Responses of soil microbial communities and enzyme activities to nitrogen and phosphorus additions in Chinese fir plantations of subtropical China

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Abstract. Nitrogen (N) and phosphorus (P) additions to forest ecosystems are known to influence various above-ground properties, such as plant productivity and composition, and below-ground properties, such as soil nutrient cycling. However, our understanding of how soil microbial communities and their functions respond to nutrient additions in subtropical plantations is still not complete. In this study, we added N and P to Chinese fir plantations in subtropical China to examine how nutrient additions influenced soil microbial community composition and enzyme activities. The results showed that most soil microbial properties were responsive to N and/or P additions, but responses often varied depending on the nutrient added and the quantity added. For instance, there were more than 30% greater increases in the activities of β-Glucosidase (βG) and N-acetyl-β-D-glucosaminidase (NAG) in the treatments that received nutrient additions compared to the control plot, whereas acid phosphatase (aP) activity was always higher (57 % and 71 %, respectively) in the P treatment. N and P additions greatly enhanced the phospholipid fatty acids (PLFAs) abundanceespecially in the N2P treatment, the bacterial PLFAs (bacPLFAs), fungal PLFAs (funPLFAs) and actinomycic PLFAs (actPLFAs) were about 2.5, 3 and 4 times higher, respectively, than in the CK. Soil enzyme activities were noticeably

higher in November than in July, mainly due to seasonal differences in soil moisture content (SMC). βG or NAG activities were significantly and positively correlated with microbial PLFAs. These findings indicate that βG and NAG would be useful tools for assessing the biogeochemical transformation and metabolic activity of soil microbes. We recommend combined additions of N and P fertilizer to promote soil fertility and microbial activity in this kind of plantation.

1 Introduction

Nutrient availability, one of the most important factors controlling tree growth in forest plantations, can be significantly modified by fertilizer applications (Tumer and Lambert, 2008). Nitrogen (N) is generally believed to be the key growth-limiting element that controls the species composition, diversity, and productivity of forest ecosystems (Weand et al., 2010). N additions to forest ecosystems can influence a number of plant and soil processes, such as litter decomposition, carbon (C) storage and greenhouse gas fluxes (Cusack, 2013). In recent decades, N inputs into forest ecosystems from atmospheric deposition have increased at both regional and global scales, especially in Asia (Lu et al., 2009; Zechmeister-Boltenstern et al., 2011). This has raised the concern that forest ecosystems on nutrient poor soils may be at threat from imbalanced nutrition inputs (Vesterdal and Raulund-Rasmussen, 2002; Weand et al., 2010).

Phosphorus (P) is another primary limiting factor in many systems, especially in subtropical and tropical regions (Esberg et al., 2010). As a result, increased N deposition in these regions will cause a greater imbalance between N and P than in other regions. Exogenous P inputs to forests in these regions can lead to fast tree growth (Chen et al., 2010). However, to date it remains unclear how soil microbial properties respond to these nutrient additions, as N and P are rarely added simultaneously to forest ecosystems (Elser et al., 2007). An improved understanding of how nutrient additions influence soil microbial properties will be beneficial to support development of effective and sustainable management strategies for these forest ecosystems.

Just as different functional groups of microorganisms respond differently to prevailing environmental conditions, forest management practices will influence the composition of the soil microbial community in a specific way (Hackl et al., 2005; Chen et al.,

2013). Phospholipid fatty acids (PLFAs) are a vital component of the cell membrane (essentially the skin) of all microbes, and their polar head groups and ester-linked side chains (i.e. FAs) vary in compositions between eukaryotes and prokaryotes, as well as among many prokaryotic groups (Drenovsky et al., 2004). These compounds rapidly degrade as cells die, making them good indicators of living organisms(Zelles, 1999).Therefore, PLFAs representing the`living' or active component of the microbial community. PLFAs analysis allows differentiation of the microbial community composition and microbial biomass of each group quantitatively.

Studies have suggested that nutrient additions can significantly impact the population, composition, and function of soil microorganisms (Mandal et al., 2007; Hopkins et al., 2008; Geisseler and Scow, 2014), and that mineral fertilizer amendments can result in increases in soil microbial activity in subtropical forests (Cao et al. 2010; Geisseler and Scow, 2014). However, other studies have demonstrated that mineral fertilizers have either had no, or a negative effect on soil microbial diversity and activities (Moore-Kucera and Dick, 2008; Feng et al., 2009). Frey et al. (2004) found that active fungal biomass was lower in the fertilized plots compared to control plots in pine stands. The response of deciduous forests may be different from that of coniferous forests, and Nilsson et al. (2003) reported that the total soil fungal biomass may not be influenced by nutrient addition. In contrast, N additions led to a significant overall increase in fungal biomass in a northern hardwood forest ecosystem (Weand et al., 2010). In addition, some studies have found that nutrient addition have the opposite effects on soil bacteria in forest ecosystem (Demoling et al., 2008). Clearly, the response of the microbial community composition to nutrient additions appears to be substrate-specific in subtropical forests (Weand et al., 2010; Chang et al., 2011).

