical analyses

All statistical analyses were performed in Vegan and MASS packages of *R* studio (Dixon 2003, Oksanen 2014). Multivariate analysis of variance (Kabacoff 2015) was performed for assessing the effect of different treatments and treatment time (first month and second month) as factors for each group, and the effect on soil organic matter. Principal component analysis (PCA) based on the Euclidean measure of distance. PCA data was used to examine individual PLFA changes of soil samples under different treatments and times. Redundancy analysis (RDA) was performed to reveal the relationships between the soil community and environment. Spearman's correlation analysis was used to analyze nonparametric data.

Results

The effect each of four treatments on the soil microbial community

By the first month after treatment (Fig. 2a, Table 2), there was no significant difference between the biomass of soil fungal, bacteria and arbuscular mycorrhizal fungi (AMF) in the four treatments. Furthermore, after one month of treatment, the G^+/G^- ratio in NL was significantly higher than NR and the control ($P<0.05$), and the F/B ratio significantly decreased in O^-L^+ treatment compared to the control ($P<0.05$). There was no significant difference in actinomycetes biomass between the control and the four treatments during the first month. Saprotrophic fungi were rarely found in any of the four treatments or in the control plots.

After two months of treatment (Fig. 2b, Table 2), the O^-L^+ plots had a significantly lower fungal, bacterial, and total community biomass, as well as a lower F/B ratio compared to the control but showed a significant increase in the G^+/G^- ratio ($P<0.001$). In addition, the total biomass of the microbial community in the O^-L^+ treatment was significantly lower than the NL, NO and NR treatments ($P<0.05$) (Table 2) and the fungal biomass was significantly lower than the NL

and NR treatments (*P<0.01*) (Table 2). The biomass of fungi, G^+/G^- and F/B had no significant difference between O^-L^+ and NO treatment (Table 2). The AMF biomass decreased significantly under the O^-L^+ treatment compared to NL and NR treatments, whereas the AMF biomass under the NL and NR treatments, significantly increased relative to the O^-L^+ and NO (*P<0.05*) treatments. The NO treatment led to a significant increase in the biomass of the G^+/G^- ratio (*P<0.05*). The biomass of actinomycetes under NL and NR treatments were significantly higher than that of O^-L^+ treatment (*P<0.05*). Furthermore, there were no significant differences between the four treatments in the biomass of saprotrophic fungi.

Table 2 Results of multivariate analysis of variance testing the effects of the five treatments on biomass of the key soil microbial groups sampled from a mixed forest dominated by *Picea* and *Abies* tree species in Yulong Snow Mountain, southwestern China.

Treatment	**One month**	**Two months**								
/*P*	G^+/G^-	OB	G^+	G^-	A	Fungi	AMF	G^+/G^-	F/B	Total
CK-NL	**0.006***	0.439	0.142	0.986	0.277	0.995	**0.050***	0.076	0.331	0.523
CK-NO	0.669	0.998	0.980	0.629	0.927	0.801	1.000	**0.014***	0.214	0.990
CK-NR	0.989	0.859	0.669	0.955	0.131	0.956	0.728	0.721	0.990	0.891
CK-O^-L^+	0.352	**0.049***	0.126	**0.003****	0.323	**0.017***	0.152	**0.000*****	**0.003****	**0.021***
NO-NL	0.101	0.295	0.349	0.3412	0.722	0.588	**0.050***	0.924	0.998	0.285
NR-NL	**0.017***	0.943	0.799	0.9997	0.991	0.997	0.470	0.558	0.588	0.958
O^-L^+-NL	0.265	**0.001****	**0.001*****	**0.000*****	**0.006****	**0.007****	**0.000*****	**0.033***	0.172	**0.000*****
NR-NO	0.905	0.712	0.931	0.2578	0.458	0.402	0.728	0.174	0.424	0.659
O^-L^+-NO	0.980	0.088	**0.042***	0.0737	0.080	0.156	0.152	0.163	0.274	**0.050***
O^-L^+-NR	0.622	**0.006****	**0.008****	**0.000*****	**0.002****	**0.003****	**0.012***	**0.001****	**0.008****	**0.003****

*$P<0.05$, **$P<0.01$, ***$P<0.001$

A: actinomycete; AMF: arbuscular mycorrhizal fungi; G^+: gram positive bacteria; G^-: gram negative bacteria; OB: nonspecific bacteria; F/B: fungi/ bacteria.

