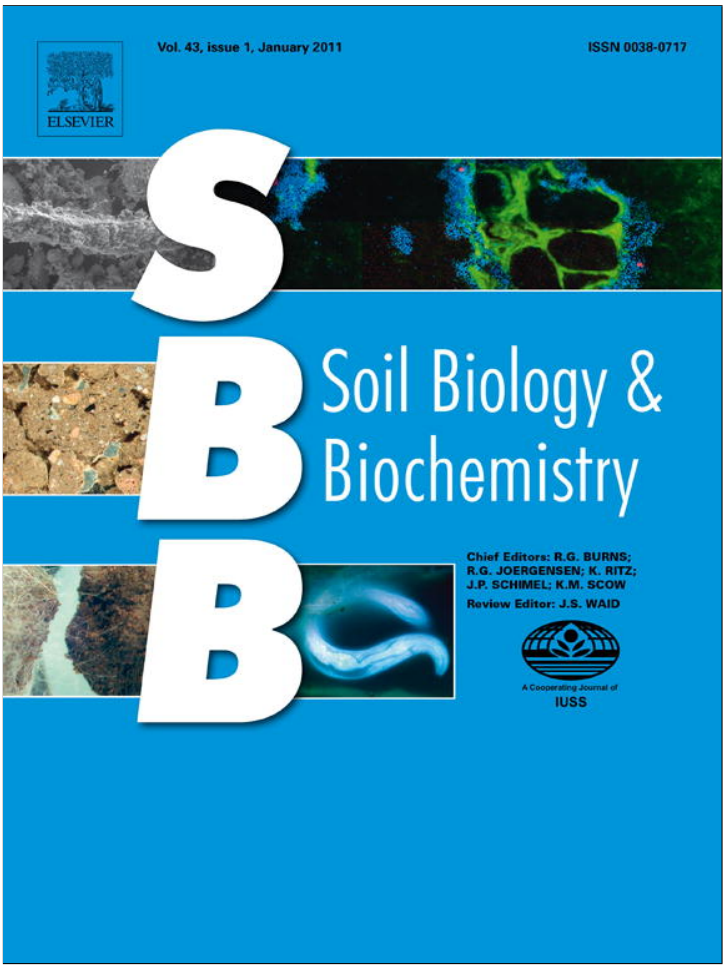

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Effects of phosphorus addition on soil microbial biomass and community composition in three forest types in tropical China

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ABSTRACT

Elevated nitrogen (N) deposition in humid tropical regions may aggravate phosphorus (P) deficiency in forest on old weathered soil found in these regions. From January 2007 to August 2009, we studied the responses of soil microbial biomass and community composition to P addition (in two monthly portions at level of 15 g P m^{-2} yr^{-1}) in three tropical forests in southern China. The forests were an old-growth forest and two disturbed forests (mixed species and pine dominated). The objective was to test the hypothesis that P addition would increase microbial biomass and change the composition of the microbial community, and that the old-growth forests would be more sensitive to P addition due to its higher soil N availability. Microbial biomass C (MBC) was estimated twice a year and the microbial community structure was quantified by phospholipid fatty acid (PLFA) analysis at the end of the experiment. Addition of P significantly increased the microbial biomass and altered the microbial community composition in the old-growth forest, suggesting that P availability is one of the limiting factors for microbial growth. This was also reflected by significant increases in soil respiration after P addition. In contrast, P addition had no effect on the microbial biomass and the microbial community composition in the pine forests. Also in the mixed forest, the microbial biomass did not significantly respond to P addition, but soil respiration and the ratio of fungal-to-bacteria was significantly increased.

1. Introduction

Nutrient limitation to primary production and other ecological processes is widespread in terrestrial ecosystems, and nitrogen (N) and phosphorus (P) are the most common limiting elements. It is generally believed that biological processes in many ecosystems on young soils may be limited by low supplies of N, whereas ecosystems with very old soils can become depleted in P (Walker and Syers, 1976). This P limitation occurs because P is derived primarily from rock weathering, which means that ecosystems begin their existence with a fixed complement of P (a certain amount of P) from which even very small losses can not readily be replenished (Walker and Syers, 1976; Vitousek et al., 2010). There is a substantial synergistic effect of joint N and P enrichment, most likely because single enrichment leads to limitation by the alternative nutrient, suggesting that N and P supplies are relatively closely balanced in most natural environments (Elser et al., 2007). However, most fertilization experimen7ts conducted in tropical forests, have resulted in a stronger response to added P than added N (Elser et al., 2007), suggesting support for the long-held belief that tropical ecosystems on old soils are predominantly P limited (Walker and Syers, 1976).