Soil microbial communities produce extracellular enzymes to acquire energy and resources from complex soil environments. These enzyme activities are also useful for detecting changes in soil quality, as they underpin nutrient cycling, and also serve as signals of altered microbial activity caused by environmental impacts (Li et al., 2009). Hydrolytic enzymes control the decomposition of many biological macromolecules that are abundant in plant litter and soil such as cellulose, hemicellulose, chitin, and protein (Allison et al., 2007). For our study we chose three enzymes that are related to the soil oganic carbon cycle, β-Glucosidase (βG) mainly releases glucose from cellulose and plays an important role in C cycling. N-acetyl-β-D-glucosaminidase (NAG) mainly releases N-acetyl-β-D-glucosamine from the terminal non-reducing

ends of chitooligosaccharides and plays an important role in N cycling. Acid phosphatase (aP) mainly releases phosphate groups, and plays an essential role in P cycling (Stone et al., 2012). The production of such enzymes by microbes is closely related to the balance between the availability of and the demand for nutrients.

Mineral fertilizers have been reported to have positive, negative, and neutral effects on soil C-, N-, and P-acquiring enzyme activities (Wang et al., 2011; Stone et al., 2012; Qian et al., 2014). It has also been pointed out that the response of soil enzymes to nutrient additions is highly context-dependent and that it varies with environmental and management related factors (Geisseler and Scow, 2014). Therefore, further studies about the effects of different fertilizers across a range of soil types and environmental conditions are needed to provide an improved understanding of these complex interactions. In recent years, the influence of nutrient additions on soil microbial communities has been intensively studied (Weand et al., 2010; Cusack, 2013). However, most studies have been carried out in subtropical broad-leaved forests (Wu et al., 2011; Tu et al., 2013; Huang et al., 2014). Since coniferous forests are a specific type of subtropical forest (Lv et al., 2014), it is important to study how N and P additions influence nutrient cycling functions in soil microbial communities in subtropical coniferous forest.

Different seasons may have a strong influence on the life cycle of microbes in subtropical forests through changes in biotic and abiotic factors. In spring, the vegetation starts to produce shoots and leaves, followed by a photosynthetically active period in summer. The growth period ends when the litter falls in autumn, providing a wealth of material for the soil decomposer community. During winter, vegetation is generally inactive, and decomposition processes are also slow because of the decelerating effect of low temperatures on soil microbial metabolism (Thoms et al., 2013). There is also an almost complete turnover of the microbial community between winter and summer, with different functions occurring in both seasons (Bardgett ea al., 2011). Soil microbial communities are likely to change as the soil temperature and moisture change (Moore-Kucera et al., 2008). July and November were two contrasting periods with hot and humid, and cold and dry conditions. The sharp contrast between the conditions in the two months suggests that the microbial communities may be different, and so findings from this study may reflect seasonal soil microbial diversity. Therefore, because we studied soils from two different months, we have obtained a limited insight into the influence of Chinese fir

plantations on soil microorganisms in two seasons with very different climatic conditions.

Chinese fir (*Cunninghamia lanceolata*), an important native conifer, has been extensively planted in subtropical China. It covers 9.11 million hectares and accounts for more than 18 % and 5 % of Chinese and global forest plantations, respectively (Huang et al., 2013). Over the past few years, Chinese fir plantations have received attention because of the decline in soil fertility and related yields; these declines are the result of successive planting, short rotation times, whole-tree harvesting, and poor site preparation (Yang et al., 2005). In order to improve soil quality and forest productivity, a number of management practices have been attempted, such as litter management, forest fertilization, and planting of broadleaved tree species (Zhang et al., 2004). Out of these measures, fertilization is the most effective and feasible. Many studies have reported findings about the effects of nutrient additions to Chinese fir plantations, but most of them were focused on the influence of nutrients on soil C, N sequestration, and nutrient cycling (Liao et al., 2014), and few studies have examined soil microbial properties and enzymes.

This study was conducted to determine the response of soil enzyme activities and microbial communities to N and P additions in different seasons in Chinese fir plantations, and to examine the linkages between soil properties, microbial community composition and soil enzyme activities. We hypothesized that soil hydrolytic enzyme activities and microbial biomass would increase under nutrient additions because of increased availability of resources from complex sources; we would also expect to find significant relationships between hydrolytic enzyme activities involved in C, N, and P transformations, soil C, N, and P contents, and the composition of the microbial communities.

2. Materials and methods

2.1 Site description

The study was conducted in the Qianyanzhou Forest Experimental Site, in Jiangxi Province, South China (26°44′52″N, 115°04′13″E, at an elevation of 102 m above sea level). The Chinese fir plantation was established in 2000. Average tree height and diameter at breast height were about 15 m and 13 cm, respectively. The site is characterized by a subtropical monsoon climate, with a mean annual temperature and

precipitation of 17.9 ℃ and 1471.2 mm, respectively (Wen et al., 2010). The mean soil temperature and precipitation in July 2013 were 29.6 ℃ and 171.0 mm, respectively, while the mean soil temperature and precipitation in November 2013 were 14.0 ℃ and 118.6 mm, respectively (Fig.1). The soil is classified as Ultisols using the USDA-NRCS soil taxonomy (1996). The soil bulk density was 1.31 g cm^{-3}, the pH value was 4.6, the soil organic carbon (SOC) content was 17.68 g kg^{-1}, total N content was 1.12 g kg^{-1}, and total P was 0.1 g kg^{-1}.