The effect of treatment time on soil microbial communities and soil properties

During the experimental period, the biomass of G^+/G^- at the second month was significantly higher than that at the first month ($P<0.05$) in the O^-L^+ plots, but other microbial groups showed no significant increase over time. The F/B ratio did not change significantly over time under all the treatments. Additionally, soil properties had no significant change among four treatments with treatment time (Table 3).

Analyses of the main factors affecting soil microbial community

PCA analysis indicated distinct differences between the control, the four treatments and treatment time. PC1 and PC2 separately captured 67.1 % and 23.8 % of the total data variability (Fig. 3). PC1 separated the samples of different treatment time. The second month of O^-L^+ treatment showed a significant difference in the soil microbial community compared to other treatments (*P<0.05*). Three treatments (NO, O^-L^+, NL) were discriminated from the control on PC2. Samples of NO and NL were characterized mainly by bacteria, fungi and AMF. O^-L^+ had a significantly lower microbial biomass than the other treatments, and this effect was more pronounced during the second month of treatment.

Correlations among soil chemical properties, treatments and soil microbial community

RDA analyses showed that RDA1 accounted for 38.3 % of the variation (Fig. 4). AMF, actinomycetes and fungi were strongly correlated with C/N, coarse grained soil, TS and pH. The O^-L^+ was separated from the control at the second month. The second axis, RDA2, accounted for 2.2 % of the variation; TC was significantly intercorrelated ($P<0.05$). We also found positive relationships among TN, TC and TH and that were strongly related to litter removal. Furthermore, C/N, TS, AP and pH were negatively correlated with coarse sand and positively correlated with litter and organic layer removal. Plots of O^-L^+ were separated from the control based on TC and

Table 3 The soil properties and meteorological data at every plot of the five treatments in mixed forest with *Picea* and *Abies* at Yulong Snow Mountain, southwestern China. Values are means (standard errors) of five replicate plots.

	One month					**Two months**				
	CK	NL	NR	NO	O^-L^+	CK	NL	NR	NO	O^-L^+
Total C (mg/kg)	574.60±13.194	615.40±10.165	545.60±76.170	569.00±77.699	586.00±77.64	521.00±45.374	612.60±111.64	524.0±59.970	556.4±87.53	576.0163.37±
