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SOIL MICROBIAL COMMUNITY RESPONSE TO NITROGEN AND PHOSPHOROUS FERTILIZATION IN A TEMPERATE FOREST NURSERY

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Abstract

Nitrogen and phosphorus application to forest nursery seedlings is known to influence various above-ground growth factors, such as plant composition and productivity, and the below-ground microbial community. In this study, we added N and P to forest nursery seedlings in a temperate forest region in China to examine how nutrient additions influence the plant biomass and soil microbial community composition through phospholipid fatty acids (PLFAs) analysis. The results showed that N had a significant effect on plant biomass, increasing the below-ground and the above-ground biomass, by 34 and 39%, respectively, and enhancing microbial community markers including fungal PLFAs (50%) and the ratio of fungal to bacterial PLFAs (35%) at 100 g nitrogen/1.3m^2, but didn't change or negatively affect total PLFAs, bacterial PLFAs, gram-positive and gram-negative PLFAs and the ratio G^-: G^+, or actinomycete PLFAs. Similarly, P increased the below-ground and above-ground biomass by 11.96 and 13%, respectively, and fungal PLFAs (35%) and the ratio of fungal to bacterial PLFAs (21%), at 80 g phosphorous/1.3m^2 but didn't change or negatively affect total PLFAs, bacterial PLFAs, gram-positive and gram-negative PLFAs and the ratio G^-:G^+, or actinomycete PLFAs. Principal component analysis explains 77% and 56% of total variations in PLFA composition in N and P treatment. In conclusion, N and P improved the seedling growth without negative effect on total PLFAs.

Key words: Nitrogen, Phosphorous, Plant biomass, Phospholipid fatty acids (PLFAs).

Introduction

Microorganisms are important living components in soil that play a vital role in the plant nutrient cycling process. These soil microbes play essential roles in the plant environment, such as plant growth promotion/ inhibition (Degens *et al*., 2000) and metabolism of organic materials, followed by a number of physical and chemical soil processes, including nutrient transformation (Burton *et al*., 2010). Thus, they influence soil sustainability and help to enable proper functioning of the ecosystem. Microbial activity, biomass and community structure can also affect soil quality in ways separately from the soil's physical and chemical properties (Sparling, 1997). Ge *et al*., (2012) noted that soil fertility, and degraded or improved, could be detected by sensitive signals received from microbial indicators.

Microbial biomass as a soil quality indicator is an important marker to induce biochemical changes and store plant nutrients such as NP in soil ecosystems (Chen *et al*., 2003; Velmourougane *et al*., 2014). According to Stewart *et al*., (2005), NP is the primary component in applied nutrients, which are mostly dependent on inorganic fertilizers. Based on the type of soil and plants, these components are applied as fertilizers in different ratios. An NP imbalance in soil can be worsened by continuous fertilization in pure plantation systems and can disturb soil ecosystem function, leading to changes in microbial community structure and abundance (Kamaa *et al*., 2011). Plant growth is affected when inorganic nutrients are applied, changing soil properties (Eo & Park, 2016). Some individual microbial groups help to utilize and degrade added nutrients in the soil (Tian *et al*., 2004). These added chemical fertilizers can influence microbial community diversity and composition (Zhang *et al*., 2013).

According to Weand *et al*., (2010), N generally acts as a key growth-limited component in controlling diversity, species composition and productivity of forest ecosystems. Cusack (2013) noted that a number of plants and soil processes are influenced when N is added in forest ecosystems. In recent decades in various parts of the world, especially in Asia, the atmospheric deposition of N inputs into forest ecosystems is on the rise (Zechmeister-Boltenstern *et al*., 2011). Weand *et al*., (2010) have observed that, due to an imbalance in nutrient inputs, forest ecosystems may be threatened in nutrient-poor soils (Vesterdal & Raulund-Rasmussen, 2002).

According to a report published by Esberg *et al*., (2010), P is an important limiting factor that induces rapid growth in trees when ingested exogenously (Chen *et al*., 2010). Due to this observation, an imbalance is created between N and P when the N deposition rate is higher. Only a few studies, such as one by Elser *et al*., (2007), have reported on the responses of soil microbial characteristics when N and P are added in parallel. Different studies conducted in this arena have shown that, when nutrients are added, a significant impact is found on population, composition (Mandal *et al*., 2007) and soil microorganism functions (Geisseler & Scow, 2014). Mineral fertilizer amendments also enhance the soil's microbial activity (Geisseler & Scow, 2014). By contrast, fertilizers either had no effect (Moore-Kucera & Dick, 2008) or resulted in an inhibitive effect on the diversity of soil microbes and their activities (Feng *et al*., 2009). However, most related studies have been carried out in forest ecosystems (Tu *et al*., 2013; Huang *et al*., 2014). Therefore, few studies hitherto have examined the effect of different chemical fertilizers on soil microbial characteristics, particularly in forest nursery seedlings. This current study aims to investigate the changes in a

soil's microbial community in a temperate forest nursery over one growing season after the addition of N and P.We hypothesize that NP additions do not change the total microbial community but do affect the individual numbers of PLFAs. This study focuses mainly on two questions: (1) Does the addition of N and P affect the biomass of *Acer mono* seedlings? and (2) What changes does the microbial community experience in temperate forest nursery soil after the addition of N and P?

Materials and Methods

Study site and soil collection: This experiment was conducted at the Maoershan experimental station for seedling growing (127°′–127°′E, 45°23′–45°26′ N, 390 m above sea level), located in the temperate forest region in the Heilongjiang Province of northeast China. The site has a cold, continental monsoon climate, with an average annual air temperature of 2.8°C. Average temperatures in January and July are -19.6°C and 20.9°C, respectively, and the annual average humidity, annual precipitation and annual evaporation are 70%, 723.8 mm and 1094 mm, and the frost-free period usually lasts from 120 to 140 d. The soil is mostly dark brown earth that belongs to the Boric Luvisols suborder in the Chinese Soil Taxonomy classification system (Gong *et al*., 2003).

Soil samples were collected from the experiment field at 0-20 cm soil depth and were thoroughly mixed to make a representative composite soil sample. Analysis found a total N, P and K content of 3.98 g kg^{-1}, 8. 2 g kg^{-1} and 14 g kg^{-1}, respectively, and an available N, P, and K content of 4 mg kg^{-1}, 7.23mg kg^{-1} and 176 mg kg^{-1}.

Plant materials and fertilization treatments: Four-year-old Acer mono Maxim seedlings of uniform size were selected in a field nursery for study (2016). Planting spacing was 20 cm× 30 cm. Each plot (1.3 m^{-2}) was treated with one of four levels of N1, N2, N3 or N4 (0, 50, 100 or 150 g) and one of four levels of P1, P2, P3 or P4 (0, 40, 60 or 80 g). Ten plants were included in each treatment, and each treatment was replicated three times (total n = 480). We applied nitrogen in two split doses during May and July, and phosphorus was applied once as a basal dose in May. Standard cultural practices were performed during the experiment (i.e., weeding, hoeing, irrigation, etc.) to produce healthy seedlings.