2.2 Experimental treatments

Thirty 20 m×20 m plots, each with an area of 400 m^2 and a buffer zone of more than 10 m between the plots were established in November, 2011. Six different treatments were used on five randomly distributed replicates as follows: control (CK), low N addition (N1: 50 kg ha^{-1} yr^{-1} of N), high N addition (N2: 100 kg ha^{-1} yr^{-1} of N), P addition (P: 50 kg ha^{-1} yr^{-1} of P), low N and P addition (N1+P: 50 kg ha^{-1} yr^{-1} of N +50 kg ha^{-1} yr^{-1} of P) and high N and P addition (N2+P: 100 kg ha^{-1} yr^{-1} of N +50 kg ha^{-1} yr^{-1} of P). N was added as NH_4NO_3 and P was added as NaH_2PO_4. The amount of N applied in the lower N treatment matched observed rates of N deposition in southern China (Lü et al., 2007), and the amount of P added was at a 1:1 ratio of the amount of the lower N application. The amount of N added for the higher N application was double the amount added for the lower application. Fertilizers were mixed with sand and were hand-scattered once every three months from March 2012 and until December 2013. Application varied according to the season, each application in the growing season accounted for 30 % of the total annual application, while each application in the non-growing season accounted for 20 % of the total annual application. Understory plants were removed manually at regular intervals and no herbicide was applied, so that potential impacts on soil organisms were avoided.

2.3 Soil sampling and analysis

Soils were sampled twice in 2013, at the end of July and November. Five soil cores (5 cm inner diameter) were collected randomly from each plot from the 0 to 10 cm soil layer, and were combined to form a composite sample. The litter layer was carefully removed before sampling. Soil pH was measured on a soil-water suspension (1:2.5 v: v) using a pH digital meter (Iovieno et al., 2010). Soil moisture content (SMC) was

measured gravimetrically on 20 g fresh soil oven dried at 105 ℃ to constant weight (Liu et al., 2012). SOC and total N were measured with an elemental analyzer (Elementar, Vario Max, Germany). Total P was analyzed with a flow injection auto analyzer following digestion with H_2SO_4-$HClO_4$ digestion (Huang et al., 2011).

The soil microbial community was characterized by phospholipid fatty acids (PLFAs) analysis. PLFAs were extracted from the soil using the procedure of Bossio and Scow (1998). After mild alkaline methanolysis to form fatty acid methyl esters (FAMEs), samples were then dissolved in hexane and analyzed with a DB-5 column in a gas chromatography mass spectroscopy (GC-MS) system (Thermo TRACE GC Ultra ISQ). Total amounts of the different PLFA biomarkers were used to represent the different groups of soil micro-organisms. The following combinations of PLFA biomarkers were considered to represent the bacterial origin: (Gram-positive bacteria were represented by i15:0, a15:0, i16:0, i17:0, Gram-negative bacteria by 16:1ω7c, cy17:0, cy19:0, and total bacteria were represented by the sum of the two types) (Frostegård and Bååth 1996). The PLFA 10Me18:0 and 10Me16:0 were used as a measure of actinomycic biomass. The PLFA 18:2ω6 and 18:1ω9c were used as markers for fungal biomass. Taken together, the combination of bacterial, fungal and actinomycic PLFAs biomarkers was considered to represent the total PLFAs of the soil microbial community. The enzyme activities of βG, NAG and aP were determined using 96-well microplates following the methods of Saiya-Cork (2002). Assay plates were incubated in the dark at 20 ℃ for 4 h. Fluorescence was measured at an excitation wavelength of 365 nm and a 450 nm emission cutoff filter by a microplate fluorometer (SynergyH4 BioTek, USA).

2.4 Statistical analysis

One-way analysis of variance (ANOVA) and Duncan's multiple comparisons were performed to identify the differences between the fertilizer treatments because of N and P additions. The paired-sample *t*- test was used to compare the seasonal variation in soil PLFAs and enzyme activities. Pearson correlations were used to determine the significance and strength of any relationships between soil properties, soil PLFAs, and enzyme activities. All statistical analyses were performed using SPSS version18.0 (SPSS Inc., C hicago, IL, USA). The level of significance was $P<0.05$.

3. Results

3.1 Soil properties

Comparison shows that, relative to the CK treatment, soil pH declined significantly after fertilizer applications (Table1). The N2P treatment had the lowest soil pH (4.4 and 4.1 for both sampling times). Further comparison with the CK treatment shows that N and P fertilizer applications resulted in improvements in SOC, total N and total P contents compared with the CK (P <0.05).The average SOC, total N and total P contents in N1P were highest in July, and were approximately 26 %, 44 % and 127 % higher than those of the CK treatment, respectively. In addition, SOC, total N, and total P concentrations in November were highest for the N2P treatment, and were 18 %, 35 % and 60 % higher than those in the CK. However, compared with the CK, the P treatments had no significant influence on soil properties in either July or November (P >0.05). Seasonally, the SMC was higher in November (25.6 % -27.9 %) than in July (18.1 %- 21.4 %).

3.2 Soil hydrolytic enzyme activities involved in C, N and P transformations

βG enzyme activity was significantly influenced by fertilizer applications (P <0.05), and the highest activities in both July and November were observed in the N2P treatment, both of which were about 93 % higher than those in the CK, respectively (Fig. 2). In addition, compared with the CK, βG activity was not influenced by P fertilizer applications (P >0.05).

