Microbial regulation of soil carbon properties under nitrogen addition and plant inputs removal

Ran Wu, Xiaoqin Cheng, Wensong Zhou and Hairong Han
Beijing Key Laboratory of Forest Resources and Ecosystem Processes, Beijing Forestry University, Beijing, China

ABSTRACT

Background. Soil microbial communities and their associated enzyme activities play key roles in carbon cycling in terrestrial ecosystems. Soil microbial communities are sensitive to resource availability, but the mechanisms of microbial regulation have not been thoroughly investigated. Here, we tested the mechanistic relationships between microbial responses and multiple interacting resources.

Methods. We examined soil carbon properties, soil microbial community structure and carbon-related functions under nitrogen addition and plant inputs removal (litter removal (NL), root trench and litter removal (NRL)) in a pure Larix principis-rupprechtii plantation in northern China.

Results. We found that nitrogen addition affected the soil microbial community structure, and that microbial biomass increased significantly once 100 kg ha\(^{-1}\) of nitrogen was added. The interactions between nitrogen addition and plant inputs removal significantly affected soil bacteria and their enzymatic activities (oxidases). The NL treatment enhanced soil microbial biomass under nitrogen addition. We also found that the biomass of gram-negative bacteria and saprotrophic fungi directly affected the soil microbial functions related to carbon turnover. The biomass of gram-negative bacteria and peroxidase activity were key factors controlling soil carbon dynamics. The interactions between nitrogen addition and plant inputs removal strengthened the correlation between the hydrolases and soil carbon.

Conclusions. This study showed that nitrogen addition and plant inputs removal could alter soil enzyme activities and further affect soil carbon turnover via microbial regulation. The increase in soil microbial biomass and the microbial regulation of soil carbon both need to be considered when developing effective sustainable forest management practices for northern China. Moreover, further studies are also needed to exactly understand how the complex interaction between the plant and below-ground processes affects the soil microbial community structure.

INTRODUCTION

In the past century, human activity has altered atmospheric nitrogen exchange in forest ecosystems (Deforest et al., 2004; Galloway, 1998), with global rates of nitrogen deposition having more than doubled (Vitousek et al., 1997). Nitrogen is an important
element controlling soil quality, plant diversity and the productivity of forest ecosystems (Sophie, Kerstin & Michael, 2011). Variation in nitrogen may affect soil carbon properties by influencing how soil microbial community structure and its functions drive soil carbon transformation and turnover (Compton et al., 2004). Soil microorganisms act as decomposers and sensitive indicators of soil quality by regulating key process of soil carbon cycling, including lignin and cellulose degradation and soil carbon turnover (Lei et al., 2018; Li et al., 2018; Mele & Crowley, 2008; Sun et al., 2017).

Soil microbial community structure is sensitive to resource availability. Depending on resource availability and supply, soil microbial growth can either be stimulated or inhibited. Nitrogen addition may alter soil microbial community structure and functions in several ways. For example, the growth of soil microorganisms can be stimulated by low-levels of nitrogen addition, because nitrogen-limitation can be ameliorated by improving soil nitrogen availability (Zhu et al., 2016). Similarly, carbon-limitation can be relieved by enhancing aboveground net primary production (NPP) and litter decomposition (Sun et al., 2016; Zhang et al., 2008). De Deyn, Quirk & Bardgett (2011) found that nitrogen addition influenced microbial community structure by directly enhancing soil nitrogen availability, as well as by indirectly affecting soil microbial functions related to carbon turnover. This was accomplished through the stimulation of specific microbial groups that influence the soil carbon process. In contrast, it has been found that nitrogen deposition negatively affects soil microbial growth, composition and function (Zhang, Chen & Ruan, 2018). Overabundance of nitrogen can cause carbon-limitation due to subsequent decreases in litter decomposition rate. This inhibition of litter decomposition is also caused by decreases in phenol oxidase, and by reductions in pH or toxicity by aluminum mobilization (Treseder, 2010). Excessive nitrogen can also elicit production of ligninase from some microorganisms, including white rot fungi (Kundu & Chatterjee, 2006). Therefore, examining of soil microbial responses along a nitrogen addition gradient may help us to understand how nitrogen influences soil carbon processes via the microbial community structure and functions.

The microbial response to shifts in multiple interacting limiting resources is not well understood because few studies have been done (Zhang et al., 2015a; Zhang et al., 2015b). Changes in litter input can help improve soil nutrient availability by alleviating resource stresses (Li et al., 2015a; Li et al., 2015b). Soil carbon processes carried out by soil microbial communities rely on complex interactions with carbon input, which in turn are mediated by nitrogen availability (Bloom, Chapin & Mooney, 1985; Bohlen et al., 2001; Fisk & Fahey, 2001). The response of soil microbes to nitrogen addition is further mediated by carbon input changes due to shifts between carbon-limitation and nitrogen-limitation. Differences in microbial responses may be related to the quality of litter and roots (Liu et al., 2014). Soil microbial functions relating to carbon acquisition under nitrogen addition may also depend on the plant litter and roots (Alster et al., 2013).

A better understanding of how carbon availability specifically affects feedback between plants and microorganisms requires further research on soil microbial community structure and functions relative to different plant inputs removal scenarios. Zhao et al. (2017) conducted a litter removal experiment to study soil microbial community structure and
function in a managed pine forest. However, it still remains unclear whether microbial community shifts are a direct result of nitrogen addition, or of the carbon inputs that are mediated by nitrogen addition (Li et al., 2015a). Previous work on nitrogen addition and plant inputs removal have suggested both positive and negative effects on soil microbial community structure and functions (Bachar et al., 2010; Shi et al., 2016; Li et al., 2015b). Therefore, the interaction between nitrogen and changes in carbon input needs to be considered further when analyzing microbial mechanisms under different nitrogen addition scenarios (Georgiou et al., 2017; Zhang et al., 2015a; Zhang et al., 2015b).

Microbial regulation of soil carbon is still not well understood. Ma et al. (2018) has explained the effect of nitrogen addition on soil total organic carbon and active carbon components. However, in this present study, soil carbon properties were used to further analyze the microbial regulation of soil carbon regulatory paths. Studying the direct and indirect effects of both microbial community structure on various functions and microbes on soil carbon properties, is necessary to better understand the belowground processes affecting carbon dynamics. For example, specific microbial groups have certain functions used to drive soil carbon decomposition and turnover (Wang et al., 2017). Likewise, soil enzyme activities are direct expression of soil microbial functions, relating to soil carbon dynamics (Wang et al., 2015). These interactions can be divided into oxidases and hydrolases activities (Bissett et al., 2011; You et al., 2014). Specifically, complex compounds like lignin are degraded by oxidases, which are produced primarily by fungi. Cellulose is degraded by hydrolases, which are produced primarily by bacteria and relate to soil carbon acquisition (You et al., 2014).

The experimental approach of the Detritus Input and Removal Treatment (DIRT) experiments is an efficient method for investigating the correlation between plant and soil microbial communities through litter removal and root exclusion treatments (Veres et al., 2015). Likewise, litter removal and root exclusion regulate soil microclimate by influencing evaporation and absorption of precipitation (Fekete et al., 2016). Larix principis-ruprechtii is a common plantation tree species in the warm, temperate Taiyue Mountains of Shanxi province. In this study, a litter and root exclusion experiment designed in the method of the DIRT project and the treatments of variable nitrogen addition were established to examine feedback among plant litter, soil nutrient availability and soil microbial communities in short-term treatment in L. principis-ruprechtii plantations. We measured hydrolase activities to assess soil microbial functions relating to cellulose and chitin degrading capacity. We also assessed oxidase activities to assess microbial functions relating to lignin degradation and their influence on soil carbon transformation. We analyzed how shifts in soil microbial community and functions drive variation in soil carbon properties. Furthermore, we investigated the direct and indirect effects of soil microbial communities on soil enzyme activities, and the direct and indirect effects of microbes on soil carbon properties under various nitrogen addition and plant inputs removal treatments. The objectives of this study were: (1) to assess the responses of soil microbial community structure and functions to nitrogen addition and plant inputs removal; and (2) to explore how nitrogen addition drives linkages among microbial community structure, functions and soil carbon properties under different plant inputs removal treatments. We
hypothesized that nitrogen addition would change soil microbial community structure and functions with and without plant inputs removal, and that the soil microbial communities would regulate soil carbon properties, controlled by microbial functions.