Soil microorganisms are essential for nutrient cycling in terrestrial ecosystems and through retention and release they regulate the supply of plant-available nutrients from the soil (Jenkinson, 1987). The biomass and activity of microbes is typically thought to be constrained by the availability and quality of carbon (Wardle, 1992; Demoling et al., 2007). However, microbial utilization of carbon might be limited by nutrients. Addition of N to temperate forests, which are often N-limited under natural conditions, did not result in consistent effects upon microbial communities; in some cases, N appears to increase the microbial biomass (Johnson et al., 1998), but in many others its addition had a negative effect, or sometimes no effect (Arnebrant et al., 1996; Deforest et al., 2004; Wallenstein et al., 2006; Treseder, 2008). P limitation to soil microbial biomass has been demonstrated in the mineral soil of at least two temperate forests (Scheu, 1990; Gallardo and Schlesinger, 1994). Gallardo and Schlesinger (1994) suggested that microbial P

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limitation may be common in highly weathered soils in which P tends to be bound in iron or aluminium sesquioxides. Phosphorus is considered to be one of the main limitations for plant production as well as human wealth in tropical areas (Sanchez, 2002).

The few studies examining nutrient limitation of microbes in highly weathered humid tropical soils all have concluded P to be the most limiting (Gnankambary et al., 2008). Cleveland et al. (2002) found the microbial activity to be limited by P availability in tropical rain forests. Ilstedt and Singh (2005) observed that N limited the maximum level of microbial respiration in an acrisol if ample carbon was available, but the initial respiration rate was limited by P in a tropical acrisol soil from Sipitang on the west coast of Sabah, Malaysia. Recently, Ehlers et al. (2010) also observed P limitation of the microbial growth after a combined C and N addition in a native soil from western Kenya. However, to our knowledge, there has been no report to date on the effects of P addition on soil microbial community structure in tropical or subtropical areas.

In Asia, the use and emission of reactive N has increased from 14 Tg N yr^{-1} in 1961 to 68 Tg N yr^{-1} in 2000 and is expected to reach 105 Tg N yr^{-1} in 2030 (Zheng et al., 2002). Currently this leads to high atmospheric N deposition ($NH_4^+ + NO_3^-$) in southern China with inputs of 30–70 kg N ha^{-1} yr^{-1} to forests in some areas (Ma, 1989; Ren et al., 2000; Xu et al., 2001; Chen and Mulder, 2007). In the tropical forests of the Dinghushan Biosphere Reserve (DHSBR) in Southern China, N deposition amounted to more than 30 kg N ha^{-1} yr^{-1} for two decades (Huang et al., 1994; Zhou and Yan, 2001; Fang et al., 2008). Such high rates of N deposition may result in P limitation in forest ecosystems.

Most of the land originally covered with primary forests in China has been degraded by human activities during the past several hundred years (Wang et al., 1982; Li, 2004). In extreme cases, the land became completely non-vegetated (He and Yu, 1984). Currently, only 2% of the nation's total forest resources remains intact (Liu, 2006). Attempts to reverse this process of land degradation have been initiated in the tropical/subtropical region of China. Over the last few decades, large areas have been reforested using the native species (*Pinus massoniana* Lamb), to prevent further degradation of the landscape (Brown et al., 1995; Mo et al., 1995). Cutting of the trees is prohibited in these forests, but harvesting of understory and litter is still allowed to satisfy human fuel needs. These reforested stands are here and in former papers referred to as *pine forests* (having experienced understory vegetation and litter removal) and *mixed forests* (reforested without such removal) (Mo et al., 2003). These secondary forests now cover more than half of the total forested area in subtropical and tropical China (Brown et al., 1995; SFA, 2007). However, the effects of these significant land-use changes on the ecosystems (including soil microbial community and plant diversity) are poorly known (Mo et al., 2006; Lu et al., 2010).