In July, NAG activity was significantly higher in fertilized plots than in the CK (P <0.05), and was about 2 times greater in the N1 treatment, and 3 times greater in the N2 treatment, than in the CK. In November, NAG activity was significantly enhanced in the N1 and N2 treatments compared with the CK. However, applications of P fertilizer inhibited NAG activity, and NAG contents were 12 % lower in N1P than in N1, and 29 % lower in N2P than in N2, respectively. The NAG content was lowest in the P treatment. In contrast to NAG, aP activity was strongly influenced by the P treatment. Compared to the control, aP activities were always higher (57 % and 71 %, respectively) in the P treatment. In particular, aP activity tended to be greater in the N1, N2P and P treatments in July, and in the N2 and P treatments in November (Fig.

2).

When the activities in the different sampling months are compared, the βG, NAG, and aP activities were significantly higher in November than in July (P<0.05, supplemental Table S1).

3.3 Soil microbial community composition

Soil total PLFAs (totPLFAs) were significantly higher in the fertilized treatments than in the CK (P<0.05). The totPLFAs were about 2.5 times greater in the N2P treatment than in the CK, and about 1.5 times higher in the N2 treatment than in the CK (Fig.3). Bacterial PLFAs (bacPLFAs), Fungal PLFAs (funPLFAs) and Actinomycic PLFAs (actPLFAs) (Fig.3) were influenced by the treatments in the same way as totPLFAs, that is, there were larger increases in the fertilized soils than in the CK (P <0.05). BacPLFAs, funPLFAs and actPLFAs were highest in N2P, and were about 2.5, 3 and 4 times higher, respectively, than in the CK. G^+ PLFAs were higher than G^- PLFAs and both were significantly influenced by different treatments, and were greatest in the N2P treatments (Fig.3).

The fungal/bacterial ratio (F/B ratio) was only significantly higher in the N2P treatments in July (P <0.05, Fig.4). The G^+/G^- ratio was not significantly influenced by fertilizer treatments (P < 0.05); values of this ratio were close to 2.5 (Fig.4).

The seasonal patterns of total, bacterial, and fungal PLFAs for all soils were similar, and there were no significant differences between July and November (P >0.05, supplemental Table S2).However, the F/B ratio was markedly higher in July than in November (P <0.05, supplemental Table S2).

3.4 Relationships between soil enzyme activities, PLFAs profiles, and measured soil properties

Table1 shows the significance and strength of the relationships between microbial community composition, enzyme activities, and soil properties. Soil pH was significantly and positively correlated with aP activity, and negatively correlated with funPLFAs. The SMC was positively correlated with all soil enzyme activities and total, bacterial, G^+, and actinomycic PLFAs. Total N and total P were positively correlated with enzyme activities and soil PLFAs, while SOC was mainly responsible for the soil microbial community composition (P<0.05).

Table 2 shows the relationships between soil PLFAs and enzyme activities. βG and NAG activity were positively correlated with totPLFAs, bacPLFAs, actPLFAs, G^+ PLFAs, and G^+/G^-. AP activity was only positively correlated with G^+ PLFAs and G^+/G^-. However, there was no significant correlation between the funPLFAs and all soil enzyme activities.

4 Discussion

Numerous studies have reported decreases in soil pH after nutrient additions due to leaching of magnesium and calcium, as well as mobilization of aluminum (Wang et al., 2011). In line with these observations, we demonstrated that the soil pH decreased to a certain extent in the N and NP treatments, but not in the P treatment. This suggests that N deposition will lead to soil acidification in this region. The relationship between fertilization and soil carbon sequestration has been examined in previous studies (Khan et al., 2007; Wei et al., 2012). Khan et al. (2007) observed a net decline in soil C after 40 to 50 year of synthetic fertilization. Conversely, our study indicated that nutrient additions may have a positive influence on the amount of C stored in forests. These contrasting results may be attributed to the factor that, unlike agricultural systems, nutrient additions to forest ecosystems often lead to changes in the composition and diversity of plant species, which in turn have an influence on the forest litter. Consistent with our research, Wei et al. (2012) reported that nutrient additions led to a significantly enhancement of soil C sequestration and nutrient status in Chinese fir forest soils. Huang et al. (2011) also considered that soil nutrient enrichment, especially N, could reduce SOC decomposition. Moreover, nutrient-induced increases in forest litter and subsequent inputs of organic matter to the forest floor, and ultimately to the mineral soil, could lead to increases in soil nutrient concentrations (Moorhead and Sinsabaugh, 2006). The litter on the forest floor acts as input – output system of nutrient and the rates at which forest litter falls and subsequently, decomposes contribute to the maintenance of soil fertility in forest ecosystems (Wang et al., 2011). Zeng et al. (2015) found that while exogenous N and P additions could promote forest ecosystem biomass and could also lead to increases in the litter on the forest floor in the form of root exudates and aboveground residues, P addition had no influence on forest biomass. Therefore, total P did not change when only P was added, there were however significant increases in total P in response to

combined applications of N and P. Besides, other related unpublished studies at our study site have demonstrated that, after P additions, P concentrations in leaves and twigs increased significantly. Soil P was largely absorbed by plants, and soil P remained unchanged.

