

Asymmetric effects of litter removal and litter addition on the structure and function of soil microbial communities in a managed pine forest

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Abstract

Aims Variation in tree litter inputs and understory vegetation caused by human disturbances and climate change in forest plantations can extend to alter forest stability and productivity over time. Here, we explore how tree litter inputs interact with understory plant

management to influence belowground processes in a managed forest plantation.

Methods We conducted a two-factor nested experimental manipulation of pine litter and understory vegetation in a nutrient-poor *Pinus sylvestris* var. *mongolica* plantation. Three levels of tree litter manipulation (ambient litter, litter removal and litter addition) were nested in two levels of understory manipulation (understory intact and understory removal). After two years of manipulation, mineral soils were analyzed for total and extractable C, N and P concentrations, N mineralization, enzyme activities, as well as the microbial community structure (as indicated by phospholipid fatty acids).

Results Litter removal had little impact on C and nutrient cycling as well as microbial biomass and community structure in this low nutrient pine plantation; however, litter addition and the removal of the understory vegetation had large impacts on these processes. Litter addition elevated soil microbial biomass, acid phosphatase and β -1, 4-glucosidase activities, by a much greater degree when the understory vegetation was intact than when the understory was removed. Litter addition also reduced soil available P by 39% when the understory vegetation was intact, and reduced soil available P by 74% and NO_3^- -N by 45% when the understory was removed. Litter addition significantly reduced the ratio of Gram-positive to Gram-negative bacteria as well as the ratio between PLFA markers cy17:0 and $16:1\omega7$. Understory removal reduced the ratio of PLFA markers cy17:0 to $16:1\omega7$.

Conclusions Our study results show that, in this managed pine plantation, soil microbial community structure and function were more sensitive to an increase

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rather than to a decrease in pine litter inputs. Further, we found that the presence of understory vegetation can increase soil microbial biomass and alleviate the reduction in available N and P concentrations induced by pine litter addition. Thus, preservation of the understory vegetation is an effective way to maintain the functional stability of managed forests on nutrient-poor soils.

Keywords *Pinus sylvestris* var. *mongolica* · Litter · Understory vegetation · Soil microbial community · Nutrient availability

Introduction

Managed forests provide numerous ecosystem services such as control of soil degradation and erosion, in addition to wood production. The impacts of forest management and disturbance can extend to alter the structure and functioning of forest ecosystems over time (Fox 2000). For example, harvesting litter from managed forests for fuel and farming was widely practiced in many countries throughout the nineteenth century, and is still occurring in some areas (Hofmeister et al. 2008; Chevasco et al. 2016); however, this practice can negatively impact nutrient cycling and forest productivity. In addition, nitrogen (N) deposition, elevated atmospheric [CO₂], and warming are expected to increase litterfall in forests via the promotion of productivity (Norby et al. 2002; Liu and Greaver 2010). Variation in litter inputs to the soil can alter the amount of carbon (C) and nutrients entering the soil system and thus the amount of nutrients internally cycled in forest plantations (Sayer 2006). This impact can be especially large in nutrient-poor forest plantations where the removal of litter reduces internal nutrient feedbacks to the trees. Managing to preserve understory vegetation may be an effective way to retain C stocks and nutrient pools in plantations as well as regulate the impacts of variable litter inputs on belowground biochemical processes. Given that forest plantations make up approximately 5% of the world's forest cover and are expanding at a rate of 5 million hectares per year (FAO 2010), understanding how management of the litter and understory vegetation layer alters forest ecosystem stability is important.

Aboveground litterfall contributes significantly to organic C and nutrient pools in forest soils, and how variation in litterfall influences soil properties in forests has been extensively studied since the 1850s (Sayer 2006; Xu et al. 2013). In general, aboveground litter

addition stimulated soil biogeochemical processes (e.g., microbial biomass, soil respiration) and increased soil C storage, while litter removal had the opposite impact in tropical and subtropical forests. However, these patterns are inconsistent in temperate and tundra regions (Xu et al. 2013). For example, several temperate forest studies found that aboveground litter addition and removal had little effect on soil organic C and N transformations and microbial biomass even after 14 years of litter manipulation (Holub et al. 2005; Brant et al. 2006; Huang and Spohn 2015). The inconclusive results of litter manipulation experiments reflect that litterfall influences on C and nutrient processes are more complex than a simple addition or removal effect, and other interacting mechanisms may be at play, such as the presence of an understory plant community (Six et al. 2002; Sayer et al. 2012; Brant et al. 2006).

Understory vegetation in managed forests usually has a higher biodiversity and turnover rate than the overstory trees, but its role in maintaining the structure and function of managed forest has been largely overlooked (Gilliam 2007). Traditionally, understory vegetation is removed in managed forests to reduce resource competition between trees and the understory community (Wagner et al. 2006). However, this may be a poor management strategy in some forests because the understory plant community can increase forest nutrient retention (Chapin 1983; Nilsson and Wardle 2005). Across temperate forests, understory herbs make up only 0.2% of aboveground biomass, but contribute approximately 16% of the total annual forest litterfall. Further, herbaceous litter decomposes more than twice as rapidly as tree litter because it has a higher foliar nutrient concentration (Muller 2003). For example, in Hubbard Brook Experimental Forest, concentrations of N and P were 30% higher, Mg was twofold and K was threefold higher in understory herb foliage than in trees (Muller 2003). Thus, managed forests with their understory intact may be more resistant to the variation in litterfall than forests with their understory removed.