Plant biomass measurement: Plants were uprooted in October, divided into root and shoot, and then taken back to the laboratory. Roots were cleaned with tap water to remove the soil particles. Plants were divided into shoots and roots and kept into tag paper bags. Root and shoot samples were dried at 65°C in an oven for 72 hours and weighed in an electronic balance to determine the below-and above-ground plant biomass, respectively. Five plants each were weighed for each treatment type and for each replication.

Soil sampling: Soil was collected (from a depth of 0–15 cm) from each treatment plot at each replication, with a soil core of diameter 3 cm, during Sept 2015. Three samples were collected from each treatment plot at each replication and bulked to provide a representative sample for each treatment plot at each replication. The soil samples were placed in sterile plastic bags and transported to the laboratory in an ice box. Soils were sieved to 2.5 mm and stored at -20°C.

PLFAs analysis: Soil PLFAs were analyzed by using the method described by Frostegayrd *et al*., (1993). The fatty acid methyl esters (FAMEs) were separated by using a HewlettPackard 5890 gas chromatograph fitted with a flame ionization detector and mass spectrometric (GCMS) system Frostegayrd *et al*., (1993). FAMEs of the samples were determined by comparison of their chromatographic retention times with that of a standard mixture composed of 37 different FAMEs, ranging from C11 to C24 (Sigma corporation, USA). The sum of PLFAs i15:0, a15:0, i17:0, 18:0, 17:0, 15:0, 11:0, 14:0, 16:0, 20:0, 16:1ω9, cy17:0, cy19:0waschosen for bacterial biomass (bacterial PLFAs (Ji *et al*., 2016); the sum of PLFAs 18:3ω6, 18:2ω6, 18:1ω9c, 18:1ω9t, was used for fungal biomass (fungal PLFAs).The sum of PLFAs i15:0, a15:0, 16:0, i17:0, 18:0 was marked for gram-positive (G+) bacteria (gram-positive PLFAs), and 16:1ω9, cy17:0, cy19:0 were marked for gram-negative (G−) bacteria (gram-negative PLFAs) and 10Me16:0, 10Me17:0, 10Me18:0 for actinomycetes (actinomycete PLFAs). The proportion of fungi to bacteria (F/B) was calculated as the ratio of sum of fungi to the sum of bacteria (Buyer *et al*., 2010). The sum of total bacterial PLFAs and fungal PLFAs was considered total PLFAs.

Statistical analysis: The experiment was conducted with a randomized complete block design, with split-plot arrangements, in order to test the effects of N and P fertilizers and their interactions on seedling biomass and soil microbial community. The data were analyzed using two-way analysis of variance (ANOVA) in the computer program SPSS 21.0. The experimental treatments were randomized and repeated three times, in order to reduce any variation caused by soil heterogeneity. Furthermore, N treatment was used as the main block, whereas P treatment was used as the sub-block. LSD test was also performed for the experiments to compare treatments with one another. Multivariate principal component analysis (PCA) was performed in Canoco for Windows 4.5.Significance levels were set at 0.05.

Results

Effect of NP addition and their interactions on plant biomass: Seedlings treated with N fertilizer exhibited significantly greater root and shoot biomass than those from untreated (i.e., 0 g N) control plots (p<0.05; S1 Table, Fig. 1A+B), and the values for both parameters were the highest in the seedling plot treated with 100 g N/1.3m^2, followed by those for the seedling plot treated with 50 and 150 g N/1.3m^2, respectively.

Similarly, seedlings treated with P fertilizer linearly(p<0.05; S1 Table, Fig. 1A+B) had greater shoot and root biomass than those from untreated (i.e., 0 g P) control plots, and values for both parameters were highest in the seedling plot treated with 80 g P, followed by 60 and 40 g/1.3m^2, respectively.

We also observed a significant (p<0.05; S1 Table, Fig. 1C+D) interactive effect of N and P for both shoot and root biomass, and found that values for both shoot and root biomass were the highest in seedling plots treated with 100 g N and 80 g P/1.3m^2.

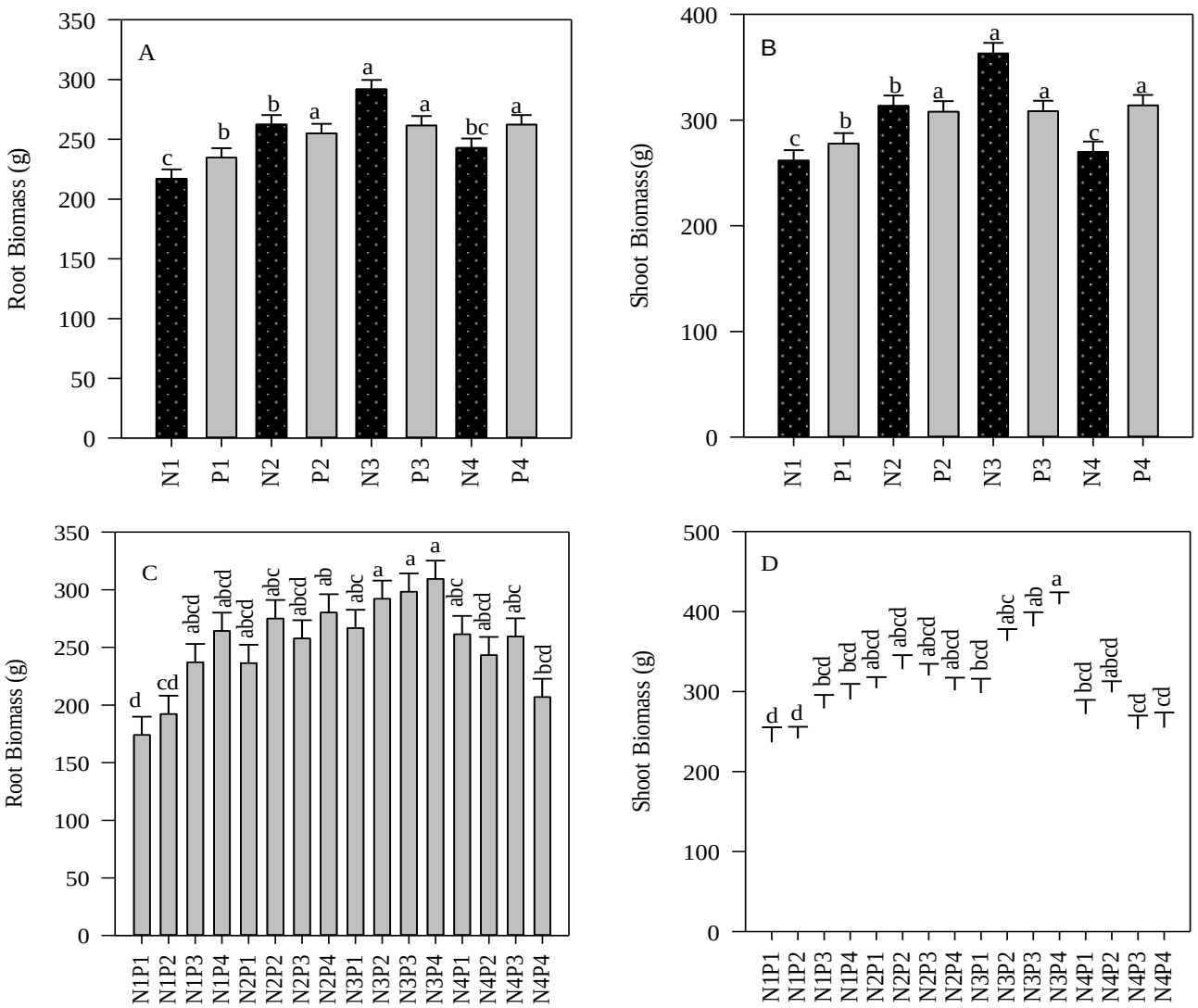

Fig. 1. Influence of N and P on root and shoot biomass of Acer mono seedling.N stand for Nitrogen levels (N1-N4); P stand for Phosphorous levels (P1-P4). Different letter show the level of significance. Error bars represent the standard error of the mean.