**MATERIAL AND METHODS**

**Study sites**

Study sites were located in *Larix principis-rupprechtii* plantations in the Taiyue Mountain (36°35′–36°53′N, 111°91′–112°04′E) of Shanxi province in northern China. A warm temperate and continental monsoon climate is characteristic of the site. Its altitude ranges from 2,100 to 2,400 m, with an average annual temperature around 8.6 °C. The growing season starts in April and lasts until October, and a rainy season is observed from June to August. Average annual precipitation is nearly 600 mm. The predominant soil type throughout the study sites is Haplic luvisol. The dominant tree species are *Larix principis-rupprechtii* and *Betula platyphylla*, which are typical temperate tree species of northern China. *Lonicera japonica*, *Corylus mandshurica*, *Rubus corchorifolius*, *Rosa xanthina*, and *Lespedeza bicolor* are common shrub species encountered in the region.

**Experimental design and treatments**

The experiment began in August 2014 in a 34-year-old *Larix principis-rupprechtii* plantation. Plots were established using a nested, two-factor design. Three replicate plots of two m × two m were established for each treatment. These plots were randomly placed, with a minimum distance of 0.5 m between plots. For the treatment, we used four nitrogen addition levels and three plant inputs removal treatments, which were nested within each nitrogen addition level. The three treatments of plant inputs removal included root trenching and litter removal (NRL), just litter removal (NL) and a control plot (C) where litter and roots were left intact. In the NRL treatment, living roots and aboveground litter were excluded, and trenches were excavated to a depth of 0.5 m. This was done to restrict roots entering the plots from nearby trees. Trenches were embedded with asbestos shingles and then refilled with soil. In the NL treatment, all aboveground inputs were removed. The aboveground litter was removed monthly during the growing season. Natural above- and below-ground litter inputs were left in the C treatment. The nitrogen addition treatments were N0 (0 kg ha⁻¹ a⁻¹), N1 (50 kg ha⁻¹ a⁻¹), N2 (100 kg ha⁻¹ a⁻¹) and N3 (150 kg ha⁻¹ a⁻¹). NH₄NO₃ was used for the nitrogen addition treatments, which were conducted once a month during the growing season from 2014 to 2016.

Soil samples were collected in August 2015 and 2016 at five random points in each plot at a depth of 0–10 cm. A metal corer with an inner diameter of five cm was used to collect samples in the field. The five soil samples from a single plot were mixed to create a composite sample. Soil samples were then stored in sealed bags and immediately taken back to the laboratory for analysis. A two mm sieve was used to remove gravel, roots, and large organic residues from the samples. Samples were then used to measure soil microbial community structure, soil enzyme activities and soil carbon properties. This data was used to assess correlations among the samples.
**Analyses of soil properties**

Soil organic carbon (SOC) was analyzed with an elemental analyzer (FLASH 2000). Soil microbial biomass carbon (MBC) was determined via the fumigation-extraction method, and each sample was fumigated for 24 h at 25 °C with alcohol-free CHCl₃. Dissolved organic carbon (DOC) and MBC were measured using a 0.5 M K₂SO₄ extracting agent and measured with a Multi N/C 3100 TOC analyzer (Chen et al., 2017). Soil microbial community structure was determined using phospholipid fatty acid (PLFA) analysis. PLFAs were calculated based on a 19:0 internal standard content. After addition of an internal standard, the phospholipid fraction was subjected to a mild alkaline methanolation, and the resulting fatty acid methyl esters were separated on a gas chromatograph (Bossio & Scow, 1998). The following soil microbial groups were classified using diagnostic fatty acids as indicators: gram-positive bacteria (GP), gram-negative bacteria (GN), Saprotrophic fungi (Sap), arbuscular mycorrhizal fungi (AMF), and actinomycete (Act) (Table S1). The activities of phenol oxidase (PO) and peroxidase (PER) were determined using DOPA (3, 4-Dihydroxy- L- phenylalamine) as the substrate. Soil suspensions (i.e., one g fresh soil with 1. five mL 50 mmol L⁻¹ sodium acetate buffer) and two mL five mmol L⁻¹ L-DOPA were mixed for the phenol oxidase assay. The same suspension was used for peroxidase analyses with an addition of 0. two mL 0.3% H₂O₂ (Baldrian, 2009). The activities of β-glucosidase (BG), N-acetyl-β-glucosaminidase (NAG) and cellobiohydrolase (CBH) were measured with p-nitrophenol assays (You et al., 2014).

**Statistics**

Analysis of variance (ANOVA) was used to assess variation in soil carbon, soil microbial communities and soil enzyme activities under various nitrogen addition and plant inputs removal treatments. Statistical analyses were performed using SPSS 19.0.

Redundancy analysis (RDA) was used to assess linkages between soil microbial composition, soil enzyme activity, and soil carbon properties. RDA was used with forward selection to filter the relative importance of individual explanatory variables, and to predict the variation in soil microbial community structure, enzyme activity and soil carbon under nitrogen addition both with and without plant inputs removal. These analyses were completed in CANOCO software for Windows 4.5.

Lastly, structural equation modelling (SEM) was used to test the hypothesis that soil microbial communities affect soil carbon properties by influencing soil enzyme activities through the combined effects of nitrogen addition and plant inputs removal. Path models were established based on existing literature, where bacteria and Sap represent the structural attributes of soil microbial communities and oxidases, and BG represents the soil microbial function driving carbon acquisition and oxidation (You et al., 2014). We combined the data from all treatments and estimated the model parameters using a maximum likelihood estimation in the Amos 22.0 software package. The adequacy of model fit was assessed by a χ² test (p > 0.05, CMIN/df < 2), comparative fit index (CFI > 0.90) and by the root square mean error of approximation (RMSEA < 0.05) (You et al., 2014). Numbers on arrows are standardized direct path coefficients. R² values represent the proportion of total variance explained for a specific dependent variable. Dash-line arrows indicate negative effects.
**RESULTS**

Variation in soil microbial biomass under nitrogen addition

In the C treatment, soil microbial biomass first increased, and then decreased with an increase in nitrogen addition level (Fig. 1). In the NL treatment, nitrogen addition enhanced soil microbial biomass. The increase in soil microbial biomass was significantly higher in the N1 level treatment than at other nitrogen levels in 2016. In the NRL treatment, soil microbial biomass increased under nitrogen addition in the first year, but decreased under the same treatment in 2016.