Understory vegetation can influence belowground biochemical processes such as microbial activity, C and nutrient stocks, which in turn can impact long-term forest productivity and stability, particularly for nutrient-poor managed forests. The effects of understory vegetation on soil C stocks can be positive or neutral, which can be dependent on species composition and the litter chemistry of understory communities (Busse et al. 1996; Nilsson and Wardle 2005; Powers et al. 2013). However, soil

microbial biomass, enzyme activities and soil respiration are generally higher when understory vegetation is intact than when it is removed (Blazier et al. 2005; Rifai et al. 2010; Wu et al. 2014). Understory vegetation can also elevate total N concentrations in top-soil (Rifai et al. 2010). While researchers know the impacts of understory removal on belowground processes generally reduce nutrient retention in forests, the mechanisms for why this occurs remain unclear. It is likely that understory plant inputs, which are more labile, are cycled more quickly than inputs from trees (Gilliam 2007). In addition, understory vegetation can interact with aboveground tree litter to shape belowground biochemical processes (Mitchell et al. 2012; Lin et al. 2013; Qiao et al. 2014). Thus, more field work is needed to understand the effects of understory vegetation and its interactions with tree litter on belowground biochemical processes.

Here, we experimentally manipulated understory vegetation and aboveground litter in a nutrient-limited temperate pine plantation to examine the impacts of variable pine litter inputs on soil processes over two years, and to test whether these impacts are affected by understory vegetation management. We predicted that: (1) soil microbial communities are likely C-limited at our site, thus litter addition would have a strong positive impact and litter removal would have a negative impact on soil microbial biomass and activity; (2) removing the understory vegetation would reduce plant nutrient uptake and thus increase soil available nutrient concentrations; (3) maintaining the herbaceous understory vegetation would accelerate the nutrient release of pine litter, thus preserving understory vegetation would lessen the negative effects of variation in litter inputs on belowground processes.

Materials and methods

Site description

We conducted this study at the Daqinggou Ecological Station (42°58' N, 122°21' E, 260 m above sea level), which belongs to Institute of Applied Ecology, Chinese Academy of Sciences and is located in southeastern Keerqin Sandy Lands, Northeast China. The climate at the site is temperate and the field site is located in the semiarid region. The mean annual temperature at the site was 6.4 °C, with the monthly mean temperature lowest in January (−12.5 °C) and highest in July (23.8 °C). Mean annual precipitation was 450 mm, with over 60%

of the precipitation occurring from June to August. The soil is nutrient-poor sandy soil developed from eolian deposit (Typic Ustipsamment), with 90.9% sand, 5.0% silt, and 4.1% clay.

We used a 13-year-old pure even-aged Mongolian pine (*Pinus sylvestris* var. *mongolica*) plantation for this experiment. Mongolian pine was widely planted in northern China in past decades mainly in soil conservation efforts. The plantation was established at a tree spacing of 2 m × 5 m on the degraded grassland with flat topography. At the start of the experiment, canopy closure was 60%, average tree height was 3.9 m, and stem diameter at breast height was 7.0 cm. In non-manipulated plots, the mean annual litterfall rate was about 305 g m^{−2}, and the concentrations of total N and P and C/N ratio in pine litter were 4.5 g kg^{−1}, 0.33 g kg^{−1} and 108, respectively. The understory vegetation mainly consisted of *Artemisia scoparia*, *Cannabis sativa*, *Setaria viridis*, *Chenopodium acuminatum*, and *Lespedeza daurica*. Understory cover was 80%, and understory aboveground biomass was approximately 337 g m^{−2}. For the dominant understory species *Artemisia scoparia*, the concentrations of total N and P, and C/N ratio in the aboveground litter were 12.4 g kg^{−1}, 3.1 g kg^{−1}, and 38, respectively. For the dominant species *Setaria viridis*, aboveground litter N and P concentrations and C/N ratio were 3.7 g kg^{−1}, 1.3 g kg^{−1}, and 116, respectively.

Field manipulations and soil sampling

The field experiment was established as a fully nested two-factor design with 4 replicates and the aboveground litter manipulation was nested within understory vegetation manipulation. The understory manipulation experiment was started in April 2011. There were two treatments: understory vegetation intact (Uc, the control) and understory vegetation removal (U-). We established 8 main plots (20 m × 30 m), where 4 plots of Uc and 4 plots of U- were randomly arranged. All plots were at least 5 m away from each other. At the beginning of the experiment, 50% (w/v) acetochlor solution was spread evenly in U- plots to kill the understory vegetation, and an equal amount of water was spread in the Uc plots as a disturbance control. Afterward, the remaining and recolonizing understory vegetation was removed by hand monthly during the growing season. The acetochlor is easily degraded and it has minimal impacts on soil ecosystems (Feng et al. 2008).