Effects of NP and their interactions on soil microbial community: There was no significant (p>0.05; S1+S2 Table, Fig. 2 A+B+C+D+E+F)effect noted in total PLFAs, bacterial PLFAs, gram-positive PLFAs, gram-negative PLFAs and the ratio of G^+/G^- PLFAs, and actinomycetePLFAs after N addition. However, there was an increase (p<0.05; S2 Table, Fig. 3 A+B) in fungal PLFAs, and the ratio of fungal to bacterial PLFAs with an increase in N levels, and the highest values were recorded in the plot treated with 100 g N/1.3m^2.

On the other hand, we didn't find any significant (p>0.05; S1+S2 Table, Fig. 2 A+B+C+D+E+F) effect of P addition on total PLFAs, bacterial PLFAs, gram-positive PLFAs, gram-negative PLFAs and the ratio of G^+/G^- PLFAs, and actinomycetePLFAs. We found a significant (p<0.05; S2 Table, Fig. 3 A+B) positive effect of P fertilization on fungal PLFAs and the ratio of fungal to bacterial PLFAs in 80 g P/1.3m^2. However, there was no significant interactive effect of NP on soil microbial community.

Principal component analysis results: Principal component analysis (PCA) showed that the soil microbial PLFA contents under different nitrogen treatments had different degrees of variations (Fig. 4A). The first two principal components accounted for approximately 32.0% and 45.0% of variation in PLFA composition in all treatments. The PLFAs 17:0, 18:0, 11:0, i17:0, and 16:1ω9 (bacterial PLFAs) had positive loadings on PC 1, while the PLFAs 18:3ω6, 18:2ω6, 18:1ω9c, 18:1ω9t, (fungal PLFAs) cy17:0, cy19:0, a15:0 and 14:00(bacterial PLFAs) had positive loadings on PC 2. The fungal PLFAs (18:3ω6, 18:2ω6, 18:1ω9c, and 18:1ω9t) had positive correlation with nitrogen fertilization N3 level (Fig. 4A).

Similarly, PCA showed soil microbial PLFA contents under different P treatments had different degrees of variation (Fig. 4B). The first two principal components accounted for approximately 19.0% and 37.0% of the variation in PLFA composition in all treatments. The PLFAs 18:0, 14:0, 16:1ω9, i17:0, and 11:0, (bacterial PLFAs) had positive loadings on PC 1, while the PLFAs 18:3ω6, 18:2ω6, 18:1ω9t, 18:1ω9c (fungal PLFAs) and 16:0, a15:0, 17:0, 20:0, cy19:0(bacterial PLFAs) and 10Me16:0 (actinomycete PLFAs) had positive loadings on PC 2. The fungal PLFAs (18:3ω6, 18:2ω6, 18:1ω9c, and 18:1ω9t) had positive correlation with P4 (Fig. 4B), while bacterial PLFAs (a15:0, 16:0) had positive correlation with P3 (Fig. 4B).

S1 Table. Summary of ANOVAs (F&P values) for the effect of fertilization on plant biomass of *Acer mono* seedlings and Soil PLFAs.

Effect		Root Biomass		Shoot biomass		Total PLFAs		BacterialPLFAs		G^+ PLFAs	
	DF	F	P	F	P	F	P	F	P	F	P
Nitrogen	3	17.91	**0.0021**	15.20	**0.0033**	3.43	NS	4.01	NS	1.44	NS
Phosphorous	3	3.40	**0.0339**	3.46	**0.0321**	1.46	NS	0.47	NS	2.20	NS
N x P	9	4.04	**0.0030**	2.64	**0.0276**	0.22	NS	1.96	NS	0.79	NS

Abbreviations: G^+ PLFAs; gram positive PLFAs. Statistically significant probabilities ($p<0.05$) are indicated in bold. NS indicates non-significant P-values

S2 Table. Summary of ANOVAs (F&P values) for the effect of fertilization on Soil PLFAs.

Effect		G^- PLFAs		G^+: G^-		Actin PLFAs		Fungal PLFAs		F: B	
	DF	F	P	F	P	F	P	F	P	F	P
Nitrogen	3	0.67	NS	45.65	NS	16.33	NS	4.66	**0.0029**	11.51	**0.0017**
Phosphorous	3	0.88	NS	3.55	NS	5.16	NS	4.10	**0.0073**	6.46	**0.0014**
N x P	9	0.93	NS	2.13	NS	1.87	NS	0.26	NS	0.16	NS

Abbreviations: G^- PLFAs; gram negative PLFAs; G^+: G^-, ratio of gram negative and positive PLFAs; F: B, ratio of fungal and bacterial; Actin PLFAs, for Actinomycete PLFAs. Statistically significant probabilities ($p<0.05$) are indicated in bold. NS indicates non-significant P-values