Several previous studies have reported that nutrient additions can have both positive and negative influences on C-, N- and P- acquiring enzyme activities depending on tree species (Stursova et al., 2006; Piotrowska and Wilczewski, 2012). Consistent with our hypothesis, our study showed that βG and NAG activity levels were obviously higher after N and NP applications than the other treatments, which demonstrates that these enzymes were easily stimulated by substrates. This is the result of increased SOC and total N from the N and NP treatments, which were significantly and positively correlated with βG and NAG activities in our study (Figure 6). Similar results were also reported by Mandal et al. (2007) and Liang et al. (2014), and they attributed the higher enzyme activity levels to higher organic matter contents and enhanced microbial activity. N additions to both labile and recalcitrant substrates are thought to allow microbes to invest N in enzyme production, which often results in increased activity of enzymes responsible for cellulose degradation (e.g., βG), for acquisition of organic N (NAG). Soil organic matter not only provides substrates for enzymes, but also plays a vital role in protecting soil enzymes by forming complexes with clay and humus (Saha et al., 2008).

The βG and NAG activities in the P fertilized plots were generally equal to or lower than those in the CK. Our results showed that higher total soil N could stimulate βG and NAG activity, but P additions had no influence on total soil N. Secondly, Turner and Wright (2014) found that P additions could lead to increases in soil microbial C and N which, in turn, would mean that microbes could reduce their investment in C- and N- acquiring enzymes such as βG and NAG. When a resource is limiting, microbes may benefit from producing enzymes to obtain it, but could be constrained by the availability of C and N required for enzyme synthesis. Similarly, aP was higher in fertilized treatments than in the control suggesting that fertilization improved soil microbial activity, which, in turn, produce enzymes to mobilize resources from complex sources (Keeler et al., 2009).

Our results clearly demonstrate that the two-season investigated micros (July and November) differed in their functional responses to nutrient additions. The microbes

demonstrated a higher capacity to degrade substrates (cellulose, plant cell walls) in November than in July, as indicated by the enhanced βG, NAG and aP activities. This was due to the higher SMC in November, which was significantly and positively correlated with soil enzyme activities in the present study (Table3). Similar results have been observed previously for other tropical forest sites, in which they considered that low soil moisture would strongly limit soil enzyme activities (Liu et al., 2012; Steinweg et al., 2012; Schaeffer et al., 2013). Furthermore, McDaniel (2013) found that simulated warming decreased both soil βG and NAG enzyme acitivities by 19 % and 21 %, respectively. In our study, the mean temperature in July was close to 30 ℃, which might suggest that the soil enzyme activity was inhibited by high temperature in July than in November (Fig.1).

A meta-analysis based on 107 datasets from 64 trials around the world showed that, compared to control unfertilized treatments, mineral fertilizer applications led to a 15.1 % increase in microbial biomass (Geisseler and Scow, 2014). Allen and Schlesinger (2004) suggested that increases in SOC and total N corresponded with increases in soil microbial biomass. Similarly in this study, we observed that, relative to CK, fertilizer applications enhanced bacterial, fungal, and actinomycic populations. Girvan et al. (2003) reported that soil properties could be a key control on the general composition of the microbial community. Studies have demonstrated that nutrient addition can increase forest productivity (Thomas et al., 2010). The higher productivity can lead to increased inputs of organic resources in the form of root exudates, decaying roots and aboveground residues, which would alleviate the C and N limitations for soil microbes (Keeler et al., 2009). The soil totPLFAs were highest in N2P and lowest in the P treatment, suggesting that the combined additions of N and P promoted synergistic positive effects on the soil microbial community.

High values of the F/B biomass ratios are thought to indicate a more sustainable ecosystem with lower environmental impacts, in which organic matter decomposition and N mineralization are the main sources of soil nutrients for plant growth (Chen et al., 2013). In our study nutrient addition to mineral soil led to significant increases in bacterial and fungal biomass. Similar results were found by Weand et al. (2010). He et al. (2008) suggested that fertilizer applications had less impact on soil bacterial community than fungi. Likewise, the higher F/B ratio in the N2P treatment was due to the degree of fungal increase was greater than that of bacteria under this treatment.

Hackl et al. (2005) found that soil moisture was an important driver of overall microbial activity. Using multivariate analysis, Steinweg et al. (2012) reported that SMC was the most closely correlated with bacterial community structure. We also found that SMC was significantly and positively correlated with bacterial PLFA signatures, and the abundance of soil bacteria biomass was higher in November compared to July. This suggests that the significantly lower F/B ratio in November was attributable to the higher SMC.

The correlations between enzyme activities and soil PLFAs were not consistent for all the enzymes assayed. The activities of βG and NAG were correlated strongly with the totPLFAs, bacPLFAs, actPLFAs and G^+ PLFAs, but only aP was correlated with G^+. Therefore, soil βG and NAG activities are more useful for reflecting the metabolic activity of soil microbes in our study region than aP. There were no consistent correlation between fungal PLFAs and enzyme activities in this study. Šnajdr et al (2008) obtained similar results, which they speculated to be due to the fungal biomass, of which the hyphal cords used for nutrients translocation were metabolically inactive. Nevertheless, there are a few limitations with PLFA analysis, which cannot reveal species-level information and archae cannot be determined using this method. The abundance and diversities of some functional genes of C, N, and P cycling can be analyzed by molecular biology technique. It will present detail information about the relationships between soil microbial diversities and enzyme activities.