The aboveground litter manipulation was initiated in October 2012. There were three treatments: litter removal (0 L), ambient litter (1 L), and litter addition (2 L), which were randomly arranged in 3 sub-plots (5 m × 5 m) within each understory manipulation plot. The litter manipulation focused on removing litter from the Mongolian pine. At the beginning of the experiment, the whole aboveground litter layer was removed from 0 L plots and added to 2 L plots. Afterward, 2 mm nylon mesh was laid on the ground in 0 L plots during the litterfall season (from October to May) when litterfall accounted for >80% of total annual litterfall, and litter on the mesh was collected and added to corresponding 2 L plots regularly. During the growing season (from June to September), the net was removed to avoid interference to the growth of understory vegetation, and pine litter on the ground was carefully raked monthly from the 0 L plots and added to the 2 L plots.

Surface mineral soil samples (0–10 cm) were collected in August 2014 using a soil corer with an inner diameter of 2.5 cm. We chose to sample in August (mid-summer) because temperature and rainfall peak and biological activities were at their maximum from July to August at the study site. Ten soil cores were collected from each experimental unit and homogenized into one sample. Soil samples were sieved through 2 mm mesh and divided into three subsamples: One subsample was air-dried for the determination of soil pH, soil organic C (SOC), total N, total P and Olsen-P; the second subsample was stored at 4 °C for less than 5 days until the measurement of soil moisture, inorganic N (the sum of $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$), potential N mineralization and nitrification rates, acid phosphatase activity and β -1,4-glucosidase activity; the third subsample was freeze-dried for phospholipid fatty acid analysis of soil microbial community.

Soil physicochemical properties

Soil water content was measured from mass loss after drying at 105 °C to constant weight. Soil pH was measured with a pH meter in a 1:2.5 soil/water suspension (Lu 1999). SOC was determined by the $\text{H}_2\text{SO}_4\text{-K}_2\text{Cr}_2\text{O}_7$ oxidation method (Nelson and Sommers 1996). Light fraction organic C was isolated by flotation on NaI solution adjusted to a density of 1.8 g cm^{-3} (Sohi et al. 2001). Soil total N and P concentrations were determined using a continuous-flow autoanalyzer (AutoAnalyzer III, Bran + Luebbe GmbH, Germany)

after digestion in 5 ml H_2SO_4 with a catalyst (mixture of CuSO_4 and K_2SO_4) (Lu 1999). Soil Olsen-P concentration was analyzed colorimetrically using the molybdate blue method after the soil was extracted with 0.5 mol L^{-1} NaHCO_3 at pH = 8.5 (Olsen et al. 1954). Concentrations of soil $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ were analyzed colorimetrically on the autoanalyzer after the soil was extracted with 2 M KCl solution. Potential net N mineralization rate was calculated as the change in soil mineral N ($\text{NO}_3^-\text{-N}$ plus $\text{NH}_4^+\text{-N}$) concentration over the 28-d aerobic incubation at 25 °C. Potential net nitrification rate was calculated as the change in $\text{NO}_3^-\text{-N}$ concentration during incubation. Soil acid phosphomonoesterase (AP) and β -1,4-glucosidase (BG) activities were assayed using disodium *p*-nitrophenyl phosphate and *p*-nitrophenyl- β -d-glucopyranoside as the substrate, respectively. The *p*-nitrophenol (*p*NP) released by enzymatic hydrolysis was measured colorimetrically, and AP and BG activities were expressed as $\text{mg pNP kg}^{-1} \text{ h}^{-1}$ (Tabatabai 1994).

Phospholipid fatty acid analysis

Soil microbial community composition was analyzed with a phospholipid fatty acid (PLFA) analysis, using a combination of methods from Frostegård and Bååth (1996) and Bossio et al. (1998). Briefly, 8 g freeze-dried soil samples were extracted in chloroform-methanol-phosphate buffer (1:2:0.8 vol/vol/vol). Extracts were sequentially fractionated and transmethylated to their fatty acid methyl esters by alkaline methanolysis. The extracted fatty acid methyl esters were identified on a gas chromatograph (Agilent 7890A) equipped with a flame ionization detector, in combination with the MIDI identification software (MIDI Inc. Newark, DE). Methyl nonadecanoate fatty acid (19:0) was used as the internal standard for quantifying the PLFAs. A total of 26 individual PLFA biomarkers were identified in this study. Total extractable PLFAs were used as an indicator of total soil microbial biomass, and specific PLFA markers were used to quantify the biomass of various taxonomic microbe groups. The PLFA 18:2 ω 6 was used to indicate fungi biomass, and 16:1 ω 5 was used to indicate arbuscular mycorrhizal fungi (AMF) biomass (Swallow et al. 2009). The PLFAs 10Me16:0 and 10Me18:0 were used to indicate soil actinomycetes. The PLFA 20:4 ω 6 was used as the indicator of protozoa. Gram-positive (G+) bacteria were considered to be comprised of PLFAs i14:0, i15:0,

i16:0, a15:0, a17:0, i15:1 ω 6 and i17:1 ω 9. Gram-negative (G-) bacteria comprised of PLFAs 16:1 ω 9, 16:1 ω 7, 17:1 ω 8, cy17:0 ω 7, 18:1 ω 5 and cy19:0 ω 7. The fungal/bacterial ratio was calculated as 18:2 ω 6/(the sum of G+ and G- bacteria and non-specific bacteria marks 14:0, 15:0, 16:0, 18:0, 24:0, i17:0, 18:3 ω 6 and 18:1 ω 9) (Frostegård and Bååth 1996; Zelles 1997, 1999). In addition, we used the ratio of cy17:0/16:1 ω 7 as an indicator of environmental stress because this ratio tends to rise as substrate availability declines (Bossio et al. 1998; Moore-Kucera and Dick 2008).