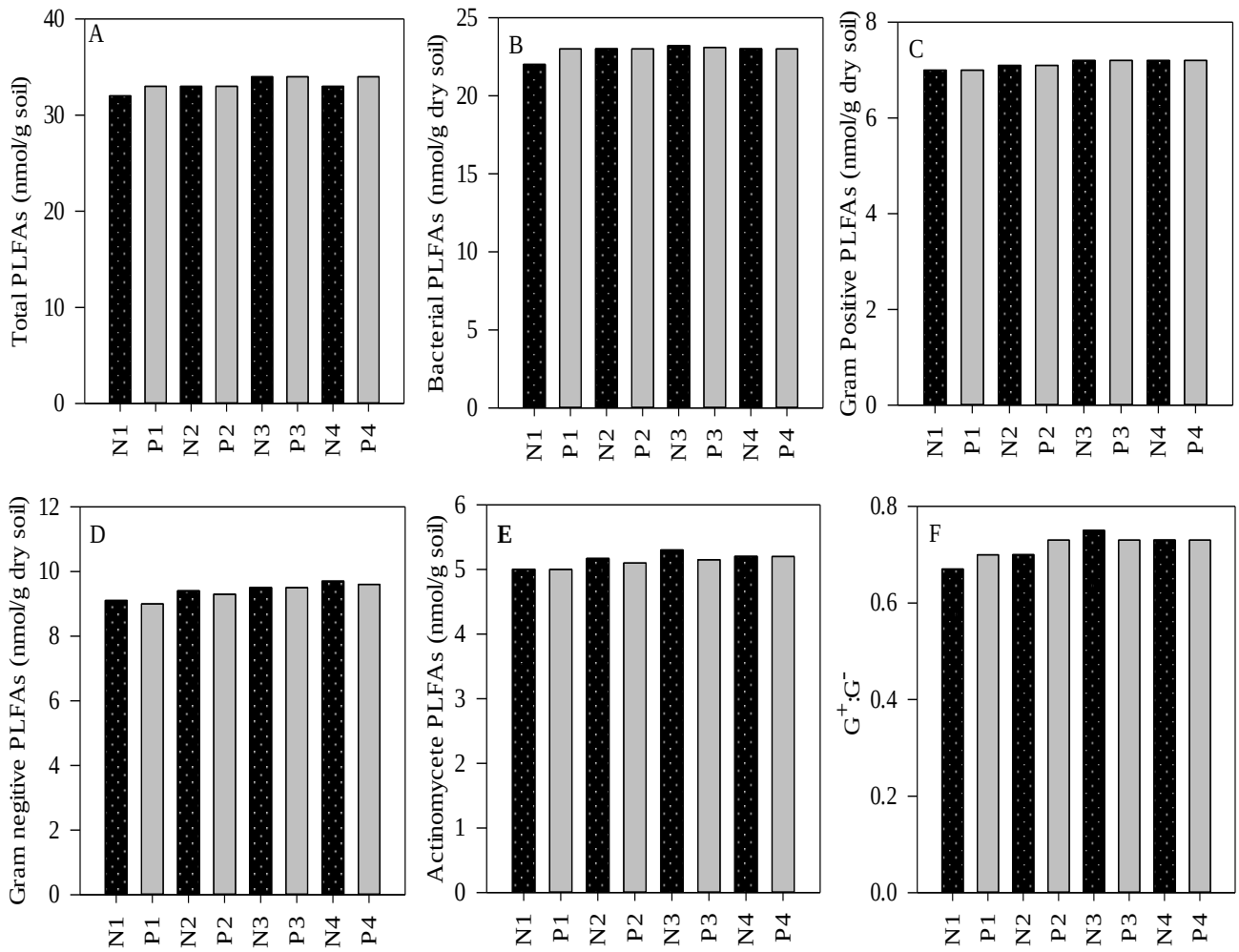

Fig. 2. Soil microbial community after nitrogen and phosphorus addition. N stand for Nitrogen levels (N1-N4); P stand for Phosphorous levels (P1-P4); G^+: G^-stand for Gram positive and Negative bacterial PLFAs ratio. All the data in fig are not significant. Error bars represent the standard error of the mean.

Discussion

N supply can result in rapid initial growth; during the initial stages of a plant growth and post stabilization, plants gain the ability to fix N from the atmosphere in large volumes. During the initial growth stage, there is less demand for N in a plant, but demand then increases multifold as growth continues (Williams & Haynes, 1995). In different environments, the N in soil is not sufficient for plants to grow, so fertilizers must be supplied to sustain optimal growth rate. In our study, among the different levels of N treatment, the highest above- and below-ground biomass values were recorded at the optimal level of N (Fig. 1A+C).

In general, the plants exposed to the highest nitrogen levels have been reported to develop toxicity that results in the hampering of regulatory plant root mechanisms necessary for the uptake of nutrients (Elmer & LaMondia, 1999). Wu *et al.,* (2008) obtained similar results, finding that nitrogen fertilization enhanced the adaptability of *Bauhinia faberi* seedling growth characteristics, with an increase in biomass production. Numerous studies reported that N compounds in low concentrations (Harrison *et al.,* 2008) can be taken up by a number of plant species (Hodge *et al.,* 2000) to increase below- and above-ground plant biomass (Näsholm *et al.,* 2009). Chaukiyal *et al.,* (2013) reported an adverse effect of nitrogen on *Dalbergiasissoo*. Aynehband *et al.,* (2012) also observed that high soil N concentration decreases and low N concentration increases biomass in *Triticuma estivum.*

We further predicted the influence of P fertilization on above- and below-ground plant biomass. The root system morphology may be impacted by phosphorus; general observations show phosphorus stimulates root growth (Williamson *et al.,* 2001, Razaq *et al.,* 2017) and also increases root length and root dry weight (Jin *et al.,* 2013). The increase in lateral root dry weight by adding P is due to longer lateral root length; a significantly positive relationship exists between root dry weight and root length (Costa *et al.,* 2000).

The results of our study confirm the findings of Dutt *et al.,* (2013) that P application has a significant positive influence on above- and below-ground dry biomass of apricot seedlings. Similarly, Kim & Li, (2016) found a dramatic increase in number of leaves and leaf surface area with P addition, subsequently leading to a disproportionate increase in shoot biomass compared to root biomass in *Lantana camara* (New Gold). However, the biomass samples were kept in an oven for short duration; there might be the possibility of water content in the tissue as samples have different water content.

According to Aynehband *et al.,* (2010), N fertilizers show a dual advantage in promoting soil mineral elements as well as growth and maximum yield in plants. Several authors stated that N additions can change the soil microbial community diversity and composition (Bates *et al.,* 2011). In the current study, the microbial community was further assessed using PLFA analysis to better understand the influence of N addition on the microbial community.

Our study results revealed that the total PLFAs and bacterial PLFAs did not change significantly (Fig. 2A). Li *et al,* (2015) conducted a study at a secondary tropical forest in China that found no influence on bacterial PLFAs when N was added. Similar results were reported by Balser, (2001) in three types of Hawaiian forest soil. In contrast to the current study results, Smolander *et al.,* (2005) reported a significant decrease in bacterial PLFAs after N fertilization.

He *et al.,* (2007) observed that N fertilization reduces the soil pH and hence decreases the soil microbial communities' abundance and/or diversity in Chinese upland red soil under long-term fertilization practices.However, McDowell *et al.,* (2004) reported that gram-negative and gram-positive PLFAs and the ratio of these PLFAs remains unchanged with N fertilization (Fig. 2B+E).Contrary to the findings of Balser, (2001), our study results showed a significant effect on fungal PLFAs from N application (Fig. 3A). Högberg *et al.,* (2007) observed that fungi are often the group of microorganisms affected most by fertilization. Tietema *et al.,* (1998) suggested that soil microbial community changes depended on soil nitrogen status, with more fungi than bacteria at N-limited sites.