Total N (mg/kg)	39.60±8.686	41.40±6.974	36.80±6.046	37.20±2.993	39.60±4.499	35.80±3.311	41.60±7.499	35.40±3.499	38.20±3.816	39.60±9.646
Total S (mg/kg)	11.88±2.210	12.74±1.986	12.42±1.754	14.60±2.839	11.820±1.933	13.06±2.333	14.50±0.867	15.20±1.789	13.12±2.093	13.86±3.822
Total H (mg/kg)	161.12±20.433	173.64±5.302	156.940±13.593	167.56±12.756	173.32±11.89	159.64±3.511	177.90±21.533	155.7±10.818	160.8±8.783	170.04±23.62
C/N (mg/kg)	14.59±1.093	14.97±1.049	14.94±1.154	15.21±1.190	14.80±0.939	14.58±0.628	14.79±0.945	14.81±0.598	14.52±1.321	14.37±1.310
pH	4.900±0.342	5.226±0.285	5.11±0.239	4.914±0.257	4.95±0.399	5.04±0.271	5.14±0.262	5.02±0.183	4.9790.190	4.91±0.287
AP (mg/kg)	7.743±1.441	10.51±6.631	7.50±3.249	8.704.218±	6.24±3.623	4.47±0.973	6.90±6.580	4.469±0.943	6.15±3.146	5.0±3.319
<1mm,%	99.62±0.763	99.01±1.961	99.85±0.303	99.59±0.828	99.75±0.495	100.00±0.000	100.00±0.000	4.47±0.943	100.0±0.000	100.0±0.00
<0.5mm,%	95.82±3.473	96.79±5.161	97.37±3.559	97.60±4.305	97.61±3.846	97.92±2.760	98.613±1.716	96.90±2.910	97.02±2.749	97.67±1.768
<0.1mm,%	75.79±3.62	77.14±5.583	77.82±4.786	78.36±5.250	77.94±4.416	78.52±2.760	79.26±2.878	76.90±3.904	76.81±3.868	77.85±4.223
<0.05mm,%	67.03±3.545	68.413±5.563	68.92±4.911	69.54±5.315	69.21±4.508	69.72±4.329	70.44±2.986	67.89±3.920	67.78±3.924	68.83±4.483
<0.01mm,%	46.69±3.493	48.152±5.548	48.26±5.259	49.08±5.507	48.96±4.769	49.29±4.778	49.98±3.407	467.97±4.021	46.79±4.124	47.90±5.102
<0.005mm,%	37.92±3.522	39.45±5.555	39.36±5.431	40.26±5.605	40.24±4.900	40.50±4.985	41.16±3.646	37.96±4.089	37.76±4.238	38.88±5.375
<0.001mm,%	17.58±3.704	19.16±5.603	18.70±5.872	19.79±5.869	19.99±5.239	20.08±5.492	20.69±4.293	17.04±4.300	16.78±4.558	17.94±6.016
Sand, %	32.58±3.124	30.61±3.828	30.93±4.67	30.04±4.537	30.54±4.046	30.28±4.329	29.56±2.986	32.11±3.920	32.23±3.924	31.17±4.483
Coarse grain,%	20.35±0.766	20.26±0.495	20.66±0.710	20.47±0.575	20.25±0.596	20.42±0.651	20.47±0.923	20.92±0.584	20.98±0.648	20.94±0.692
Fine clay,%	17.578±3.704	19.17±5.603	18.70±5.872	19.80±5.869	19.99±5.239	20.08±5.492	20.69±4.293	17.04±4.300	16.78±4.558	17.94±6.016
Mean precipitation (mm)		134.9					341.4			
Mean lowest Temperature (℃)		14.2					14.4			
Mean highest Temperature (℃)		23.8					22.1			
Mean Temperature (℃)		18.2					17.4			
Mean relatively humidity (%)		71					82			