Soil microbial biomass was significantly influenced by sampling year (Table 1). There were also significant interactive effects between plant inputs removal and sampling year.
Table 1  Result (F value) of a three—way ANOVA on the effects of nitrogen addition, plant inputs removal and year on soil microbial biomass.

| Treatments | GP        | GN        | AMF      | Sap       | Act       |
|------------|-----------|-----------|----------|-----------|-----------|
| N          | 7.673***  | 1.863     | 6.644*   | 8.020***  | 1.117     |
| L          | 34.228*** | 6.409***  | 5.641**  | 4.524     | 1.643     |
| Y          | 225.004***| 1144.864***| 511.431***| 15.818*** | 171.889***|
| N * Y      | 1.365     | 0.117     | 0.606    | 0.975     | 3.473*    |
| L * Y      | 10.023*** | 10.483*** | 0.046    | 0.442     | 8.476**   |
| N * L      | 3.027*    | 2.612*    | 1.131    | 2.237     | 3.030*    |
| N * L * Y  | 2.814*    | 3.151*    | 1.339    | 0.690     | 1.705     |

Notes.  
GP, Gram-positive bacteria; GN, Gram-negative bacteria; AMF, arbuscular mycorrhizal fungi; Sap, Saprotrophic fungi; Act, actinomycete; N, nitrogen addition; L, plant inputs removal; Y, year.  
Each of these variables were fitted in following statistical model, e.g., four levels of nitrogen addition * three plant inputs treatments * two years.  
* p < 0.05.  
** p < 0.01.  
*** p < 0.001.

on the biomass of GP, GN and Act. Interactive effects were also found between nitrogen addition and plant inputs removal on the biomass of GP and GN, though the effects differed with year.

Variation in soil enzyme activity under nitrogen addition

In the C treatment in 2015, nitrogen addition enhanced the activities of PO, PER, BG and NAG (Fig. 2). In contrast, the activity of PO had a decreased trend in 2016. In the NL and NRL treatment, PO and PER activities increased under nitrogen addition in 2015, but they decreased in 2016. Moreover, the N1 level treatment enhanced the activity of NAG in the C and NL treatments.

Nitrogen addition, plant inputs removal, and sampling year all significantly affected soil enzyme activities (Table 2). The effect of plant inputs removal or nitrogen addition on the activities of PER, NAG and CBH were also dependent on year. There were interactive effects between nitrogen addition and plant inputs removal on the activities of PER and BG in all years.

Variation in soil carbon properties under nitrogen addition

In the C treatment in 2015, the N1 and N2 level treatments lowered SOC and DOC. However, there was no significant variation in SOC and DOC in the N1 and N2 level treatments in 2016 (Table 3). In the NL treatment in 2015, SOC and MBC first increased and then declined with nitrogen addition. DOC declined gradually with nitrogen addition in 2015. In the NRL treatment in 2015, SOC had a trend of decrease at the N3 levels. At the N2 level treatment, MBC was significantly higher than at the other nitrogen levels in 2015, but the opposite was true in 2016. DOC had a trend of decrease under nitrogen addition in the NRL treatment during both sampling years.
Figure 2  Soil enzyme activities under nitrogen addition in the NRL (A–B), NL (C–D) and C (E–F) treatments. PO, phenol oxidase; PER, peroxidase; BG, β-glucosidase; NAG, N-acetyl-β-glucosidase; CBH, cellobiohydrolase; NRL, root trench and remove litter; NL, litter removal; C, the control plot. N0, nitrogen addition at 0 kg ha$^{-1}$; N1, nitrogen addition at 50 kg ha$^{-1}$; N2, nitrogen addition at 100 kg ha$^{-1}$; N3, nitrogen addition at 150 kg ha$^{-1}$. * indicates that soil enzyme activities differs significantly from the N0 treatment at each nitrogen level ($p < 0.05$).

Full-size DOI: 10.7717/peerj.7343/fig-2

Microbial regulatory pathways on soil enzymes under nitrogen addition

The pathway showed that GN and Sap had a direct and indirect effect on soil enzyme activities under nitrogen addition (Table 4). The biomass of GN had relatively strong total effects on the soil enzyme activities. For the C treatment, the biomass of GN had a direct and positive effect on the activities of PER and BG, while Sap had a direct effect on the activity of PER. In the NL and NRL treatments, the activities of PER and BG were directly affected by the biomass of GN. Compared to the total effects of plant inputs removal,
Table 2  Result (F value) of a three-way ANOVA on the effects of nitrogen addition, plant inputs removal and year on soil enzyme activities.

| Treatments | PO  | PER  | BG   | NAG  | CBH  |
|------------|-----|------|------|------|------|
| N          | 1.066 | 10.504 | 16.875 | 20.183 | 13.420 |
| L          | 33.173 | **111.121*** | 79.646 | 54.570 | 72.586 |
| Y          | 215.231*** | 382.211*** | 165.531*** | 97.786*** | 22.437*** |
| N * Y      | 6.979 | **19.828*** | 6.987 | 6.723 | 6.003 |
| L * Y      | 1.944 | 25.067*** | 2.482 | 13.460*** | 16.278*** |
| N * L      | 2.723 | 14.129*** | 12.178*** | 1.189 | 6.613*** |
| N * L * Y  | 1.699 | 3.936** | 4.254 | 1.592 | 1.922 |

Notes. PO, phenol oxidase; PER, peroxidase; BG, β-glucosidase; NAG, N-acetyl-β-glucosidase; CBH, cellobiohydrolase; N, nitrogen addition; L, plant inputs removal; Y, year. Each of these variables were fitted in following statistical model, e.g., four levels of nitrogen addition * three plant inputs treatments * two years.

* p < 0.05.
** p < 0.01.
*** p < 0.001.

Table 3  The variation in soil carbon properties under nitrogen addition in the NRL, NL and C treatments.

| Soil carbon | Plant inputs removal | Years     | The levels of nitrogen addition |
|-------------|----------------------|-----------|---------------------------------|
|             |                      | N0        | N1                              | N2                              | N3                              |
| SOC (g kg⁻¹) | NRL 2015             | 53.45 ± 7.42a | 55.27 ± 13.37a | 52.7 ± 3.52a | 40.13 ± 6.1a  |
|             | NL 2015              | 42.6 ± 6.42b | 65.6 ± 8.43a | 46.7 ± 2.85b | 40.65 ± 0.35b |
|             | C 2015               | 51.23 ± 7.57a | 44.37 ± 3.72ab | 39.97 ± 1.01b | 37.7 ± 6.01b  |
|             | NRL 2016             | 41.63 ± 3.43a | 37.85 ± 2.51a | 37.7 ± 1.75a | 41.19 ± 2.23a |
|             | NL 2016              | 42.36 ± 1.91a | 45.13 ± 4.4a | 40.71 ± 1.37a | 39.96 ± 3.16a |
|             | C 2016               | 45.04 ± 4.67ab | 50.57 ± 0.94a | 49.14 ± 1.71a | 43.33 ± 3.5b  |
| MBC (mg kg⁻¹)| NRL 2015             | 1490.08 ± 49.36c | 1115.13 ± 64.48d | 2022.12 ± 26.69a | 1675.74 ± 45.3b |
|             | NL 2015              | 1287.53 ± 19.1d | 2166.46 ± 27.79a | 1798.6 ± 46.93b | 1581.09 ± 124.8ac |
|             | C 2016               | 1492.85 ± 33.98b | 1474.8 ± 38.83b | 1423.57 ± 83.89b | 1671.82 ± 48.68a |
|             | NRL 2016             | 902.13 ± 81.86a | 885.61 ± 82.73a | 692.32 ± 306.33a | 887.68 ± 49.31a |
|             | NL 2016              | 1022.38 ± 152.33a | 822.7 ± 157.68a | 985.09 ± 51.54a | 920.88 ± 10.91a |
|             | C 2016               | 1064.01 ± 124.2a | 934.6 ± 52.31a | 1031.53 ± 159.29a | 988.17 ± 28.51a |
| DOC (mg kg⁻¹)| NRL 2015             | 138.25 ± 25.84a | 133.39 ± 12.51a | 135.07 ± 0.07a | 126.25 ± 2.42a |
|             | NL 2016              | 140.02 ± 2.94a | 132.52 ± 16.28ab | 125.19 ± 4.76ab | 115.01 ± 6.6b  |
|             | C 2016               | 193.9 ± 3.74a | 165.3 ± 6.93b | 159.63 ± 23.77b | 181.5 ± 2.42ab |
|             | NRL 2016             | 173.5 ± 8.24a | 161.59 ± 10.26a | 154.72 ± 10.75b | 158.67 ± 10.02ab |
|             | NL 2016              | 165.02 ± 7.88a | 159.19 ± 7.31a | 159.93 ± 10.39a | 162.78 ± 15.69a |
|             | C 2016               | 179.92 ± 6.89a | 179.51 ± 16.25a | 191.83 ± 9.14a | 173.69 ± 13.21a |

Notes. Different letters indicate significant differences under different nitrogen addition (p < 0.05). Values are mean ± standard errors (n = 3).
SOC, soil organic carbon; MBC, soil microbial biomass carbon; DOC, dissolved organic carbon; NRL, root trench and remove litter; NL, litter removal; C, the control plot; N0, zero rate of nitrogen addition; N1, nitrogen addition at 50 kg ha⁻¹ a⁻¹; N2, nitrogen addition at 100 kg ha⁻¹ a⁻¹; N3, nitrogen addition at 150 kg ha⁻¹ a⁻¹.