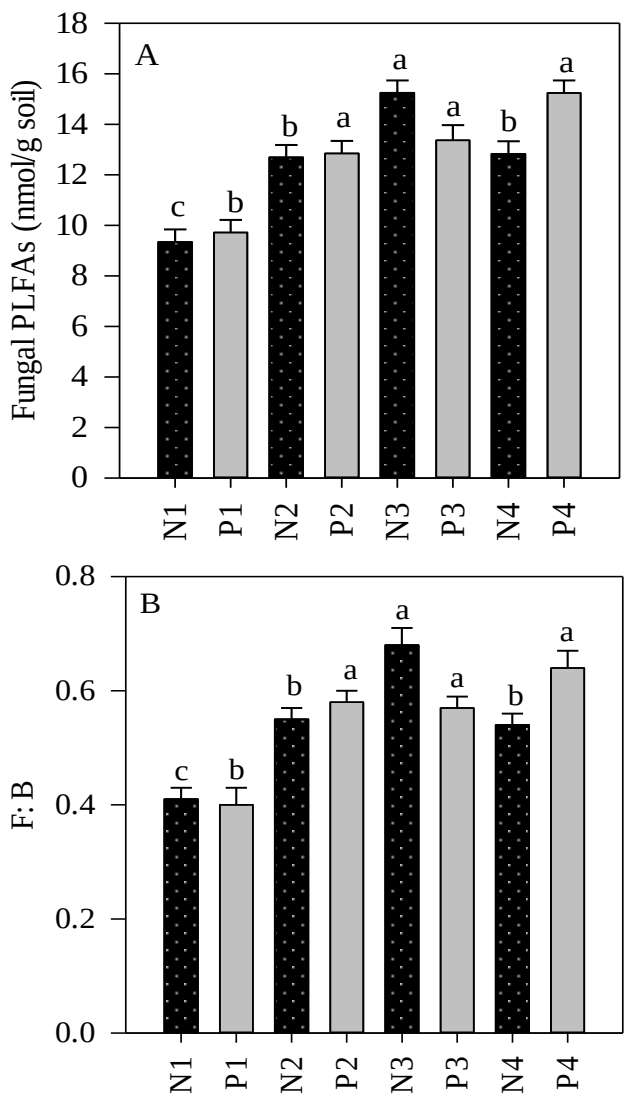

Fig. 3. Soil microbial community after nitrogen and phosphorus addition.N stand for Nitrogen levels (N1-N4); P stand for Phosphorous levels (P1-P4); F: Bstand for fungal and bacterial PLFAs ratio. Different letter show the level of significance. Error bars represent the standard error of the mean.

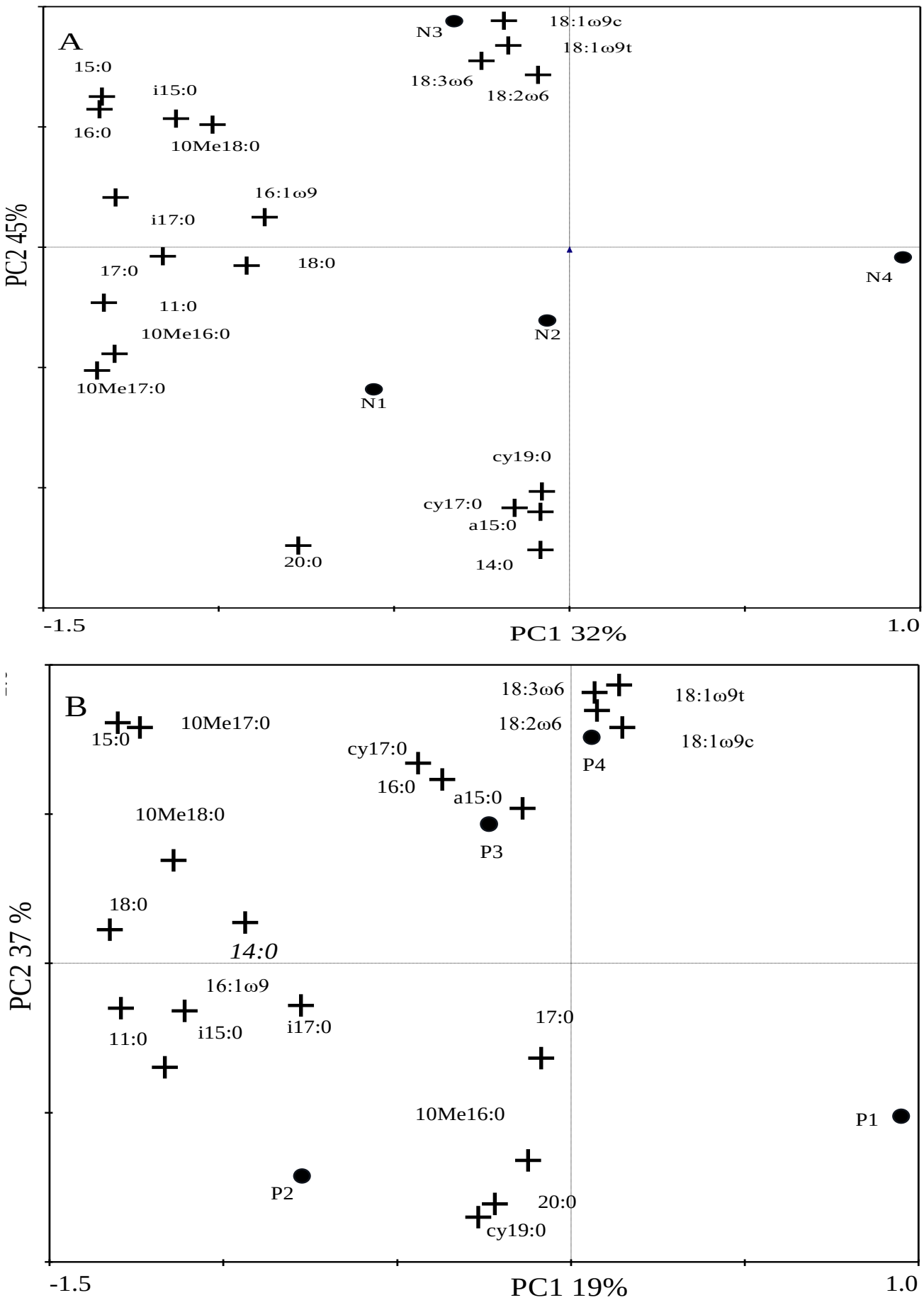

Fig. 4. Principal component analysis showed soil microbial PLFA contents under different levels of nitrogen and phosphorous treatment. (A) for nitrogen levels and (B) for phosphorous levels.

Fungal biomarkers have been reported to increase in N manipulation experiments in northern temperate forest soils (Gallo *et al.,* 2004).In contrast, several authors have reported that N fertilization reduced soil fungal PLFAs (Demoling *et al.,* 2008; Fraterrigo *et al.,* 2006). Nilsson *et al.,* (2007) noted that when natural N was deposited along with fertilizer applications of nitrogen, the total fungal biomass in the soil of oak forests did not seem to be affected. Furthermore, studies have also noted an improvement in the number of total PLFAs as well as in fungal PLFAs in rice paddies and vegetable fields, but no effect on total bacterial PLFAs (Yuan *et al.,* 2015). Because organic carbon is used as a carbon and energy source byheterotrophic soil microbes, microbial growth is stimulated by supplying N as a fertilizer (Ya-Juan *et al.,* 2012).