Statistical analysis

The main effects of litter manipulation (L) and understory manipulation (U) on soil variables were analyzed with a two-way nested analysis of variance (ANOVA). Further, one-way ANOVA was used to test the effects of litter manipulation on soil variables for the understory intact plots and understory removal plots separately. Post-hoc multiple comparisons of means were then used to compare differences among treatments using the least significant difference (LSD) test. We focused on the differences between the ambient litter treatment and two manipulated treatments (litter removal and litter addition). All data were tested for normality and homogeneity of error variance prior to ANOVA, and log transformed when necessary. Significant level was set as 0.05. We did not apply a correction to the *P* values (e.g. Bonferroni) though we conducted ANOVA for lots of soil variables, because Bonferroni correction required independence between tests while all variables we

measured were more or less correlated with one another (Bland and Altman 1995). To determine effects of experimental treatments on the structure of microbial communities, data from PLFA profiles were analyzed with a two-way analysis of similarity (ANOSIM) using a matrix of Euclidian distance on PRIMER 7.0 (Clarke 1993). *R* values from ANOSIM range from -1 to 1, with the higher value indicating the greater difference between or among groups. In addition, to visualize differences in microbial community structure, principal components analysis (PCA) of the microbial community composition, defined by the PLFA profile (Frequency \geq 50%), were performed using CANOCO software.

Results

Soil properties

Soils were more acid in the understory removal plots than in the understory intact plots; however, there were no effects of litter manipulation on soil pH (Table 1). The concentration of SOC was 52% (in the understory intact plots) and 34% (in the understory removal plots) higher in the litter addition treatment than in the ambient litter treatment, and it was not affected by either understory removal or litter removal. The concentrations of light fraction organic C, total N and total P did not vary among any of our treatments. There were no significant effects of treatment on soil moisture, though it tended to increase with litter addition and decline with understory removal (Table 1 and Supplementary material 1).

Table 1 Soil physicochemical properties in 0–10 cm soil layer in a Mongolian pine plantation as affected by understory removal and litter manipulation

Treatment		Moisture (%)	pH	SOC (g kg ⁻¹)	Total N (g kg ⁻¹)	Total P (g kg ⁻¹)	Light fraction C (g kg ⁻¹)
Uc	0 L	4.91(0.46)	6.75(0.35)	3.60(0.58)	0.37(0.05)	0.10(0.01)	0.20(0.00)
	1 L	4.52(0.38)	6.36(0.21)	3.27(0.03)	0.34(0.04)	0.10(0.00)	0.20(0.01)
	2 L	5.28(0.41)	6.78(0.47)	4.96(0.56)*	0.41(0.06)	0.10(0.01)	0.21(0.00)
U-	0 L	3.95(0.31)	5.96(0.08)	3.15(0.05)	0.35(0.06)	0.10(0.00)	0.20(0.02)
	1 L	4.06(0.28)	6.13(0.14)	3.25(0.58)	0.29(0.04)	0.10(0.00)	0.20(0.01)
	2 L	4.91(0.49)	6.02(0.07)	4.35(0.10)*	0.40(0.08)	0.10(0.00)	0.20(0.04)

Values are means \pm standard error in parenthesis, *n* = 4

Asterisks denote significant differences (*P* < 0.05) in comparison to 1 L, within Uc and U- plots separately, using a LSD post-hoc test following a one-way ANOVA

Uc understory intact, U- understory removal, 0 L litter removal, 1 L ambient litter, 2 L litter addition

Soil P and N cycling

Litter addition significantly reduced extractable soil P and N concentrations, and this reduction was much smaller in the understory intact plots than in the understory removal plots (Fig. 1). Soils where litter was added had 26% (in the understory intact plots) and 61% (in the understory removal plots) lower Olsen-P concentration than soils in the ambient litter treatment (Fig. 1a). Soil NO_3^- -N concentration in the understory intact plots was not affected by litter addition, but in the understory removal plots it was significantly lower in the litter addition treatment

(0.18 mg kg^{-1}) than in the ambient litter treatment (0.33 mg kg^{-1}). Soil NH_4^+ -N concentration did not show significant differences among all treatments (Fig. 1c). In general, litter removal did not affect soil P or N availability (Fig. 1). Understory removal had no significant effect on soil NO_3^- -N or NH_4^+ -N concentrations, but significantly affected Olsen-P that was 30% higher in understory removal plots relative to the understory intact plots (Supplementary material 1).