Wu et al. (2019), PeerJ, DOI 10.7717/peerj.7343
Table 4. Path coefficients of the effects of soil microbes on soil enzyme activities under nitrogen addition in the NRL, NL and C treatments.

| Factors      | Effect   | Soil enzyme |
|--------------|----------|-------------|
|              |          | PER         | BG     |
| In the NRL treatment | Direct   | 0.716       | 0.733  |
| GN           | Indirect | 0           | 0      |
|              | Total    | 0.716       | 0.733  |
| Sap          | Indirect | 0.204       | 0.210  |
|              | Total    | 0.204       | 0.210  |
| In the NL treatment | Direct   | 0.844       | 0.749  |
| GN           | Indirect | 0           | 0      |
|              | Total    | 0.844       | 0.749  |
| Sap          | Indirect | 0.384       | 0.341  |
|              | Total    | 0.384       | 0.341  |
| In the C treatment | Direct   | 0.635       | 0.526  |
| GN           | Indirect | 0.091       | 0      |
|              | Total    | 0.726       | 0.526  |
| Sap          | Indirect | 0.177       | 0.146  |
|              | Total    | 0.506       | 0.146  |

Notes. PER, peroxidase; BG, β-glucosidase; GN, Gram-negative bacteria; Sap, Saprotrophic fungi; NRL, root trench and remove litter; NL, litter removal; C, the control plot.

we found that the total effect of GN augmented the activity of PER in the NL and NRL treatments, while the total effect of Sap reduced the activity of PER. The total effects of GN and Sap increased the activity of BG in the NL and NRL treatments.

**Soil enzyme regulatory pathways on soil carbon properties under nitrogen addition**

The pathway showed that soil enzymes had both direct and indirect effects on soil carbon under nitrogen addition (Table 5). The activity of PER had relatively strong total effects on soil carbon. In the C treatment, SOC was directly and negatively affected by the activity of PER, while MBC was directly and positively influenced by PER activity. There was no direct effect of BG activity on soil carbon properties. In the NL treatment, the activities of PER and BG directly affected MBC, but only PER directly affected SOC. In the NRL treatment, MBC was directly affected by BG activity. However, PER and BG did not directly influence SOC. Compared to the total effects under nitrogen addition and plant inputs removal, we found that the total effect of PER reduced MBC and SOC in the NRL treatments. The total effect of BG augmented MBC and SOC in the NL treatments.
Table 5. Path coefficients of the effects of soil enzyme activities on soil carbon under nitrogen addition in the NRL, NL and C treatments.

| Factors   | Effect   | Soil carbon |
|-----------|----------|-------------|
|           |          | MBC         | SOC         |
| In the NRL treatment |          |             |             |
| PER       | Direct   | 0           | 0           |
|           | Indirect | 0.437       | 0           |
|           | Total    | 0.437       | 0           |
|           | Direct   | 0.691       | 0           |
| BG        | Indirect | 0           | 0           |
|           | Total    | 0.691       | 0           |
| In the NL treatment |          |             |             |
| PER       | Direct   | 0.556       | 0.479       |
|           | Indirect | 0.305       | 0           |
|           | Total    | 0.861       | 0.479       |
|           | Direct   | 0.399       | 0           |
| BG        | Indirect | 0.425       | 0.366       |
|           | Total    | 0.824       | 0.366       |
| In the C treatment |          |             |             |
| PER       | Direct   | 0.825       | −0.495      |
|           | Indirect | 0           | 0           |
|           | Total    | 0.825       | −0.495      |
|           | Direct   | 0           | 0           |
| BG        | Indirect | 0.305       | −0.183      |
|           | Total    | 0.305       | −0.183      |

Notes.
PER, peroxidase; BG, β-glucosidase; MBC, soil microbial biomass carbon; SOC, soil organic carbon; NRL, root trench and remove litter; NL, litter removal; C, the control plot.

Regulation of soil carbon by the soil microbial communities and enzymes under nitrogen addition

Among soil microbial community and soil enzyme variables, RDA ordination indicated that the biomass of GN and the activity of PER significantly affected soil carbon properties under nitrogen addition (Table 6). The path of the variables under the interaction between nitrogen addition and plant inputs removal passed the statistical test for adequacy ($\chi^2 = 4.468, p = 0.614$, $\text{CNMI/df} = 0.745$, $\text{CFI} = 1.000$, $\text{GFI} = 0.979$, $\text{RMSEA <0.001}$) and the non-significant pathways were deleted (Fig. 3). The model explained 56% and 9% of the variance in the activities of PER and BG, respectively. Likewise, 66% and 27% of the variance in MBC and SOC were explained by this model. The path analysis indicated that GN and Sap regulated soil oxidases, hydrolases, and soil carbon properties under nitrogen addition and plant inputs removal. SOC was negatively and directly affected by PER activity, which was positively influenced by GN and Sap. GN and Sap directly and positively influenced soil carbon.
Table 6  Marginal and conditional effects of soil microbial composition and soil enzyme activities on soil carbon obtained from forward selection in Redundancy analysis (RDA) under nitrogen addition.

| Variables | Lambda-A$^a$ | Lambda-B$^b$ | p$^c$ | F-ratio$^d$ |
|-----------|--------------|--------------|-------|-------------|
| GN        | 0.515        | 0.515        | 0.002 | 72.223      |
| PER       | 0.234        | 0.028        | 0.020 | 4.082       |

Notes.

$^a$Describe marginal effects, which show the variance explained when the variable is used as the only factor.

$^b$Describe conditional effects, which show the additional variance each variable explains when it is included in the model.

$^c$The level of significance corresponding to Lambda-B when performing Monte Carlo test at the 0.05 significance level.

$^d$The Monte Carlo test statistics corresponding to Lambda-B at the 0.05 significance level.

PER, peroxidase; GN, Gram-negative bacteria.

Figure 3  The structural equation model depicting the regulation of soil carbon by enzyme activities and the soil microbial community under the combined effects of nitrogen addition and plant inputs removal. Numbers on arrows are standardized direct path coefficients. $R^2$ value represents the proportion of total variance explained for the specific dependent variable. Dash-line arrows indicate negative effects. PER, peroxidase; BG, β-glucosidase; GN, Gram-negative bacteria; Sap, Saprotrophic fungi. SOC, soil organic carbon; MBC, soil microbial biomass carbon.