5 Conclusions

N additions increased soil nutrient contents, with more pronounced effects with combined N and P applications. The average SOC, total N and total P contents in N1P were highest in July, and were approximately 26 %, 44 % and 127 % higher than those of the CK treatment, respectively. Soil pH tended to decrease when nutrients were added, indicating that nutrient inputs, especially N deposition, were the main cause of soil acidification in this region.

The C (βG) and N (NAG) related hydrolase were more sensitive to N and NP additions than the P (aP) related hydrolase, and their contents were higher in the fertilizer applied plots compared to the CK. P additions stimulated the aP activity and inhabited βG and NAG activity. Compared to the control, aP activities were always higher (57 % and 71 %, respectively) in the P treatment. The three enzyme activities

were obviously higher in November than in July, and reflect the higher SMC in November.

The response of the soil microbial community composition was more significant for the combined N and P additions than for single additions of either N or P. Fertilizer applications resulted in increased bacterial, fungal, actinomycic, and total PLFAs in this study region, especially in the N2P treatment, the bacterial PLFAs (bacPLFAs), fungal PLFAs (funPLFAs) and actinomycic PLFAs (actPLFAs) were about 2.5, 3 and 4 times higher, respectively, than in the CK. However, there were no significant differences between the response for July and November.

The βG and NAG were strongly correlated with different soil PLFAs, and so would be useful tools for assessing the biogeochemical transformation and metabolic activity of soil microbes. Since microbial activities are considered to be important components of soil biological activity, we would recommend simultaneous additions of N and P fertilizer to promote soil fertility in Chinese fir plantations.

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Table 1. Response of soil properties to N and P additions to Chinese fir plantations in July and November (means±standard errors).

	Treatment	pH	SMC (%)	Total N ($g•kg^{-1}$)	SOC ($g•kg^{-1}$)	Total P ($g•kg^{-1}$)
July	CK	4.6 (0.06)a	18.1 (1.5)ns	0.9(0.03)b	21.6 (0.75)b	0.11(0.00)c
	N1	4.2 (0.06)b	18.7 (2.4)ns	0.9(0.01)b	24.3 (0.15)ab	0.12(0.01)c
	N2	4.2 (0.13)b	20.8 (2.3)ns	1.1(0.06)a	25.8 (1.20)a	0.16(0.01)abc
	N1P	4.2 (0.05)ab	21.4 (2.1)ns	1.3(0.11)a	27.2 (0.70)a	0.25(0.03)a
	N2P	4.1 (0.06)b	19.9 (1.7)ns	1.3(0.07)a	26.7 (1.28)a	0.18(0.01)ab
	P	4.4 (0.07)a	20.4 (1.4)ns	0.9(0.02)b	22.1 (0.95)b	0.16(0.03)bc
November	CK	4.8 (0.11)a	25.0 (0.92)ns	1.1 (0.04)b	22.9 (0.51)b	0.15(0.01)b
	N1	4.4 (0.05)b	27.9 (0.82)ns	1.3 (0.07)b	23.5 (0.63)ab	0.16(0.01)ab
	N2	4.4 (0.16)b	25.6 (0.67)ns	1.6 (0.02)a	25.8 (1.47)a	0.18(0.01)ab
	N1P	4.6 (0.04)ab	25.9 (1.16)ns	1.6 (0.06)a	24.5 (1.35)a	0.22(0.01)ab
	N2P	4.4 (0.06)b	30.2 (1.25)ns	1.7 (0.07)a	27.0 (2.61)a	0.24(0.02)a
	P	4.8 (0.07)a	26.1 (1.07)ns	1.6(0.06)a	23.3 (0.58)b	0.18(0.01)ab

Note: Numbers in brackets represent the standard errors of the means. Different lower-case letters in the same column indicate significant differences when $P<0.05$; ns: no significant difference between treatments. CK: control; N1: 50 $kg•ha^{-1}•yr^{-1}$ of N; N2: 100 $kg•ha^{-1}•yr^{-1}$ of N, N1P: 50 $kg•ha^{-1}•yr^{-1}$ of N +50 $kg•ha^{-1}•yr^{-1}$ of P; N2+P: 100 $kg•ha^{-1}•yr^{-1}$ of N +50 $kg•ha^{-1}•yr^{-1}$ of P; P: 50 $kg•ha^{-1}•yr^{-1}$ of P, the same below.

Table 2. Pearson correlations between soil properties, soil enzyme activities and microbial variables.

	pH	SMC	Total N	SOC	Total P
βG	0.31ns	0.82**	0.72**	0.16ns	0.37**
NAG	0.24ns	0.71**	0.71**	0.12ns	0.36*
aP	0.59**	0.73**	0.71**	0.05ns	0.30ns
Tot PLFAs	-0.24ns	0.39**	0.67**	0.65**	0.60**
BacPLFAs	-0.17ns	0.49**	0.71**	0.62**	0.61**
FunPLFAs	-0.44**	-0.17ns	0.18ns	0.49**	0.27ns
ActPLFAs	-0.07ns	0.50**	0.67**	0.57**	0.55**
G^+PLFAs	-0.10ns	0.59**	0.73**	0.55**	0.60**
G^-PLFAs	-0.34ns	0.14ns	0.53**	0.68**	0.52**
F/B	-0.36ns	-0.47**	-0.27ns	-0.10ns	-0.12ns
G^+/G^-	0.20ns	0.59**	0.34**	0.10ns	0.15ns