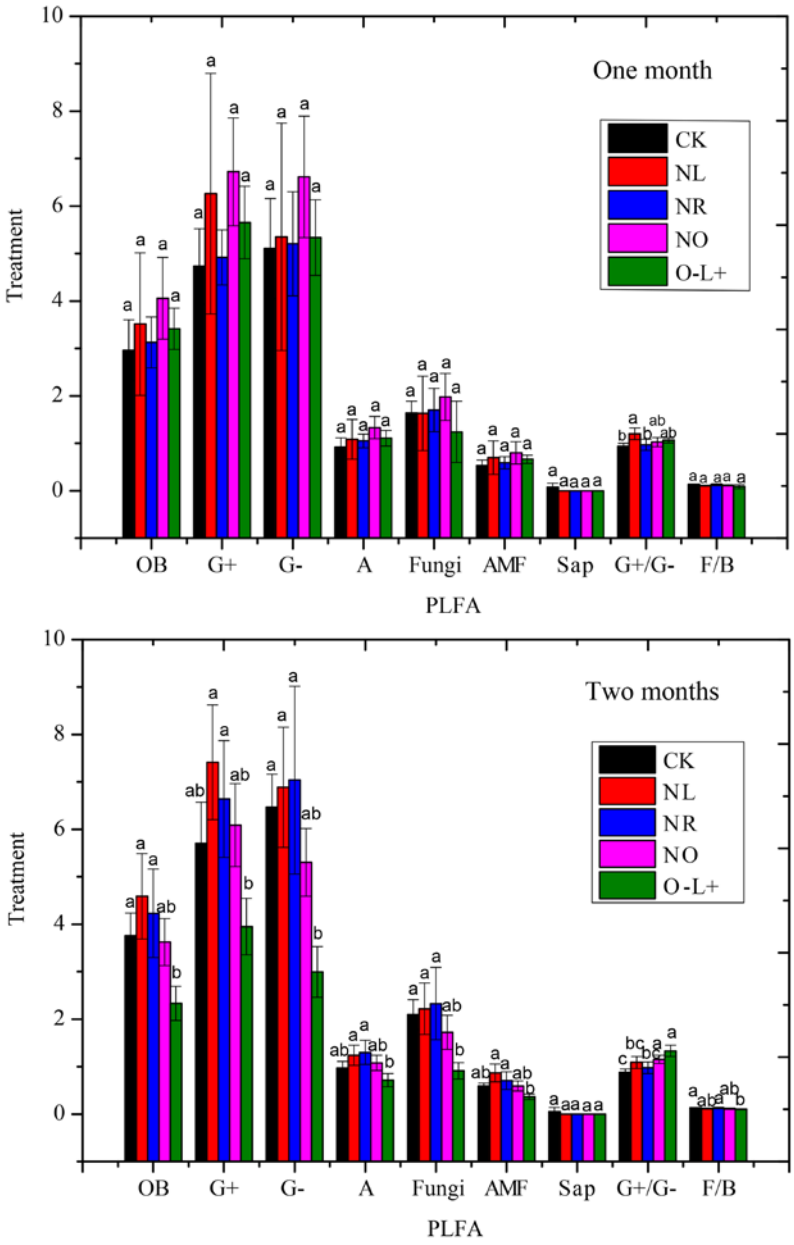

Fig. 2 – Total soil microbial biomass for the five treatments and different treatment times (one month and two months) in a mixed forest with *Picea* and *Abies* at Yulong Snow Mountain, southwestern China. The different letters (a, b, c) represent significant difference ($P<0.05$) among all treatments. CK means control. O^-L^+ means organic horizon removal and cover with litter. NR means the cutting external root connections. NL means litter removal. NO means organic horizon removal. A: actinomycete; AMF: arbuscular mycorrhizal fungi; G^+: gram-positive bacteria; G^-: gram-negative bacteria; OB: non-specific bacteria; F/B: fungi/bacteria; Sap: saprotrophic fungi.

TN. Fungi, AMF and saprotrophic fungi were affected strongly by C/N. The G^+/G^- ratio was highly correlated with AP, TC, TN and TH.

Discussion

The effect of litter and O horizon on soil microbial communities

Litter removal led to an increased biomass of bacteria, AMF and G^+/G^- ratio in the forest soils studied. Sharma et al. (2016) showed that coniferous litter inhibited the growth of soil microbial

communities by releasing phenolic compounds into the soil environment. However, the O horizon, found just below the litter layer, provides a richer source of nutrients than the other soil layers, so the soil microbial communities mainly obtain their nutrition from this layer (van Leeuwen et al. 2017). The availability of resources was thus greatly reduced for the microbial communities following the O horizon removal. However, the removal of both the litter layer and O horizon did not affect the AMF, bacterial or total soil fungal biomass within the two months periods of our study. The combined effect of removing the litter layer, and thus the inhibitory effect of the litter, and the resource rich of O horizon resulted in no significant changes in the biomass of the soil microbes in this treatment. However, although there were no notable changes in microbial biomass in this treatment, there was an increase in the G^+/G^- ratio. The observed increase in the G^+/G^- ratio is indicative of a change from copiotrophic to oligotrophic conditions and increasing environmental stress caused by the O horizon removal (Willers et al. 2015). Based on our results, it appears that resource availability in the O horizon is more important than the litter for maintaining soil microbial communities.