Full-size DOI: 10.7717/peerj.7343/fig-3
**DISCUSSION**

**The responses of soil microbial communities and functions to nitrogen addition and plant inputs removal**

An important finding in our study include that soil microbial biomass increased under nitrogen addition and that microbial biomass was significantly higher in the N2 level treatment than the other levels. This result is consistent with previous observations showing increases in soil bacterial biomass and diversity after nitrogen addition (Nguyen et al., 2018), as well as increases in bacterial biomass in a lower-elevation forest and in fungal biomass in an upper-elevation forest after nitrogen addition (Cusack et al., 2011). This result shows that the N1 treatment enhanced the activity of NAG, which degrades chitin. This suggests that microbial turnover occurs more rapidly at the N1 level treatment, possibly because microbial cell walls consist of fungal chitin and bacterial peptidoglycan. Our findings also suggest that soil oxidase activities increased after the first year of nitrogen addition, and then decreased after the second year. Similar results were also observed in an Alaskan boreal forest, where carbon-degrading enzyme activities increased after short-term nitrogen addition (Allison, Czimczik & Treseder, 2008). However, declines in soil bacterial biomass have also been reported after three years of nitrogen addition in a secondary tropical forest of China and one year of nitrogen addition in North America (Li et al., 2015a; Li et al., 2015b; Ramirez, Craine & Fierer, 2012). It has also been found that long-term nitrogen addition reduces soil fungi via nitrogen saturation in temperate ecosystems (Demoling, Nilsson & Bååth, 2008). Our results show that nitrogen addition reduced SOC and DOC, and increased soil microbial biomass in the first year of treatment. It has been suggested that soil microbial biomass correlates negatively with SOC under nitrogen addition. This result was supported by the literature showing a negative relationship between soil bacterial biomass and SOC over three years of nitrogen addition in a temperate needle-broadleaved forest (Cheng, Fang & Yu, 2017). Similar results were reported by other studies that found declines in soil organic carbon under nitrogen addition in grasslands (Moinet et al., 2016).

It has been theorized that changes in soil organic matter may result from variation in soil nutrient availability following plant inputs removal Lietal2015. Our results show that nitrogen addition lowered soil microbial biomass and soil oxidases activities in the NRL treatment in 2016. The response of soil microbial communities to nitrogen addition did not align with the response of communities to the plant inputs removal. It has been indicated that plant inputs removal could potentially alter nutrient limitation under nitrogen addition (Sayer et al., 2012). Likewise, after the first year of root trenching and litter removal, soil microbial biomass and oxidase activities increased under nitrogen addition because they had been previously nitrogen-limited. However, these metrics declined in the second year due to a shift from nitrogen-limitation to carbon-limitation (Averill & Waring, 2018; Traoré et al., 2016). Microbial biomass was much higher at the N1 level than at other nitrogen addition levels in the second year of litter removal. This may have been because litter removal caused nitrogen-limitation, and nitrogen addition positively affected soil microbes at the N1 level. We also found that SOC and DOC decreased with nitrogen addition in the NRL treatments due to the shift in nutrient limitation. Our
result is inconsistent with previous work showing that soil organic matter decomposition does not differ under nitrogen addition between root exclusion and natural states. This is because the presence of roots mediates the response of soil organic carbon decomposition to nitrogen addition (Moinet et al., 2016).

Plant inputs removal altered soil bacterial and fungal communities, which aligned with previous literature (Brant, Myrold & Sulzman, 2006; Kramer et al., 2010). These studies theorized that root-derived carbon was the predominant source of carbon for microbes. However, this theory was not supported by a study in a mixed-wood forest of northern China, where microbes and soil enzyme activities were not significantly affected by the interaction between nitrogen addition and plant inputs removal (Sun et al., 2016). As resources for fungal decomposers, nitrogen, litter and roots were the main factors influencing AMF and Sap. Meanwhile, bacteria mediated soil carbon processes via the combined effects of nitrogen addition and plant inputs removal. This was inconsistent with results from M. laosensis soils, where root exclusion did not change the biomass of AMF (Wan et al., 2015). Our results show that the biomass of GP was significantly influenced by nitrogen addition and plant inputs removal, while the biomass of GN was significantly affected by plant inputs removal and not by nitrogen addition. It is likely that GN first utilizes recent plant material with a lower C/N ratio as its carbon source, while GP tends to utilize more recalcitrant carbon first (Kramer & Gleixner, 2006).

Nitrogen addition drives linkage between soil microbial community structure and soil carbon in different plant inputs removal

It has been theorized that the availability of nitrogen can modulate the microbial structure-function relationship (Nguyen et al., 2018). Here, we found that nitrogen addition lead to the direct and positive effect of GN on oxidases and hydrolases. Meanwhile, fungi (Sap) did not directly affect hydrolases. This result was also observed in the literature, where soil microbial community structure was not correlated with hydrolases under land-use change conditions (Tischer, Blagodatskaya & Hamer, 2015). GN had a strong total effect on soil oxidases and hydrolases under nitrogen addition, while Sap had little effect. This result was inconsistent with previous studies that suggest that soil enzyme activities are not always related to soil microbial community structure, such as those in the area of P-limitation (Tischer, Blagodatskaya & Hamer, 2014). After plant inputs removal lowered the carbon input, the direct effects of bacteria and fungi varied under nitrogen addition. However, the GN in each plant inputs removal directly affected the oxidase activities. It has been theorized that substrate complexity leads to the connection between soil microbial community and function (Baldrian, 2009). We also found that the total effect of GN increased PER activity and that the effect of Sap decreased PER activity in both the NL and NRL treatments. It has been suggested that decreases in carbon input weaken the effects of oxidases on complex carbon sources, while the carbon decreases enhance the effects of hydrolases on easily-decomposed carbon sources. This idea has been supported by previous studies showing that litter input increases lignin oxidase (Sun et al., 2016). Meanwhile, GN grew quickly to compete for soil organic compounds after altering carbon and nitrogen resources. This was consistent with previous studies (Bell et al., 2009; Waldrop, Balser &
Firestone, 2000) that found that GN was correlated with hydrolases that degrade simple organic compounds.

Presently, understanding the linkages between soil enzyme activities and soil carbon properties is necessary for predicting shifts in soil microbial community and functions (Weand et al., 2010). Previous work has reported that soil enzyme activities are significantly correlated to SOC and MBC (Wang et al., 2013), which was consistent with our result that PER and BG activities directly and indirectly affected MBC and SOC under nitrogen addition with and without plant inputs removal. After the plant inputs removal, the direct effect on MBC and SOC changed. This was likely due to the functional shift from degrading complex to simple carbon resources. These results show that soil oxidases greatly influenced soil carbon properties under nitrogen addition. However, plant inputs removal enhanced the total effect of BG on soil carbon properties, which suggests that carbon acquisition was enhanced. Our finding is consistent with previous studies theorizing that nitrogen addition enhances linkages between oxidases and the loss of aliphatic carbon, which are related to decreases in soil carbon storage (Cusack et al., 2011). Meanwhile, the interaction between nitrogen addition and plant inputs removal converted poorer-quality soil (i.e., high lignin and low N) to higher-quality soil (i.e., low lignin and high N), which increased microbial substrate utilization and strengthened the correlation between the hydrolases and soil carbon properties (Ostertag et al., 2008; Gallo et al., 2005).

The regulation of soil carbon properties under treatments of nitrogen addition with plant inputs removal

Research on the response of soil microbial community structure to ecosystem function has been growing rapidly (Lewis et al., 2010; Malchair et al., 2010). An increasing number of studies have been published in recent years on the linkages between soil microbial community structure, functions and soil carbon dynamics (Cusack et al., 2011; Ren et al., 2018; Sun et al., 2017; Veres et al., 2015). Variation in soil microbial communities is likely to lead to microbial function shifts. Specific soil microbial composition is involved in regulating specific soil enzyme activities, which are used to assess the function of soil carbon transformation and turnover (Waldrop & Firestone, 2004). Our results suggest that shifts in soil microbial community structure mediated microbial functions, and that their interactions regulated soil carbon turnover under treatments of nitrogen addition with plant inputs removal. Our results are an important addition to the literature on this topic (You et al., 2014; Brockett, Prescott & Grayston, 2012).