At the same time, plant growth is also promoted by providing root exudates as C sources for the growth of microbes (Lu *et al.,* 2002). F:B ratios were significantly higher in the N-addition plots, compared to the control plots (Fig. 3B). This is consistent with the results reported by Balser (2001), which showed that F:B increased with N fertilization in tropical forests. The PLFAs 18:3ω6, 18:2ω6, 18:1ω9c, and 18:1ω9t (fungal PLFAs) had positive loadings on PC 2; these PLFAs showed a positive correlation with nitrogen fertilization N3 level (Fig. 4A).

For instance, plants adapted to growing in fertile soils produce not only high-quality litters but also nourish the bacterial-based soil microbial community; this may not be the case with plants that grow in less-fertile soils (Eskelinen *et al.,* 2009). As shown in Fig. 2B, the current study results indicate there is no association between P application and microbial community; this aligns with the study results of Groffman& Fisk, (2011), who conducted their study in northern hardwood forests. Our results are also consistent with those of Hamel *et al.,* (2006).

Contrary to our results, Zhang *et al.,* (2007) found a significant relationship with G^+/G^- PLFAs under PK treatment (Fig. 2F). However, Wei-Dong *et al.,* (2008) conducted an experiment at the Hailun Agricultural and Ecological Station of the Chinese Academy of Sciences in Northeast China, and reported no significant difference between soil microbial biomass and microbial functional diversity/distribution when treated with different fertilizers.

Our study results about soil microbial biomass are in line with those of Liu *et al*., (2015). He *et al.,* (2008) found the application of fertilizers influenced fungi more than the soil bacterial community.Weand *et al.,* (2010) also found notable increases in fungal biomass with P application, thus confirming our results (Fig. 3A). When P was added on a long-term basis, soil respiration was found to increase significantly. From this, one understands that when P is sufficiently present, there is a high microbial activity that further enhances the decomposition of organic matter in soil (Liu *et al.,* 2013). P addition may increase mycorrhizal growth (Liu *et al.,* 2013). Mycorrhizal fungi scavenge nutrients from soil more efficiently than roots, and mycorrhizal fungi have lower nutrient limitations than plants (Treseder & Allen, 2002).

Another possible reason was that the addition of P increases the soil pH, which shows an association with high below-ground biomass (Rousk *et al.,* 2010).Several studies also reported that P addition had a significant positive influence on fungal growth in P-poor forests (Tornberg *et al.,* 2003; Nilsson & Wallander, 2003). However, Fageria& Moreira, (2011) found that P addition had no effect on microbial biomass and activity in a northern hardwood forest. In this study, the principal component analysis (Fig. 4B) indicated that the P treatment increased fungal PLFAs (18:3ω6, 18:2ω6, 18:1ω9c, and 18:1ω9t) and had a positive correlation with high level P4.

In the current study, N and P were added in parallel and didn't produce interactive effects in the soil microbial community in a temperate forest nursery. Likewise, Liu *et al.,* (2013) did not note any interactive effects of NP on the soil microbial community. A study conducted by Fanin *et al.,* (2015) in Amazon rain forests found no evidence of NP interactions in the soil microbial community. However, when compared to the literature, our experiment was for a shorter duration than other studies.

In conclusion, we found that N and P alone significantly increase plant root shoot biomass and soil fungal PLFAs and the ratio of fungal to bacterial PLFAs. However, they did not change or negatively affect total PLFAs, bacterial PLFAs, gram-positive PLFA, gram-negative bacteria PLFAs or the ratio of G^+/G^-. Similarly, N and P interaction did not exhibit any effect on soil microbial biomass, but had a significant impact on plant biomass. It appears that an optimal level of NP application in a temperate forest nursery is beneficial for and improves root shoot biomass, buthas no notable negative effect on the soil microbial community. However, further study is warranted for better understanding the long-term effects of continuous fertilization in forest nurseries faced with a rising future need for healthy seedling production.

Acknowledgments

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References

Aynehband, A., F. Gerami and E. Fateh. 2012. Effect of different green manure crops and nitrogen levelson biomass production efficiency and nitrogen concentration in wheat (*Triticum aestivum* L.) and soil. *Adva. in Environ. Biol.,* 6(1): 362-367.

Aynehband, A., M. Tehrani, and D.A. Nabati. 2010. Effects of residue management and N-splitting methods on yield and biological and chemical characters of canola ecosystem. *J. Food Agri. & Environ.,* 8(2): 317-324.

Balser, T.C. 2001. The impact of long-term nitrogen addition on microbial community composition in three Hawaiian forest soils. *The Scient. World J.,* 1(1): 500-504.

Bates, S.T., D. Berg-Lyons, J.G. Caporaso, W.A. Walters, R. Knight and N. Fierer. 2011. Examining the global distribution of dominant archaeal populations in soil. *The ISME J.*, 5(5): 908-917.

Burton, J., C. Chen, Z. Xu and H. Ghadiri. 2010. Soil microbial biomass, activity and community composition in adjacent native and plantation forests of subtropical Australia. *J. Soils & Sediments*, 10(2): 1267-1277.

Buyer, J.S., J.R. Teasdale, D.P. Roberts, I.A. Zasada and J.E. Maul. 2010. Factors affecting soil microbial community structure in tomato cropping systems. *Soil Biol. & Biochem.*, 42(1): 831-841.

Chaukiyal, S.P., R.A. Mir and T.C. Pokhriyal. 2013. Effect of nitrogen fertilizer on biomass production and nodulation behavior of Pongamia pinnata Pierre seedlings under nursery conditions. *J. Fores. Res.*, 24(1): 531-538.

Chen, F.S., T.J. Fahey, M.Y. Yu and L. Gan. 2010. Key nitrogen cycling processes in pine plantations along a short urbanâ rural gradient in Nanchang, China. *Forest Ecol. & Manag.*, 259(1): 477-486.

Chen, G., Z. He and Y. Wang. 2003. Impact of pH on microbial biomass carbon and microbial biomass phosphorus in red soils. *Pedosphere.*, 14(1): 9-15.

Costa, C., L.M. Dwyer, R.I. Hamilton, C. Hamel, L. Nantais and D.L. Smith. 2000. A sampling method for measurement of large root systems with scanner-based image analysis. *Agron. J.*, 92(10): 621-627.

Cusack, D.F. 2013. Soil nitrogen levels are linked to decomposition enzyme activities along an urban-remote tropical forest gradient. *Soil Biol. Biochem.*, 57(14): 192-203.

Degens, B.P., L.A. Schipper, G.P. Sparling and M. Vojvodic-Vukovic. 2000. Decreases in organic C reserves in soils can reduce the catabolic diversity of soil microbial communities. *Soil Biol. Biochem.*, 32(5): 189-196.

Demoling, F., L.O. Nilsson and E. Bayayth. 2008. Bacterial and fungal response to nitrogen fertilization in three coniferous forest soils. *Soil Biol. Biochem.*, 40(2): 370-379.

Dutt, S., S.D. Sharma and P. Kumar. 2013. Inoculation of apricot seedlings with indigenous arbuscular mycorrhizal fungi in optimum phosphorus fertilization for quality growth attributes. *J. Plant Nutr.*, 36(1): 15-31.