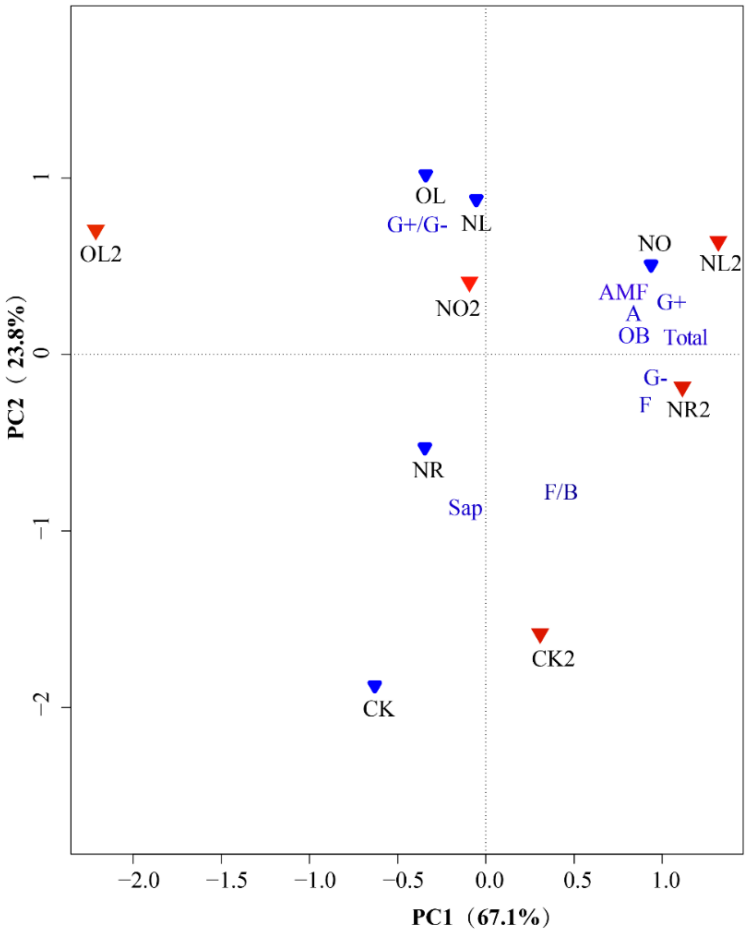

Fig. 3 – Principal component analyses (PCA) of PLFAs showing regular and un-regular triangle plots of five treatments at different treatment time and every microbial group, in mixed forest with *Picea* and *Abies* at Yulong Snow Mountain, southwestern China. *Blue un-regular triangle plots*: one month after treatment, *Red regular triangle plots*: two months after treatment.

The removal of the O horizon, but with the retention of the litter layer, led to a decline in the fungal, bacterial and the total microbial biomass, as well as a decrease in the F/B ratio after two months, yet with no observed changes after one month. It is likely that during the first month enough nutrients remained in the soil to sustain the microbial groups. Once this nutrient source was depleted, the treatment effect became notable (in the second month). The results of O^-L^+ provide further evidence that litter and O horizon have different effects on the soil microbial communities.

Our results indicate that the O^-L^+ treatment had the greatest negative impact on the soil microbial communities in short term, this impact stemming from two differences sources. First, the suggested decline in available nutrition in the soil is because of the removal of the organic layer, and secondly, there is the aforementioned negative impact by the coniferous litter layer on the microbial groups. The fungal and AMF biomass in O^-L^+ plots were significantly lower than that in the NL plots, which indicates that the O horizon plays an important role in AMF and fungal development. These results are in agreement with those of Toljander et al. (2006), who found that nutrient-rich soils in the O horizon support plants in their production of easily degradable litter and the ability of their roots to be easily colonized by AMF. Furthermore, F/B ratio was significantly decreased compared with the control, which may be due to the fact that fungi are more sensitive to disturbance than bacteria and they tend to respond more quickly than bacteria to changes (D'Ascoli et al. 2005, Mamilov & Dilly 2002).