In this paper, the biomass of GN and the activity of PER were found to significantly affect soil carbon properties under nitrogen addition. The activity of PER increased with the biomass of GN at higher levels of nitrogen addition, which enhanced the response of soil carbon to linkages between oxidases and bacteria (Cusack et al., 2011). The changes in carbon inputs, in the form of litter and root exudates, altered soil microbial community utilization and influenced functional shifts in soil enzymes (Baumann et al., 2013; Tardy et al., 2015).

Our results also suggest that the interactions between nitrogen addition and plant inputs removal significantly affected GN. Furthermore, the biomass of GN and Sap directly
regulated soil enzyme activities and soil carbon properties, because Sap was correlated with below- and above-ground carbon decomposition under plant inputs removal (Boldiburisch et al., 2018; Bennett et al., 2017). GN and Sap regulated SOC by affecting on the activity of PER, which was involved in lignin degradation. However, some previous have reported that oxidases are produced directly by fungi (Jiang, Cao & Zhang, 2014; Zhang et al., 2014).

**CONCLUSION**

Our study revealed that moderate nitrogen addition increased soil microbial biomass. The interaction between nitrogen addition and plant inputs removal significantly affected soil microbial community structure and function. The biomass of gram-negative bacteria and saprotrophic fungi directly affected how soil microbial function relates to carbon turnover. Altogether, microbial community structure and function, the biomass of gram-negative bacteria and peroxidase activity were the key factors regulating soil carbon dynamics. This study suggests that nitrogen addition and plant inputs removal could alter soil enzyme activities and further affect soil carbon turnover via microbial regulations. These findings have important implications for forest management. The increase in soil microbial biomass and the microbial regulation on soil carbon both need to be considered when developing sustainable forest management practices for northern China. Moreover, further studies are also needed to more precisely understand the complex interaction between the plant and the below-ground processes and how it affects soil microbial community structure.

**ADDITIONAL INFORMATION AND DECLARATIONS**

**Funding**

This study was financed by the National Key Research and Development Program of China (2016YFD0600205). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**Grant Disclosures**

The following grant information was disclosed by the authors:

National Key Research and Development Program of China: 2016YFD0600205.

**Competing Interests**

The authors declare there are no competing interests.

**Author Contributions**

- Ran Wu conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, approved the final draft.
- Xiaqin Cheng and Hairong Han conceived and designed the experiments, contributed reagents/materials/analysis tools, authored or reviewed drafts of the paper, approved the final draft.
- Wensong Zhou performed the experiments, approved the final draft.
Data Availability
The following information was supplied regarding data availability:

The raw measurements are available in the Supplemental Files.

Supplemental Information
Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.7343#supplemental-information.

REFERENCES

Allison SD, Czimczik CI, Treseder K. 2008. Microbial activity and soil respiration under nitrogen addition in Alaskan boreal forest. Global Change Biology 14:1156–1168 DOI 10.1111/j.1365-2486.2008.01549.x.

Alster CJ, German DP, Lu Y, Allison SD. 2013. Microbial enzymatic responses to drought and to nitrogen addition in a southern California grassland. Soil Biology and Biochemistry 64:68–79 DOI 10.1016/j.soilbio.2013.03.034.

Averill C, Waring B. 2018. Nitrogen limitation of decomposition and decay: how can it occur? Global Change Biology 24:1417–1427 DOI 10.1111/gcb.13980.

Bachar A, Al-Ashhab A, Soares MI, Sklarz MY, Angel R, Ungar ED, Gillor O. 2010. Soil microbial abundance and diversity along a low precipitation gradient. Microbial Ecology 60:453–461 DOI 10.1007/s00248-010-9727-1.

Baldrian P. 2009. Microbial enzyme-catalyzed processes in soils and their analysis. Plant Soil and Environ—UZEI 55:370–378 DOI 10.17221/134/2009-PSE.

Baumann K, Dignac MF, Rumpel C, Bardoux G, Sarr A, Steffens M, Maron PA. 2013. Soil microbial diversity affects soil organic matter decomposition in a silty grassland soil. Biogeochemistry 114:201–212 DOI 10.1007/s10533-012-9800-6.

Bell CW, Acosta-Martinez V, McIntyre NE, Cox S, Tissue DT, Zak JC. 2009. Linking microbial community structure and function to seasonal differences in soil moisture and temperature in a chihuahuan desert Grassland. Microbial Ecology 58:827–842 DOI 10.1007/s00248-009-9529-5.

Bennett JA, Maherali H, Reinhart KO, Lekberg Y, Hart MM, Kliromonomos J. 2017. Plant-soil feedbacks and mycorrhizal type influence temperate forest population dynamics. Science 355:181–184 DOI 10.1126/science.aai8212.

Bissett A, Richardson AE, Baker G, Thrall PH. 2011. Long-term land use effects on soil microbial community structure and function. Applied Soil Ecology 51:66–78 DOI 10.1016/j.apsoll.2011.08.010.

Bloom AJ, Chapin FS, Mooney HA. 1985. Resource limitation in plants—an economic analogy. Annual Review of Ecology and Systematics 16:363–392 DOI 10.1146/annurev.es.16.110185.002051.

Bohlen PJ, Groffman PM, Driscoll CT, Fahey TJ, Siccama TG. 2001. Plant-soil-microbial interactions in a Northern Hardwood Forest. Ecology 82:965–978 DOI 10.2307/2679896.
Boldtburisch K, Naeth MA, Schneider U, Schneider B, Hüttl RF. 2018. Plant growth and arbuscular mycorrhizal development in oil sands processing by-products. *Science of the Total Environment* 621:30–39 DOI 10.1016/j.scitotenv.2017.11.188.

Bossio DA, Scow KM. 1998. Impacts of carbon and flooding on soil microbial communities: phospholipid fatty acid profiles and substrate utilization patterns. *Microbial Ecology* 35:265–278 DOI 10.1007/s002489900082.

Brant JB, Myrold DD, Sulzmann EW. 2006. Root controls on soil microbial community structure in forest soils. *Oecologia* 148:650–659 DOI 10.1007/s00442-006-0402-7.

Brockett B, Prescott CE, Grayston SJ. 2012. Soil moisture is the major factor influencing microbial community structure and enzyme activities across seven biogeoclimatic zones in western Canada. *Soil Biology and Biochemistry* 44:9–20 DOI 10.1016/j.soilbio.2011.09.003.

Chen ZM, Wang HY, Liu XW, Zhao XL, Lu DJ, Zhou JM, Li CZ. 2017. Changes in soil microbial community and organic carbon fractions under short-term straw return in a rice-wheat cropping system. *Soil & Tillage Research* 165:121–127 DOI 10.1016/j.still.2016.07.018.

Cheng S, Fang H, Yu G. 2017. Threshold responses of soil organic carbon concentration and composition to multi-level nitrogen addition in a temperate needle-broadleaved forest. *Biogeochemistry* 137:1–15 DOI 10.1007/s10533-017-0412-z.

Compton JE, Watrud LS, Porteous LA, Degrood S. 2004. Response of soil microbial biomass and community composition to chronic nitrogen additions at Harvard forest. *Forest Ecology and Management* 196:143–158 DOI 10.1016/s0378-1127(04)00197-5.

Cusack DF, Silver WL, Torn MS, Burton SD, Firestone MK. 2011. Changes in microbial community characteristics and soil organic matter with nitrogen additions in two tropical forests. *Ecology* 92:621–632 DOI 10.1890/10-0459.1.