Note: The values are correlation coefficients. *P <0.05, **P <0.01; ns: no significant differences. pH: soil acidity, SMC: soil moisture content, SOC: soil organic carbon, βG: β-Glucosidase, NAG: N-acetyl-β-glucosaminidase, aP: acid phosphatase; Tot PLFAs: Total PLFAs, BacPLFAs: Bacterial PLFAs, ActPLFAs: Actinomycete PLFAS, G^+ PLFAs: Positive gram bacterial PLFAs, G^- PLFAs: Negative gram bacterial PLFAs, F/B: ratios of fungal PLFAs to bacterial PLFAs, the same below.

1 Table 3. Pearson correlations between soil enzyme activities and microbial PLFAs.

	TotPLFAs	BacPLFAs	FunPLFAs	ActPLFAs	G^+PLFAs	G^-PLFAs	F/B	G^+/G^-
βG	0.39*	0.51**	-0.22ns	0.49**	0.59**	0.17ns	-0.59**	0.57**
NAG	0.35*	0.46**	-0.19ns	0.43**	0.56**	0.09ns	-0.53**	0.62**
aP	0.23ns	0.33ns	-0.26ns	0.37ns	0.42**	0.07ns	-0.53ns	0.54**

Note：The values are the correlation coefficients. *P <0.05, **P <0.01. ns: no significant differences.

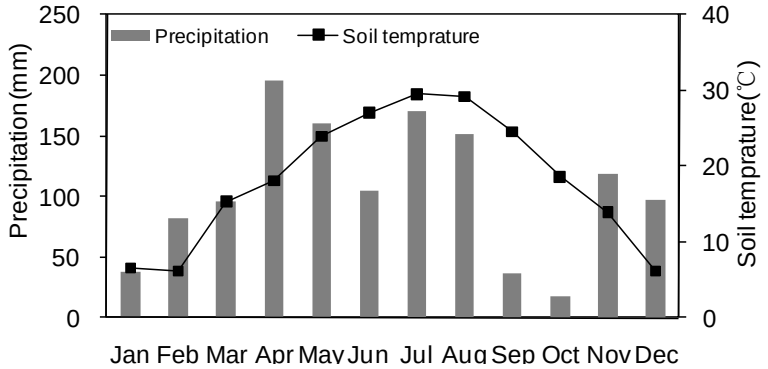
Figure captions

Figure 1. Mean monthly soil temperature and precipitation in the study area during 2013.

Figure 2. Responses of soil enzyme activities to N and P additions in Chinese fir plantations in July and November (Different lower-case letters in different bars indicate significant differences when $P<0.05$).

Figure 3. Responses of soil microbial PLFAs to N and P additions in Chinese fir plantations in July and November (Different lower-case letters in different bars indicate significant differences when $P<0.05$.Tot PLFAs: Total PLFAs, BacPLFAs: Bacterial PLFAs, ActPLFAs: Actinomycete PLFAs, G^+ PLFAs: Positive gram bacterial PLFAs, G^- PLFAs: Negative gram bacterial PLFAs, the same below).

Figure 4. Ratio of F/B and G^+/G^- to N and P additions to Chinese fir plantations (F/B: ratios of fungal PLFAs to bacterial PLFAs, G^+/G^-: ratios of positive gram bacterial PLFAs to gram negative bacterial PLFAs. Different lower-case letters in different bars indicate significant differences when $P<0.05$).



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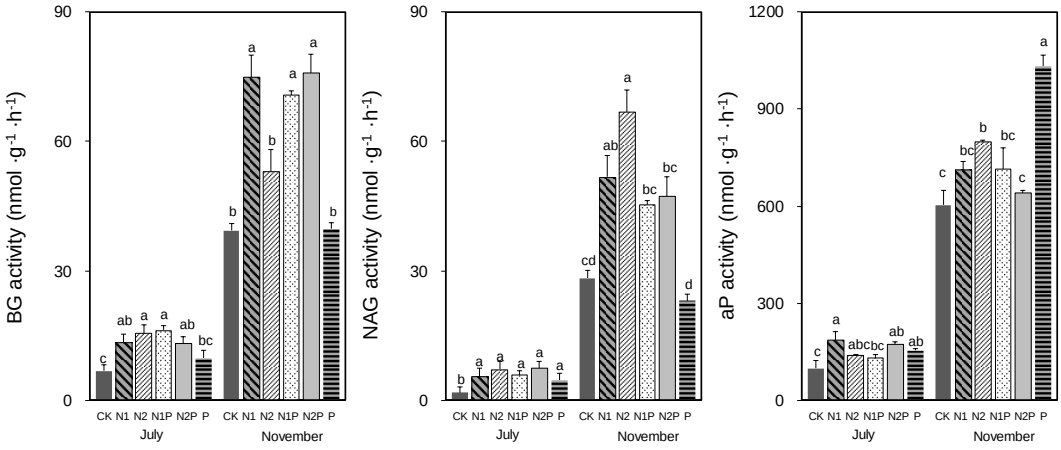