The impacts of litter manipulation on soil P and N mineralization were contingent on the presence of the understory vegetation (Fig. 1). Soil AP activity

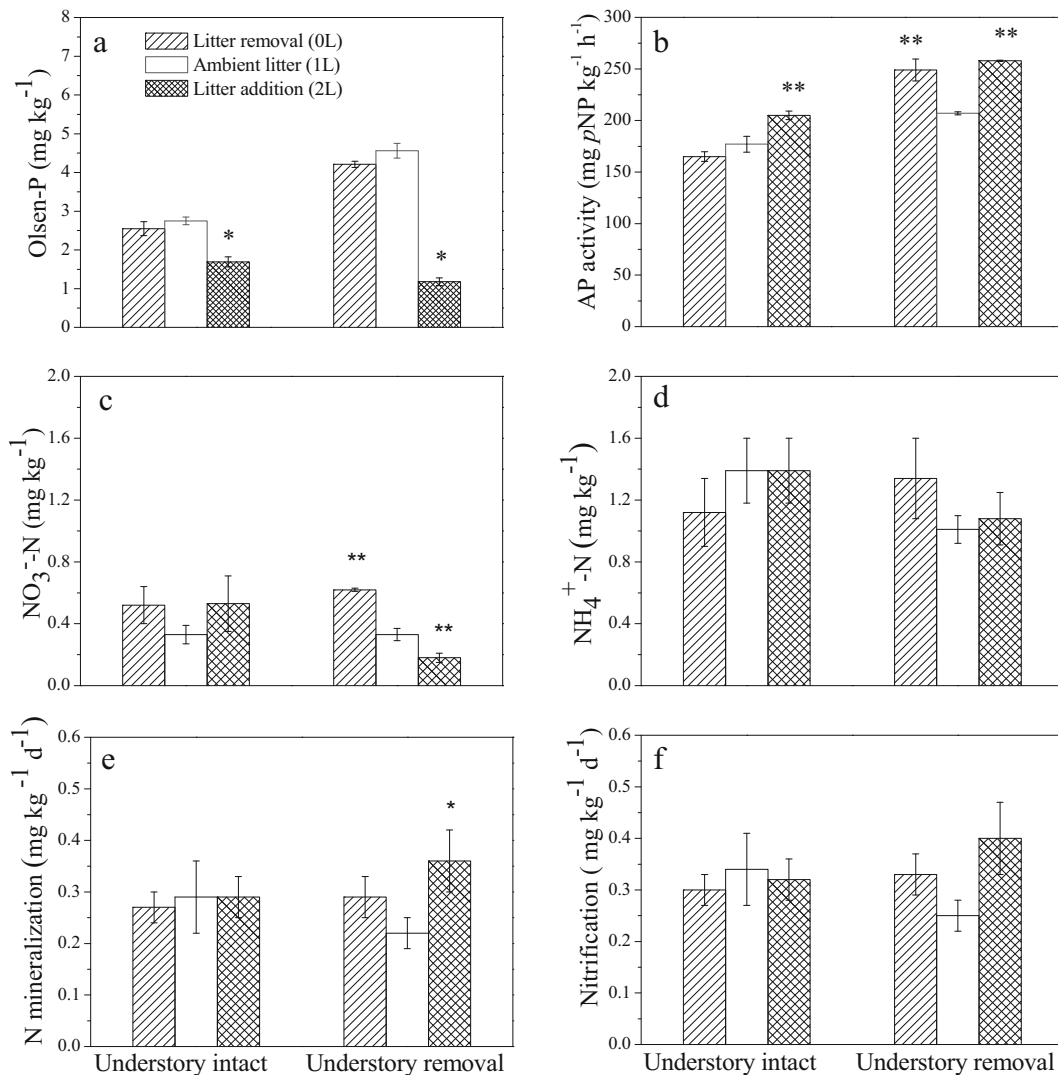


Fig. 1 Changes in soil available P and N concentrations, AP activity and potential net N mineralization and nitrification rates in response to understory and litter manipulation in a Mongolian pine plantation. Values are means \pm standard error ($n = 4$).

Asterisks denote significant differences ($* P < 0.05$ and $** P < 0.01$) in comparison to ambient litter treatment, within the understory intact and understory removal plots separately (using a LSD post-hoc test after a one-way ANOVA)

was 16% (in the understory intact plots) to 25% (in the understory removal plots) higher in the litter addition treatment than in the ambient litter treatment. Soil AP activity was also significantly elevated by understory removal and litter removal (Fig. 1b and Supplementary material 1). Net N mineralization and nitrification rates were not changed by litter addition in the understory intact plots, but were approximately 38% greater in the litter addition treatment relative to the ambient litter treatment in the understory removal plots ($P = 0.050$ and $P = 0.057$) (Fig. 1e, f). Neither understory removal nor litter removal significantly affected net N mineralization and nitrification rates.

Soil microbial activity and community composition

Soil BG activity was 57% higher in the litter addition treatment relative to the ambient litter treatment in the understory intact plots, and was only 21% higher in the understory removal plots (Fig. 2). Soil microbial biomass, as indicated by total PLFAs, was 48% higher in the litter addition treatment relative to the ambient litter treatment in the understory intact plots, and was 32% higher in the understory removal plots (Fig. 2). Soil BG activity and microbial biomass were not affected by litter removal, but was significantly affected by understory removal (Fig. 2 and Supplementary material 1). They were 15% and 38% lower in the understory removal plots than in the understory intact plots. Biomass of all microbial groups tended to vary in the similar pattern as the total PLFA. Specifically, Gram-negative bacteria was

85% higher, Gram-positive bacteria was 48% higher and fungi were 40% higher in the litter addition treatment than in the ambient litter treatment, across the understory intact and removal plots. Biomasses of all microbial groups were significantly lower in the understory removal plots than in the understory intact plots (Table 2 and Supplementary material 1). The fungal/bacterial ratio did not differ among treatments. The Gram-positive/Gram-negative bacteria ratio was lower in the litter removal and the litter addition treatments than in the ambient litter treatment, but was not affected by understory removal (Table 2 and Supplementary material 1). The *cy17:0/16:1 ω 7* ratio was also significantly higher when the understory or litter was removed (Table 2).