De Deyn GB, Quirk H, Bardgett RD. 2011. Plant species richness, identity and productivity differentially influence key groups of microbes in grassland soils of contrasting fertility. *Biology Letters* 7:75–78 DOI 10.1098/rsbl.2010.0575.

Deforest JL, Zak DR, Pregitzer KS, Burton AJ. 2004. Atmospheric nitrate deposition, microbial community composition, and enzyme activity in Northern Hardwood Forests. *Soil Science Society of America Journal* 68:132–138 DOI 10.2136/sssaj2004.1320.

Demoling F, Nilsson LO, Bååth E. 2008. Bacterial and fungal response to nitrogen fertilization in three coniferous forest soils. *Soil Biology and Biochemistry* 40:370–379 DOI 10.1016/j.soilbio.2007.08.019.

Fekete I, Varga C, Biró B, Tóth JA, Várbiró G, Lajtha K, Szabó G, Kotorczo Z. 2016. The effects of litter production and litter depth on soil microclimate in a central european deciduous forest. *Plant Soil* 398:291–300 DOI 10.1007/s11104-015-2664-5.

Fisk MC, Fahey TJ. 2001. Microbial biomass and nitrogen cycling responses to fertilization and litter removal in young northern hardwood forests. *Biogeochemistry* 53:201–223 DOI 10.18297/etd/1759.
Gallo ME, Lauber CL, Cabaniss SE, Waldrop MP, Sinsabaugh RL, Zak DR. 2005. Soil organic matter and litter chemistry response to experimental N deposition in northern temperate deciduous forest ecosystems. *Global Change Biology* 11:1514–1521 DOI 10.1111/j.1365-2486.2005.01001.x.

Galloway JN. 1998. The global nitrogen cycle: changes and consequences. *Environmental Pollution* 102:15–24 DOI 10.1016/b978-0-08-043201-4.50008-3.

Georgiou K, Abramoff RZ, Harte J, Riley WJ, Torn MS. 2017. Microbial community-level regulation explains soil carbon responses to long-term litter manipulations. *Nature Communications* 9:1223 DOI 10.1038/s41467-017-01116-z.

Jiang X, Cao L, Zhang R. 2014. Effects of addition of nitrogen on soil fungal and bacterial biomass and carbon utilisation efficiency in a city lawn soil. *Soil Research* 52:97–105 DOI 10.1071/sr13210.

Kramer C, Gleixner G. 2006. Variable use of plant- and soil-derived carbon by microorganisms in agricultural soils. *Soil Biology and Biochemistry* 38:3267–3278 DOI 10.1016/j.soilbio.2006.04.006.

Kramer C, Trumbore S, Fröberg M, Dozal LMC, Zhang DC, Xu XM, Santos GM, Hanson PJ. 2010. Recent (4 year old) leaf litter is not a major source of microbial carbon in a temperate forest mineral soil. *Soil Biology and Biochemistry* 42:1028–1037 DOI 10.1016/j.soilbio.2010.02.021.

Kundu A, Chatterjee NC. 2006. Cellulase and ligninase production by white rot fungi associated with bamboo degradation. *Journal of Mycopathological Research* 44:289–292 DOI 10.14711/thesis-b589620.

Lei L, Xiao W, Zeng L, Zhu J, Huang Z, Cheng R, Gao S, Li M. 2018. Thinning but not understory removal increased heterotrophic respiration and total soil respiration in *Pinus massoniana* stands. *Science of the Total Environment* 621:1360–1369 DOI 10.1016/j.scitotenv.2017.10.092.

Lewis DE, White JR, Wafula D, Athar R, Dickerson T, Williams HN, Chauhan A. 2010. Soil functional diversity analysis of a bauxite-mined restoration chronosequence. *Microbial Ecology* 59:710–723 DOI 10.1007/s00248-009-9621-x.

Li J, Li Z, Wang F, Zou B, Chen Y, Zhao J, Mo Q, Li Y, Li X, Xia H. 2015a. Effects of nitrogen and phosphorus addition on soil microbial community in a secondary tropical forest of China. *Biology and Fertility of Soils* 51:207–215 DOI 10.1007/s00374-014-0964-1.

Li L, Zhu-Barker X, Ye R, Doane TA, Horwath WR. 2018. Soil microbial biomass size and soil carbon influence the priming effect from carbon inputs depending on nitrogen availability. *Soil Biology and Biochemistry* 119:41–49 DOI 10.1016/j.soilbio.2018.01.003.

Li X, Liu J, Fan J, Ma Y, Ding S, Zhong Z, Wang D, Rennenberg H. 2015b. Combined effects of nitrogen addition and litter manipulation on nutrient resorption of *Leymus chinensis* in a semi-arid grassland of northern China. *Plant Biology* 17:9–15 DOI 10.1111/plb.12172.
Liu D, An SS, Cheng Y, Keiblimger K, Huang YM. 2014. Variability in soil microbial biomass and diversity among different aggregate-size fractions of different land use types. *Soilence* 179:242–249 DOI 10.1097/ss.0000000000000064.

Ma JY, Kang FF, Cheng XQ, Han HR. 2018. Response of soil organic carbon and nitrogen to nitrogen deposition in a *Larix principis-rupprechtii* plantation. *Scientific Reports* 8:1–10 DOI 10.1038/s41598-018-26966-5.

Malchair S, De Boeck HJ, Lemmens CMHM, Ceulemans R, Merckx R, Nijs I, Carnol M. 2010. Diversity—function relationship of ammonia-oxidizing bacteria in soils among functional groups of grassland species under climate warming. *Applied Soil Ecology* 44:15–23 DOI 10.1016/j.apsoil.2009.08.006.

Mele PM, Crowley DE. 2008. Application of self-organizing maps for assessing soil biological quality. *Agriculture, Ecosystems & Environment* 126:139–152 DOI 10.1016/j.agee.2007.12.008.

Moinet GYK, Cieraad E, Rogers GND, Hunt JE, Millard P, Turnbull MH, Whitehead D. 2016. Addition of nitrogen fertiliser increases net ecosystem carbon dioxide uptake and the loss of soil organic carbon in grassland growing in mesocosms. *Geoderma* 266:75–83 DOI 10.1016/j.geoderma.2015.12.004.

Nguyen LTT, Osanai Y, Lai K, Anderson IC, Bange MP, Tissue DT, Singh BK. 2018. Responses of the soil microbial community to nitrogen fertilizer regimes and historical exposure to extreme weather events: flooding or prolonged-drought. *Soil Biology and Biochemistry* 118:227–236 DOI 10.1016/j.soilbio.2017.12.016.

Ostertag R, Marin-Spiotta E, Silver WL, Schulten J. 2008. Litterfall and decomposition in relation to soil carbon pools along a secondary forest chronosequence in Puerto Rico. *Ecosystems* 11:701–714 DOI 10.1007/s10021-008-9152-1.

Ramirez KS, Craine JM, Fierer N. 2012. Consistent effects of nitrogen amendments on soil microbial communities and processes across biomes. *Global Change Biology* 18:1918–1927 DOI 10.1007/s10021-008-9152-1.

Ren C, Wang T, Xu Y, Deng J, Zhao F, Yang G, Han X, Feng Y, Ren G. 2018. Differential soil microbial community responses to the linkage of soil organic carbon fractions with respiration across land-use changes. *Forest Ecology and Management* 409:170–178 DOI 10.1016/j.foreco.2017.11.011.

Sayer EJ, Wright SJ, Tanner EVJ, Yavitt JB, Harms KE, Powers JS, Kaspari M, Garcia MN, Turner BL. 2012. Variable responses of Lowland tropical forest nutrient status to fertilization and litter manipulation. *Ecosystems* 15:387–400 DOI 10.2307/41507786.