Elmer, W.H. and J.A. Lamondia. 1999. Influence of ammonium sulfate and rotation crops on strawberry black root rot. *Plant Disease.*, 83(1): 119-123.

Elser, J.J., M.E.S. Bracken, E.E. Cleland, D.S. Gruner, E. W. Seabloom, J.B., Shurin and J. E. Smith. 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecol. Lett.*, 10(5): 1135-1142.

Eo, J. and K.C. Park. 2016. Long-term effects of imbalanced fertilization on the composition and diversity of soil bacterial community. *Agri. Ecol. Environ.*, 231(1): 176-182.

Esberg, C., B. Du Toit, R. Olsson, U. Ilstedt and R. Giesler. 2010. Microbial responses to P addition in six South African forest soils. *Plant & Soil.*, 329(1): 209-225.

Eskelinen, A., S. Stark and M. Mannista. 2009. Links between plant community composition, soil organic matter quality and microbial communities in contrasting tundra habitats. *Oecologia*, 161(1): 113-123.

Fageria, N.K. and A. Moreira. 2011. 4 The role of mineral nutrition on root growth of crop plants. *Adv.Agron.*, 110(1): 251-331.

Fanin, N., S. Hattenschwiler, H. Schimann and N. Frominm. 2015. Interactive effects of C, N and P fertilization on soil microbial community structure and function in an Amazonian rain forest. *Fun. Ecol.*, 29(5): 140-150.

Feng, W., X. Zou and D. Schaefer. 2009. Above-and belowground carbon inputs affect seasonal variations of soil microbial biomass in a subtropical monsoon forest of southwest China. *Soil Biol. Biochem.*, 41(1): 978-983.

Fraterrigo, J.M., T.C. Balser and M.G. Turner. 2006. Microbial community variation and its relationship with nitrogen mineralization in historically altered forests. *Ecol.*, 87(1): 570-579.

Frostegayrd A.S., A. Tunlid and E. Bayath. 1993. Phospholipid fatty acid composition, biomass, and activity of microbial communities from two soil types experimentally exposed to different heavy metals. *App. & Environ. Microbiol*., 59: e36053617.

Gallo, M., R. Amonette, C. Lauber, R.L. Sinsabaugh and D.R. Zak. 2004. Microbial community structure and oxidative enzyme activity in nitrogen-amended north temperate forest soils. *Micro. Ecol.*, 48(2): 218-229.

Ge, T.D., S.A. Nie, H.L. Qin, H.A. Xiao and C.L. Tong. 2012. Microcalorimetric studies for microbial activity of three vegetable soils. *J. Food Agri. Environ.*, 10(1): 930-932.

Geisseler, D. and K.M. Scow. 2014. Long-term effects of mineral fertilizers on soil microorganismsâ A review. *Soil Biol. Biochem.*, 75(1): 54-63.

Gong, Z., X. Zhang, J. Chen and G. Zhang. 2003. Origin and development of soil science in ancient China. *Geoderma.*, 115(12): 3-13.

Groffman, P.M. and M.C. Fisk. 2011. Phosphate additions have no effect on microbial biomass and activity in a northern hardwood forest. *Soil Biol. Biochem.*, 43(1): 2441-2449.

Hamel, C., K. Hanson, F. Selles, A. F.Cruz, R. Lemke, B. Mcconkey and R. Zentner. 2006. Seasonal and long-term resource-related variations in soil microbial communities in wheat-based rotations of the Canadian prairie. *Soil Bio. Biochem.*, 38(1): 2104-2116.

Harrison, K.A., R. Bol and R.D. Bardgett. 2008. Do plant species with different growth strategies vary in their ability to compete with soil microbes for chemical forms of nitrogen? *Soil Biol. Biochem.*, 40(1): 228-237.

He, J. Z., J.P. Shen, L.M. Zhang, Y.G. Zhu, Y.M. Zheng, M.G. Xu and H. Di. 2007. Quantitative analyses of the abundance and composition of ammoniaoxidizing bacteria and ammonia • oxidizing archaea of a Chinese upland red soil under longaterm fertilization practices. *Environ. Microbiol.*, 9(1): 2364-2374.

He, J.Z., Y. Zheng, C.R. Chen, Y.Q. He and L.M. Zhang. 2008. Microbial composition and diversity of an upland red soil under long-term fertilization treatments as revealed by culture-dependent and culture-independent approaches. *J. Soils Sedim.*, 8(1): 349-358.

Hodge, A., D. Robinson and A. Fitter. 2000. Are microorganisms more effective than plants at competing for nitrogen? *Trends Plant Sci.*, 5(1): 304-308.

Hogberg, M.N., P. Hogberg. and D.D. Myrold. 2007. Is microbial community composition in boreal forest soils determined by pH, C-to-N ratio, the trees, or all three? *Oecologia.*, 150(1): 590-601.

Huang, X., S. Liu, H. Wang, Z. Hu, Z. Li and Y. You. 2014. Changes of soil microbial biomass carbon and community composition through mixing nitrogen-fixing species with *Eucalyptus urophylla* in subtropical China. *Soil Biol. Biochem.*, 73(3): 42-48.

Ji, L., Salahuddin, J. Zhang, L. You, C. He and L. Yang. 2016. Larch (*Larix gmelinii*) bulk soil phenolic acids promote manchurian walnut (juglans manshurica) growth and soilmicroorganism biomass. *Pak. J. Bot.*, 48(4): 2549-2556.

Jin, J., X. Liu, G. Wang, X. Chen, Z. Yu and S.J. Herbert. 2013. Effect of phosphorus application on hierarchical lateral root morphology and phosphorus acquisition in soybean. *J. Plant Nutr.*, 36(3): 1578-1589.

Kamaa, M., H. Mburu, E. Blanchart, L. Chibole, J.L.Chotte C. Kibunja and D. Lesueur. 2011. Effects of organic and inorganic fertilization on soil bacterial and fungal microbial diversity in the Kabete long-term trial, Kenya. *Bio. Fertil. Soils.*, 47(1): 315-321.

Kim, H.J. and X. Li. 2016. Effects of phosphorus on shoot and root growth, partitioning, and phosphorus utilization efficiency in lantana. *Hort. Sci.*, 51(2): 1001-1009.

Li, J., Z. Li, F. Wang, B. Zou, Y. Chen, J. Zhao, Q. Mo, Y. Li, X. Li and H. Xia. 2015. Effects of nitrogen and phosphorus addition on soil microbial community in a secondary tropical forest of China. *Biol. Fertil. Soils*, 51(1): 207-215.

Liu, L., P. Gundersen, W. Zhang, T. Zhang, H. Chen and J. Mo. 2015. Effects of nitrogen and phosphorus additions on soil microbial biomass and community structure in two reforested tropical forests. *Sci. Reports*, 5.

Liu, L., T. Zhang, F.S. Gilliam, P. Gundersen, W. Zhang, H. Chen and J. Mo. 2013. Interactive effects of nitrogen and phosphorus on soil microbial communities in a tropical forest. *PLoS One.*, 8: e61188.