The results from the ANOSIM and PCA analyses were similar. Using ANOSIM, we found that both the understory removal ($R = 0.573$, $P = 0.001$) and the litter manipulation ($R = 0.515$, $P = 0.001$) significantly affected the soil microbial communities. Using the pairwise test of ANOSIM, we found that there were no differences in microbial composition between the litter removal treatment and the ambient litter treatment ($R = -0.036$, $P = 0.55$), but there were significant differences between the litter addition and the ambient litter treatments ($R = 0.693$, $P = 0.001$). Our PCA analysis showed that the first principal component (PC1) explained 88.2% of the total variances in the microbial community (Fig. 3). All PLFAs were highly correlated with PC1, and varied in the same direction. The understory intact plots with litter addition had the highest scores on PC1 among all treatments.

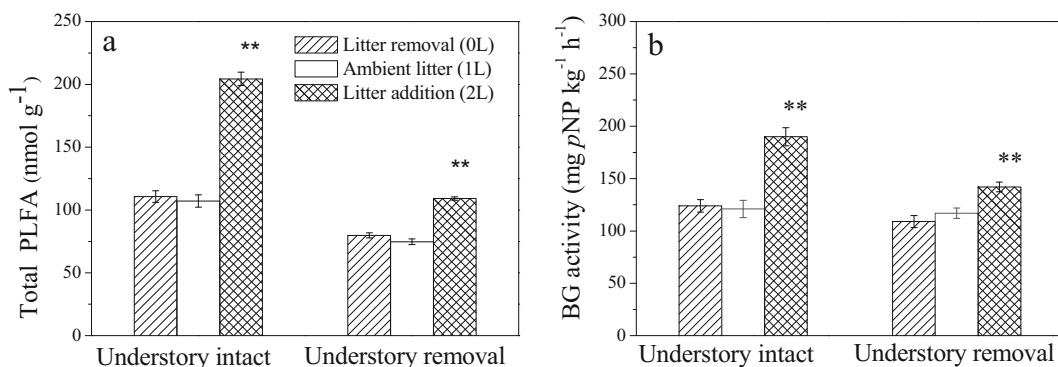


Fig. 2 Changes in soil total PLFA and BG activity in response to understory and litter manipulation in a Mongolian pine plantation. Values are means \pm standard error ($n = 4$). Asterisks denote significant differences ($P < 0.01$) in comparison to ambient litter

treatment, within the understory intact and understory removal plots separately (using a LSD post-hoc test after a one-way ANOVA)

Table 2 Microbial biomass (nmol g^{-1}) and some key ratios of PLFA biomarkers in soil (0–10 cm) impacted by understory removal and litter manipulation

Treatment	Fungi	G+ bacteria	G- bacteria	Actinomycetes	Protozoa	AMF	F/B ratio	G+/- ratio	cy17:0/16:1 ω 7
Uc 0 L	4.77(0.81)	16.1(2.04)	15.4(1.75)	6.15(0.76)	0.68(0.09)*	2.23(0.17)	0.08(0.01)	1.06(0.12)*	0.40(0.03)
1 L	5.23(0.76)	19.8(1.33)	16.1(1.78)	5.89(0.77)	0.97(0.14)	2.01(0.26)	0.09(0.01)	1.25(0.07)	0.44(0.03)
2 L	7.78(1.12)*	31.1(2.53)**	29.5(2.63)**	9.55(1.57)*	1.69(0.15)**	3.80(0.89)*	0.07(0.01)	1.07(0.09)*	0.35(0.02)*
U- 0 L	3.68(0.36)	12.2(0.26)	10.8(0.45)	4.49(0.25)	0.72(0.02)	1.26(0.04)	0.09(0.01)	1.13(0.03)**	0.69(0.07)
1 L	3.54(0.69)	12.7(1.14)	9.14(0.57)	4.66(0.91)	0.54(0.03)	1.08(0.06)	0.07(0.01)	1.38(0.06)	0.56(0.01)
2 L	4.67(0.39)	17.8(0.29)**	17.2(0.27)**	6.09(0.12)*	0.89(0.01)**	1.89(0.12)**	0.08(0.01)	1.04(0.02)**	0.49(0.02)

Values are means \pm standard error in parenthesis, $n = 4$

Asterisks denote significant differences (* $P < 0.05$ and ** $P < 0.01$) in comparison to 1 L, within Uc and U- plots separately, using a LSD post-hoc test following a one-way ANOVA
Uc understory intact, U- understory removal, 0 L litter removal, 1 L ambient litter, 2 L litter addition