Shi L, Zhang H, Liu T, Zhang W, Shao Y, Ha D, Li Y, Zhang C, Cai X, Rao X, Lin Y, Zhou L, Zhao P, Ye Q, Zou X, Fu S. 2016. Consistent effects of canopy vs. understory nitrogen addition on the soil exchangeable cations and microbial community in two contrasting forests. *Science of the Total Environment* 553:349–357 DOI 10.1016/j.scitotenv.2016.02.100.

Sophie ZB, Kerstin M, Michael P. 2011. Soil microbial community structure in European forests in relation to forest type and atmospheric nitrogen deposition. *Plant Soil* 343:37–50 DOI 10.1007/s11104-010-0528-6.
Sun S, Zhao H, Xing F, Bai Z, Gao Y, Dong Y, Zhou J, Wu Y, Yang Y. 2017. Response of soil microbial community structure to increased precipitation and nitrogen addition in a semiarid meadow steppe. *European Journal of Soil Science* 68:524–536 DOI 10.1111/ejss.12441.

Sun XL, Zhao J, You YM, Sun OJ. 2016. Soil microbial responses to forest floor litter manipulation and nitrogen addition in a mixed-wood forest of northern China. *Scientific Reports* 6:19536 DOI 10.1038/srep19536.

Tardy V, Spor E, Mathieu O, Eque JLE, Terrat SE, Plassart P, Regnier T, Bardgett RD, Putten WHVD, Roggero P. 2015. Shifts in microbial diversity through land use intensity as drivers of carbon mineralization in soil. *Soil Biology and Biochemistry* 89:204–213 DOI 10.1016/j.soilbio.2015.08.010.

Tischer A, Blagodatskaya E, Hamer U. 2014. Extracellular enzyme activities in a tropical mountain rainforest region of southern Ecuador affected by low soil P status and land-use change. *Applied Soil Ecology* 74:1–11 DOI 10.1016/j.apsoil.2013.09.007.

Tischer A, Blagodatskaya E, Hamer U. 2015. Microbial community structure and resource availability drive the catalytic efficiency of soil enzymes under land-use change conditions. *Soil Biology and Biochemistry* 89:226–237 DOI 10.1016/j.soilbio.2015.07.011.

Traoré OYA, Kiba DI, Arnold MC, Fliessbach A, Oberholzer HR, Nacro HB, Lompo F, Oberson A, Frossard E, Bünemann EK. 2016. Fertilization practices alter microbial nutrient limitations after alleviation of carbon limitation in a Ferric Acrisol. *Biology and Fertility of Soils* 52:177–189 DOI 10.1007/s00374-015-1061-9.

Treseder KK. 2010. Nitrogen additions and microbial biomass: a meta-analysis of ecosystem studies. *Ecology Letters* 11:1111–1120 DOI 10.1111/j.1461-0248.2008.01230.x.

Veres Z, Kotorczo Z, Fekete I, Tóth JA, Lajtha K, Townsend K, Tóthmérész B. 2015. Soil extracellular enzyme activities are sensitive indicators of detrital inputs and carbon availability. *Applied Soil Ecology* 92:18–23 DOI 10.1016/j.apsoil.2015.03.006.

Vitousek PM, Aber JD, Howarth RW, Likens GE, Matson PA, Schindler DW, Schlesinger WH, Tilman DG. 1997. Human alteration of the global nitrogen cycle: sources and consequences. *Ecological Applications* 7:737–750 DOI 10.1890/1051-0761(1997)007[0737:haotgn]2.0.co;2.

Waldrop MP, Balser TC, Firestone MK. 2000. Linking microbial community composition to function in a tropical soil. *Soil Biology and Biochemistry* 32:1837–1846 DOI 10.1016/s0038-0717(00)00157-7.

Waldrop MP, Firestone MK. 2004. Altered utilization patterns of young and old soil C by microorganisms caused by temperature shifts and N additions. *Biogeochemistry* 67:235–248 DOI 10.1023/b:biog.0000015321.51462.41.

Wan X, Huang Z, He Z, Yu Z, Wang M, Davis MR, Yang Y. 2015. Soil C:N ratio is the major determinant of soil microbial community structure in subtropical coniferous and broadleaf forest plantations. *Plant Soil* 387:103–116 DOI 10.1007/s11104-014-2277-4.
Wang Q, Xiao F, Zhang F, Wang S. 2013. Labile soil organic carbon and microbial activity in three subtropical plantations. *Forestry* **86**:569–574 DOI 10.1093/forestry/cpt024.

Wang R, Dorodnikov M, Yang S, Zhang Y, Filley TR, Turco RF, Zhang Y, Xu Z, Li H, Jiang Y. 2015. Responses of enzymatic activities within soil aggregates to 9-year nitrogen and water addition in a semi-arid grassland. *Soil Biology and Biochemistry* **81**:159–167 DOI 10.1016/j.soilbio.2014.11.015.

Wang Y, Hu N, Ge T, Kuzyakov Y, Wang ZL, Li Z, Tang Z, Chen Y, Wu C, Lou Y. 2017. Soil aggregation regulates distributions of carbon, microbial community and enzyme activities after 23-year manure amendment. *Applied Soil Ecology* **111**:65–72 DOI 10.1016/j.apsoil.2016.11.015.

Wenand MP, Arthur MA, Lovett GM, Mcculley RL, Weathers KC. 2010. Effects of tree species and N additions on forest floor microbial communities and extracellular enzyme activities. *Soil Biology and Biochemistry* **42**:2161–2173 DOI 10.1016/j.soilbio.2010.08.012.

You Y, Wang J, Huang X, Tang Z, Liu S, Sun OJ. 2014. Relating microbial community structure to functioning in forest soil organic carbon transformation and turnover. *Ecology and Evolution* **4**:633–647 DOI 10.1002/ece3.969.

Zhang H, Ding W, Yu H, He X. 2015a. Linking organic carbon accumulation to microbial community dynamics in a sandy loam soil: result of 20 years compost and inorganic fertilizers repeated application experiment. *Biology and Fertility of Soils* **51**:137–150 DOI 10.1007/s00374-014-0957-0.

Zhang N, Wan S, Guo J, Han G, Gutknecht J, Schmid B, Yu L, Liu W, Bi J, Wang Z. 2015b. Precipitation modifies the effects of warming and nitrogen addition on soil microbial communities in northern Chinese grasslands. *Soil Biology and Biochemistry* **89**:12–23 DOI 10.1016/j.soilbio.2015.06.022.

Zhang N, Wan S, Li L, Bi J, Zhao M, Ma K. 2008. Impacts of urea N addition on soil microbial community in a semi-arid temperate steppe in northern China. *Plant Soil* **311**:19–28 DOI 10.1007/s11104-008-9650-0.

Zhang TA, Chen HYH, Ruan H. 2018. Global negative effects of nitrogen deposition on soil microbes. *ISME Journal* **12**:1817–1825 DOI 10.1038/s41396-018-0096-y.

Zhang X, Wei H, Chen Q, Han X. 2014. The counteractive effects of nitrogen addition and watering on soil bacterial communities in a steppe ecosystem. *Soil Biology and Biochemistry* **72**:26–34 DOI 10.1016/j.soilbio.2014.01.034.

Zhao Q, Classen AT, Wang WW, Zhao XR, Mao B, Zeng DH. 2017. Asymmetric effects of litter removal and litter addition on the structure and function of soil microbial communities in a managed pine forest. *Plant Soil* **414**:81–93 DOI 10.1007/s11104-016-3115-7.

Zhu C, Ma YP, Wu HH, Sun T, Kimberly JL, Sun ZW, Yu Q. 2016. Divergent effects of nitrogen addition on soil respiration in a semiarid Grassland. *Scientific Reports* **6**:1–8 DOI 10.1038/srep33541.