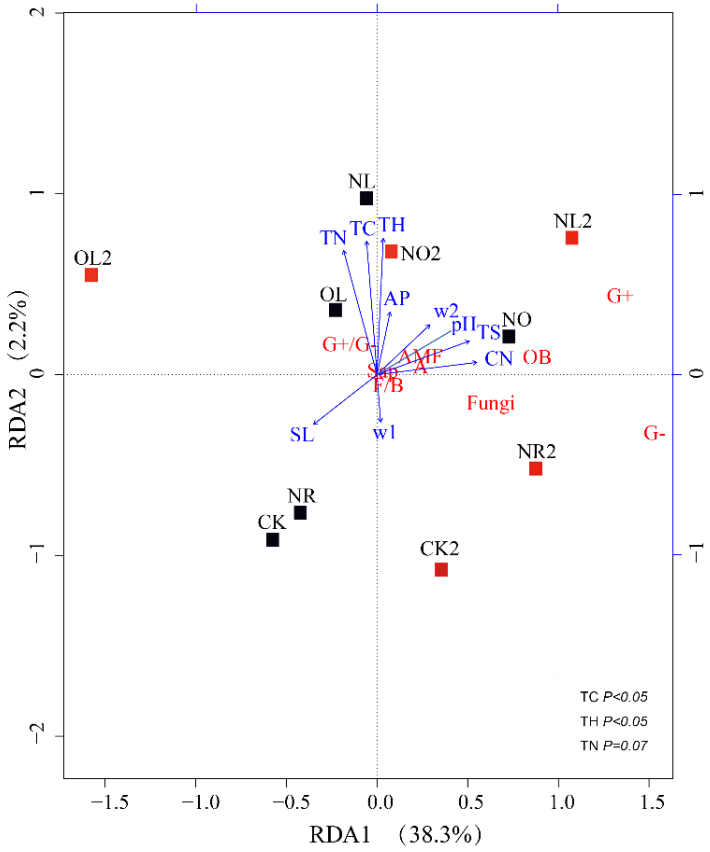

Fig. 4 – Redundancy analyses (RDA) of PLFA profiles for each soil microbial group and the five treatments (CK, NL, NR, NO and O^-L^+) at different treatment time and 11 environmental parameters. Vectors represent environmental variables. *TC*: total carbon, *TN*: total nitrogen, *TH*: total hydrogen, *TS*: total Sulphur, *pH*: *AP*: available phosphorus, *CN*: the ratio between total carbon and total nitrogen, *w1*: diameter of soil pellet < 1 mm, *w2*: diameter of soil pellet < 0.5 mm, *SL*: coarse grained soil.

The effect of tree root on soil microbial communities

Digging a trench around the plots prevented any new roots entering the plots. There was a positive relationship between root cutting and the biomass of all key microbial groups observed in our study. In comparison, the removal of the organic layer, while retaining the litter layer caused a decline in the key microbial groups, indicating that soil nutrition and litter had a greater impact on the microbial communities than do tree roots. Trenching, and thus severing of roots entering the plots, had no effect on the development of fungi, actinomycetes and bacteria. However, it is worth

mentioning that this lack of observed differences may result from the influence of roots running into the plot deeper in the soil than the 40cm trench, and that future studies should including trenching that runs to a greater depth.

The effect of soil nutrition on soil microbial communities

Soil carbon directly influences microbial community dynamics and the balance between formation and degradation of microbial byproducts (Six et al. 2006). Our study highlighted the impact of carbon on microbial biomass and the observed relationship between TC and the biomass of microbial communities has also been shown in the studies conducted by van Leeuwen et al. (2017), Schmidt et al. (2017). Soil carbon provides available carbon and energy for the microbial communities and is one of the most important soil characteristics shaping the microbial community structure and function (Yang et al. 2017, Zabaloy et al. 2016). The lack of a measureable relationship between TN and microbial biomass in our study may be due to the short-term treatment employed in our study. In order to provide an understanding of the relationships between soil nutrition and microbial biomass, further work should focus on the effect of nitrogen addition or removal on biomass of soil microbial communities, as well as a longer time frame to elucidate the long-term impacts of the treatments.

Conclusion

Our results showed that the effect of the four treatments observed on microbial communities became gradually stronger with increased treatment time. Furthermore, our work highlights how the different components of the soil system (litter layer, O horizon, roots) can influence the soil microbial groups in different ways. Moreover, the biomass of the soil microbial community was shown to be sensitive to changes in the soil environmental, and suggests that a more integrated approach investigating both the short- and long-term responses of soil communities to litter removal is required.

Acknowledgements

We would like to thank the National Science Foundation of China (NSFC) for funding this work (the project codes 41761144055 and 41771063). Kevin D. Hyde thanks the Chinese Academy of Sciences (project number 2013T2S0030) for the award of Visiting Professorship for Senior International Scientists at Kunming Institute of Botany. We wish to thanks Prof. Junchao Huang' group at Kunming institute of botany, Chinese Academy of science for soil freeze drying. We would also like to thank the staff of the Lijiang Alpine Botanic Garden for their considerable help during the field work. We thank Yunju Li and some stuffs from Kunyang Phosphate Rock, Yunnan Province, China for analyses of soil properties. The authors also would like to thank Lingling Shi, Mingxia He, Shujiao Yang for soil treatment and data analyses. We thank Fiona Worthy and Andrew Stevenson for English checking.

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