Lu, Y., A. Watanabe and M. Kimura. 2002. Contribution of plant-derived carbon to soil microbial biomass dynamics in a paddy rice microcosm. *Biol. Fertil. Soils*, 36(112): 136-142.

Mandal, A., A.K. Patra, D. Singh, A. Swarup and R.E. Masto. 2007. Effect of long-term application of manure and fertilizer on biological and biochemical activities in soil during crop development stages. *Biores.Tech.*, 98(1): 3585-3592.

Mcdowell, W.H., A.H. Magill, J.A. Aitkenhead-Peterson, J.D. Aber, J.L. Merriam and S.S. Kaushal. 2004. Effects of chronic nitrogen amendment on dissolved organic matter and inorganic nitrogen in soil solution. *Forest Ecol. Manag.*, 196(1): 29-41.

Moore-Kucera, J. and R.P. Dick. 2008. PLFA profiling of microbial community structure and seasonal shifts in soils of a Douglas-fir chronosequence. *Micro. Ecol.*, 55(1): 500-511.

Nasholm, T., K. Kielland and U. Ganeteg. 2009. Uptake of organic nitrogen by plants. *New Phytol.*, 182(1): 31-48.

Nilsson, L.O. and H.K. Wallander. 2003. Production of external mycelium by ectomycorrhizal fungi in a Norway spruce forest was reduced in response to nitrogen fertilization. *New Phytol.*, 158(1): 409-416.

Nilsson, L.O., E. Bayayth, U. Falkengren-Grerup and H.K. Wallander. 2007. Growth of ectomycorrhizal mycelia and composition of soil microbial communities in oak forest soils along a nitrogen deposition gradient. *Oecologia*, 153(1): 375-384.

Razaq, M., P. Zhang, H.L. Shen and Salahuddin. 2017. Influence of nitrogen and phosphorous on the growth and root morphology of Acer mono. *PLoS ONE*, 12(2): e0171321. doi:10.1371/*journal. pone.*0171321

Rousk, J., P.C. Brookes and E. Bayayth. 2010. The microbial PLFA composition as affected by pH in an arable soil. *Soil Biol. Biochem.*, 42(1): 516-520.

Smolander, A., J. Loponen, K. Suominen and V. Kitunen. 2005. Organic matter characteristics and C and N transformations in the humus layer under two tree species, Betula pendula and Picea abies. *Soil Biol. Biochem.*, 37(1): 1309-1318.

Sparling, G.P. 1997. Soil microbial biomass, activity and nutrient cycling as indicators of soil health. *Biol. Ind. Soil Health*, 6(1): 97-119.

Stewart, W.M., D.W. Dibb, A.E. Johnston and T.J. Smyth. 2005. The contribution of commercial fertilizer nutrients to food production. *Agron. J.*, 97(1): 1-6.

Tian, G., J.M. Vose, D.C. Coleman, C.D. Geron and J.T. Walker. 2004. Evaluation of the effectiveness of riparian zone restoration in the southern Appalachians by assessing soil microbial populations. *App. Soil Ecol.*, 26(1): 63-68.

Tietema, A., B.A. Emmett, P. Gundersen, K.O.J. Naas and C.J. Koopmans. 1998. The fate of 15 N-labelled nitrogen deposition in coniferous forest ecosystems. *Forest Ecol. Manag.*, 101(11): 19-27.

Tornberg, K., E. Bayayth and S. Olsson. 2003. Fungal growth and effects of different wood decomposing fungi on the indigenous bacterial community of polluted and unpolluted soils. *Biol. Fertil. Soils*, 37(1): 190-197.

Treseder, K.K. and M.F. Allen. 2002. Direct nitrogen and phosphorus limitation of arbuscular mycorrhizal fungi: a model and field test. *New Phytol.*, 155(1): 507-515.

Tu, L.H., T.X. Hu, J. Zhang, X.W. Li, H.L. Hu, L. Liu and Y.L. Xiao. 2013. Nitrogen addition stimulates different components of soil respiration in a subtropical bamboo ecosystem. *Soil Biol. Biochem.*, 58(1): 255-264.

Velmourougane, K., M.V. Venugopalan, T. Bhattacharyya, D. Sarkar, D.K. Pal, A. Sahu, P. Chandran, S.K. Ray, C. Mandal and K.M. Nair. 2014. Microbial biomass carbon status in agro-ecological sub regions of black soils in India. *Biol. Sci.*, 84(1):519-529.

Vesterdal, L. and K.R. Rasmussen. 2002. Availability of nitrogen and phosphorus in Norway spruce forest floors fertilized with nitrogen and other essential nutrients. *Soil Biol. Biochem.*, 34(1): 1243-1251.

Weand, M.P., M.A. Arthur, G.M. Lovett, R.L. Mcculley and K.C. Weathers. 2010. Effects of tree species and N additions on forest floor microbial communities and extracellular enzyme activities. *Soil Biol. Biochem.*, 42(1): 2161-2173.

Wei-Dong, K., Z.H.U. Yong-Guan, F.U. Bo-Jie, H.A.N. Xiao-Zeng, L. Zhang and H.E.J. Zheng. 2008. Effect of long-term application of chemical fertilizers on microbial biomass and functional diversity of a black soil. *Pedosphere.*, 18(1): 801-808.

Williams, P.H. and R.J. Haynes. 1995. Nitrogen in plant environment. *Nitrogen Nutrition in Higher Plants. New Delhi: Asso. Publishing Company.*, 1-20.

Williamson, L.C., S.P.C.P. Ribrioux, A.H. Fitter and H.M.O. Leyser. 2001. Phosphate availability regulates root system architecture in Arabidopsis. *Plant Physiol.*, 126(1): 875-882.

Wu, F., W. Bao, F. Li and N. Wu. 2008. Effects of drought stress and N supply on the growth, biomass partitioning and water-use efficiency of Sophora davidii seedlings. *Environ. Exp. Bot.*, 63(1): 248-255.

Ya-Juan, L.I., C. Xing, I.H. Shamsi, F. Ping and Y.L. Xian. 2012. Effects of irrigation patterns and nitrogen fertilization on rice yield and microbial community structure in paddy soil. *Pedosphere*, 22(1): 661-672.

Yuan, Y., X. Dai, M. Xu, H. Wang, X. Fu and F. Yang. 2015. Responses of microbial community structure to land-use conversion and fertilization in southern China. *Europ. J. Soil Biol.*, 70(1): 1-6.

Zechmeister-Boltenstern, S., K. Michel and M. Pfeffer. 2011. Soil microbial community structure in European forests in relation to forest type and atmospheric nitrogen deposition. *Plant & Soil.*, 343(1): 37-50.

Zhang, Q.C., G. H. Wang and H.Y. Yao. 2007. Phospholipid fatty acid patterns of microbial communities in paddy soil under different fertilizer treatments. *J. Environ. Sci.*, 19(1): 55-59.

Zhang, X., W. Liu, M. Schloter, G. Zhang, Q. Chen, J.L. Huang, E.J.J. Li and X. Han. 2013. Response of the abundance of key soil microbial nitrogen-cycling genes to multi-factorial global changes. *PLoS One*, 8: e76500.

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