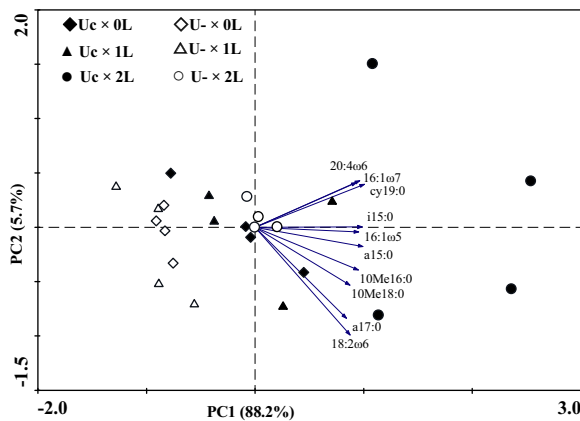


Fig. 3 Principal components analysis (PCA) of soil microbial PLFA profiles in a Mongolian pine plantation under understory vegetation and litter manipulations. Uc: understory intact; U-: understory removal; 0 L: litter removal; 1 L: ambient litter; 2 L: litter addition

Discussion

Asymmetric effects of litter removal and addition

Contrary to what we predicted, litter removal had little impact on C and nutrient cycling as well as microbial biomass and community composition in this low nutrient pine plantation. Our results are similar to those found at other temperate forest sites (Holub et al. 2005; Brant et al. 2006; Huang and Spohn 2015), but are different from most studies in tropical and subtropical regions showing lower soil C storage, nutrient availability, and microbial biomass after 2–10 years of litter removal (Fisk and Fahey 2001; Sayer 2006; Kotrocó et al. 2014). Temperate forests were often less sensitive to litter manipulation than tropical and sub-tropical forests, due to much slower soil C turnover and lower microbial biomass and activity (Xu et al. 2013; Leff et al. 2012). Soil microbial biomass (<120 nmol PLFA g^{-1} soil) was low in this study, likely due to the nutrient-poor sandy soil and semi-arid temperate climate. We do not know the exact mechanisms underlying the insignificant effect of litter removal without further study; however, one possible explanation is that belowground C sources (soil C pools, root turnover and exudates) could support the microbial growth when there are no aboveground C inputs. Many studies show that belowground C supply can exert a greater control on soil microbial biomass and activity than aboveground litter inputs (Brant et al. 2006; Feng et al. 2011; Kotrocó et al. 2014). In addition, soil microorganisms can shift their substrate use

toward recalcitrant and older soil organic matter when simple C substrates decline (Streit et al. 2014).

Soil microbial biomass and activity were more sensitive to an increase than a decrease in aboveground C inputs in our managed ecosystem. In contrast to our litter removal results, litter addition strongly and positively influenced soil microbial biomass and function — soil microbial biomass and enzyme activities were up to 48% higher in the litter addition treatment than in the ambient litter treatment. So far, studies that have explored the impacts of litter addition on soil microbial biomass found an insignificant effect of litter addition in both temperate and tropical forests, even though litter addition tended to increase soil organic C and soil respiration in these systems (Park and Matzner 2003; Brant et al. 2006; Leff et al. 2012; Sayer et al. 2011). Clearly the nutrient-poor sandy soils in our study caused the soil community to be C-limited. Given that litter addition provided a large amount of labile C substrates to these soils, it would also promote microbial growth and thus the mineralization of nutrients. Contrary to the response of soil microbial biomass and function, available N and P tended to be lower in the litter addition treatments. This likely results from the microbial immobilization of nutrients and would be a consequence of the expanding microbial biomass pool in the litter addition plots. Our previous study found that N and P immobilization occurred during the decomposition of Mongolian pine litter at this site (Zhao et al. 2013). This pattern was not surprising given that the pine litter from this plantation had the high C/N and C/P ratios (108 and 1281, respectively). Simultaneously elevated soil microbial biomass and reduced nutrient availability by litter addition suggest that increased litterfall increased N and P competition among soil microbial groups and trees in this nutrient-poor Mongolian pine plantation. This competition between the soil communities and trees may increase tree nutrient limitation and thus decrease growth.

Impacts of understory removal

Unlike the insignificant effect of litter removal on soil properties, the removal of understory vegetation had a large impact on belowground processes. Microbial biomass was significantly lower at our site when the understory vegetation was removed, a pattern observed in other studies (Blazier et al. 2005; Busse et al. 2006; Rifai et al. 2010). Reduced soil C inputs from litterfall, root turnover and exudates of the understory vegetation likely contributed to lower microbial biomass in

the understory removal treatments (Busse et al. 2006). Consistent elimination of understory vegetation had the potential to reduce soil C pools in forest plantations (Rifai et al. 2010), but the present study showed no difference in soil organic C between vegetation removal and control plots. These results were also observed in other plantation studies across a wide range of climates and site qualities, even after 20 years of manipulation (McFarlane et al. 2009; Slesak et al. 2011; Powers et al. 2013), suggesting that diversified and easily-decomposable detritus produced by understory vegetation made the C inputs from understory vegetation rapidly consumed in situ. Previous studies at our study site found that aboveground litter of dominant understory herbaceous plants have higher nutrient concentrations, lower C/N ratios, and faster decomposition rates, than litter from Mongolian pine (Lin et al. 2013; Zhao et al. 2013). Thus, understory vegetation contributed substantially to soil microbial biomass, but contributed little to soil C storage in the present study.