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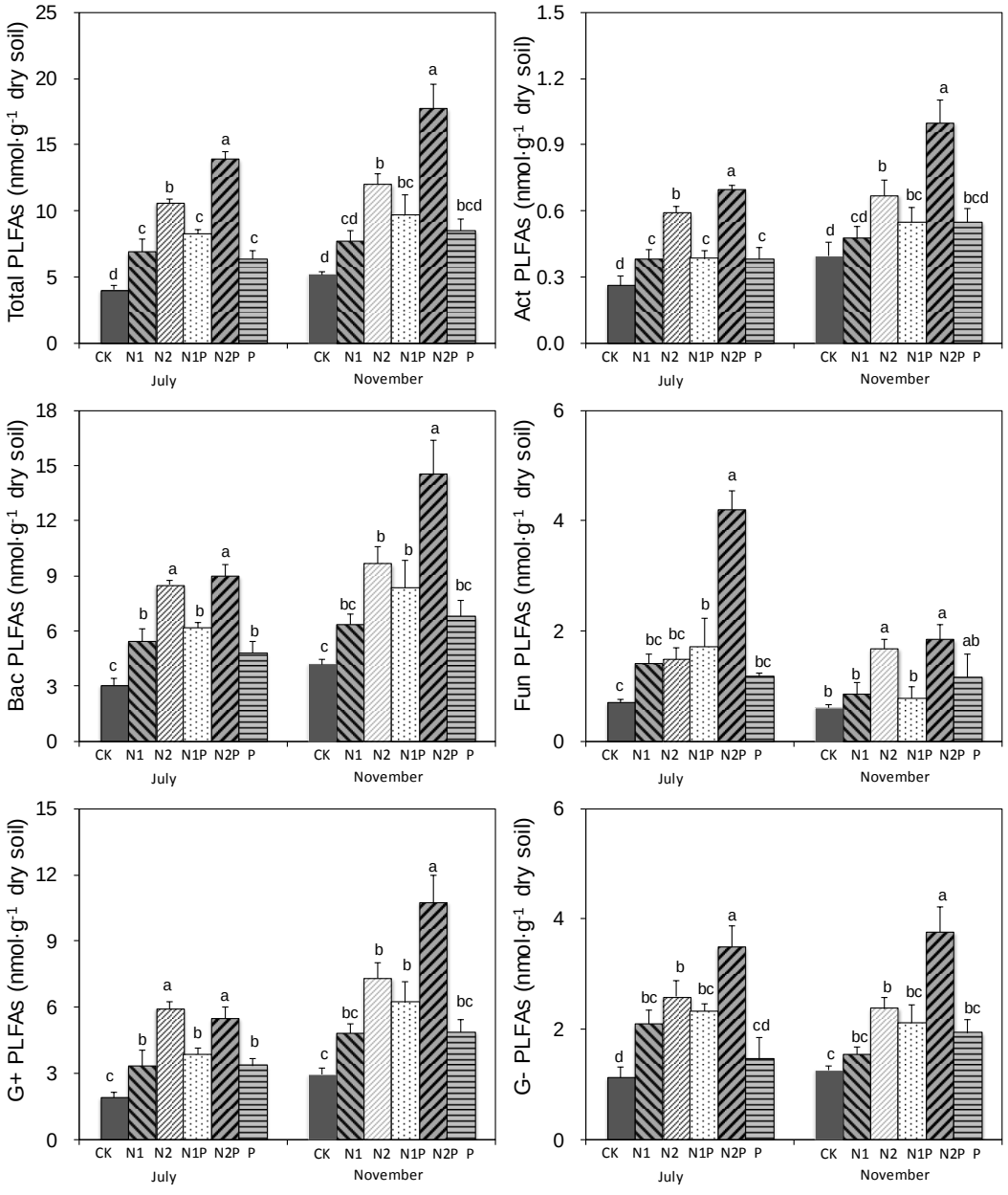

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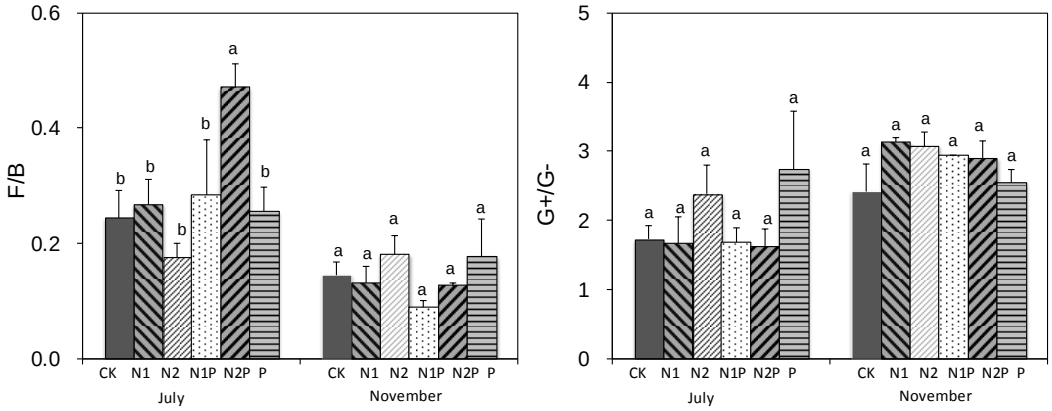

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