Understory vegetation manipulation had a large impact on soil P transformation and availability, but had no impact on soil available N concentration and net N mineralization, presumably because P cycling in forest ecosystems is more closed than N cycling (Attiwill and Adams 1993). Reduced uptake of P by the understory vegetation is probably the main reason for the higher Olsen-P in the understory removal plots. In addition, mineralization of organic P caused by elevated soil phosphatase activity and the dissolution of calcium phosphate with decreased soil pH may have contributed to increased available P concentration. At our study site, organic P accounted for over 50% of total P in the topsoil, and calcium phosphate was the predominant form of inorganic P (Zhao et al. 2009). Similar to the present study, available N and N mineralization did not change in a temperate deciduous forest on nutrient-rich soils after 14 years of understory removal (Elliott et al. 2015). In contrast, increased net N mineralization rate and available N by elimination of understory vegetation were found in a temperate white spruce plantation, a loblolly pine plantation, and a subtropical *Eucalyptus* plantation (Gurlevik et al. 2004; Matsushima and Chang 2007; Wu et al. 2011). Soil N cycling is controlled by many interacting biotic and abiotic factors, thus it is difficult to explain this variability among studies without an in-depth and synthetic study of soil N cycling processes at each of the sites.

Understory vegetation mediates the impacts of litter addition

As we predicted, the impacts of litter addition on soil microbial biomass and activity, and N and P cycling were influenced by the presence of the understory vegetation. Impacts of tree litter addition on soil microbial biomass and BG activity were stronger when the understory vegetation was intact. These results can be attributed to the accelerated pine litter decomposition when the understory vegetation was present. A previous laboratory mixed-litter decomposition study using litter collected from our study site indicated that litter of understory species stimulated the decomposition of Mongolian pine litter (Lin et al. 2013). The transfer of nutrients from rapidly decaying, higher quality litter likely stimulated the decay of more recalcitrant litter (Gartner and Cardon 2004). Enhanced decomposition of pine litter increased the release of C and nutrients to mineral soils, which in turn, increased the soil microbial biomass and biological transformation of N and P. The effects of litter addition on total C concentration in top soil appeared to be stronger when the understory vegetation was intact; however, these processes are difficult to tease apart without a quantitative study of C inputs and losses in the ecosystem. Overall, increased litter biomass production and aggregate formation induced by the presence of understory vegetation could be one possible explanation for these observed patterns (Busse et al. 1996; Elliott et al. 2015). For example, a recent study found that the presence of understory shrubs increased pine litter decomposition in *Pinus ponderosa* plantation, and induced aggregate formation and accumulation of litter-derived C and N in mineral soils (Qiao et al. 2014). In short, the rapid turnover of understory vegetation itself and accelerated decomposition of pine litter increased plant available nutrients, and consequently diminished the reductions in available N and P caused by litter addition. These results imply that the presence of the understory vegetation can alleviate nutrient limitation caused by litter addition.

Soil microbial community response to litter addition and removal

Similar to the soil microbial biomass response, the soil microbial community structure was only weakly affected by litter removal, but was significantly affected by litter addition. In this nutrient-poor pine plantation, the

soil microbial community could be relatively well buffered to a reduction in C and nutrient inputs. Few studies have examined the effects of forest litter removal on the soil community, and these studies have found that the bacterial community shifted when litter was removed (Prevost-Boure et al. 2011; Wang et al. 2013; Sun et al. 2016). Interestingly, while our multivariate statistical analysis showed insignificant effects of the litter removal on soil microbial community, the ratio between Gram-positive and Gram-negative bacteria was significantly decreased. Because Gram-negative bacteria are generally faster growing and more dependent on labile C (Waldrop and Firestone 2004), litter removal may have increased labile C inputs through root turnover or deposition, and thus slightly increased the proportion of Gram-negative bacteria (Huang and Spohn 2015).

As an indicator of environmental stress, the ratio of $cy17:0/16:1\omega7$ usually increases as substrate availability decreases (Moore-Kucera and Dick 2008). So, the decreased Gram-positive/Gram-negative bacteria and $cy17:0/16:1\omega7$ ratios with litter addition in the present study reflect that litter addition alleviated the C limitation of soil microbial growth (Waldrop and Firestone 2004). Understory removal significantly increased the ratio of $cy17:0/16:1\omega7$, suggesting that C inputs from understory vegetation is an important C source for soil communities in this ecosystem. In contrast to the present study, understory removal in tropical eucalyptus plantations reduced the ratio between fungi and bacteria, but it did not change the bacteria community structure (Wu et al. 2011). These relatively inconsistent results suggest that more work is needed to reveal how context dependent the responses of soil microbial communities are to forest management.

Conclusions

Our manipulative experiment found that soil microbial biomass, community structure and function in this pine forest plantation were resistant to a short-term decrease in aboveground litter inputs. However, these processes and communities were strongly influenced by increases in aboveground litter inputs coupled with the alteration of the understory vegetation. Litter addition in this nutrient-poor pine plantation elevated microbial biomass but reduced available N and P concentrations and thus has the potential to intensify the nutrient limitation of tree growth. Maintaining the understory vegetation

increased microbial biomass, but only had a minor influence on soil organic C accrual. Furthermore, our results point to the positive role of understory vegetation in mediating the impacts of tree litter addition on soil processes, particularly in alleviating the nutrient limitation caused by reduced N and P availability in litter addition treatment. Thus, preserving understory vegetation is important for maintaining the soil microbial activity and functional stability of managed forests on nutrient-poor soils, under the condition of human disturbance and climate change.

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