The objective of this study was to experimentally examine the effects of P addition on soil microbes in these three tropical forests of the DHSBR in southern China. Earlier studies in the DHSBR showed that no N retention occurred, but rather a net loss of 8–16 kg N ha^{-1} yr^{-1} from the soil was estimated in the old-growth forest. In total up to 60 kg N ha^{-1} yr^{-1} was leached from the old-growth forest, indicating that this forest was completely N saturated (Fang et al., 2008) from the chronic elevated N deposition in southern China. This interpretation is also supported by the results on litterfall production, which revealed no significant effects of N additions on total litterfall production in the old-growth forest (Mo et al., 2008). Previous results from the old-growth forest showed that experimental N addition significantly decreased litter decomposition, soil respiration and soil microbial biomass (Mo et al., 2006, 2008; Fang et al., 2007; Wang et al., 2008). In contrast, N addition in the two more disturbed forests had positive effects on litter decomposition rates, and no significant effect on soil respiration and soil microbial biomass, suggesting that these two forests may be N limited (Mo et al., 2006, 2008; Wang et al., 2008). As mentioned above, N addition significantly decreases soil microbial biomass in the old-growth forest and P could be one of the main nutrient limitations for microbes in highly weathered humid tropical soils. Thus, we expect that P addition would increase microbial biomass and change the composition of the microbial community, and that the old-growth forests would be more sensitive to P addition due to its higher N availability.

2. Materials and methods

2.1. Site description

This study was conducted in the Dinghushan Biosphere Reserve (DHSBR) in the central part of the Guangdong Province, southern China (112°10′ E, 23°10′ N) (Mo et al., 2003). The reserve occupies an area of approximately 1200 ha and includes three major forest types within 3–4 km distance of each other: an old-growth monsoon evergreen broadleaf forest (named old-growth forest hereafter); a mixed pine and broadleaf forest (mixed forest) and a pine forest. The old-growth forest, at about 250–300 m above sea level, occupies 20% of the reserve. This forest is a typical forest type in tropical China and at this location it has been protected by monks for more than 400 years suggesting that direct human impacts have been minimal (Mo et al., 2003). The major species in the old-growth forest are *Castanopsis chinensis*, *Schima superba*, *Cryptocarya chinensis* (Hance) Hemsl., *Cryptocarya concinna*, *Machilus chinensis* (Champ. Ex Benth.) Hemsl., *Syzygium rehderianum* Merr. & Perry in the tree layer (Table 1) and *Calamus rhabdicladus* Burret, *Ardisia quinquegona* Bl. and *Hemigramma decurrens* (Hook.) Copel in the understory layer. Both the mixed and the pine forests originate from 1930s clear-cuts and subsequent pine plantation establishment. They were under continuous human disturbance (generally the harvesting of understory and litter) from 1930 to 1956 (mixed forest) and 1998 (pine forest). In the mixed forest, after cease of the disturbance, colonization from the natural dispersal of regional broadleaf species altered its plant community. Dominant tree species in the mixed forest are *Pinus (P) massoniana*, *S. superba*

Table 1
Indices of the tree structure in three tropical forest types. The survey was conducted in February 2007 (before the start of P fertilization).

Species	Stem density (tree hm^{-2})	Mean height (m)	Mean of diameter breast height (cm)	Basal area (m^2 hm^{-2})	Percentage of basal area to total (%)
Old-growth forest					
Castanopsis chinensis	268	9.8	26.0	18.7	49.0
Machilus chinensis	131	9.0	14.8	4.0	10.6
Schima superba	185	9.9	18.3	6.4	16.7
Cryptocarya chinensis	270	8.3	14.3	4.4	11.5
Syzygium rehderianum	185	8.5	12.9	1.2	3.1
Other plants	1587	4.3	4.4	3.5	9.1
Total	2625			38.2	100
Mixed forest					
Pinus massoniana	240	9.1	20.5	9.9	38.9
S. superba	1600	3.9	4.2	4.2	16.5
Other plants	1307	4.3	7.6	11.3	44.6
Total	3147			25.4	100
Pine forest					
P. massoniana	560	7.0	19.3	22.0	88.7
Other plants	1707	3.5	3.3	2.8	11.3
Total	2267			24.8	100

Chardn. & Champ., *C. chinensis* Hance, *Craibiodendron kwangtungense* S. Y. Hu, *Lindera metcalfiana* Allen, and *C. concinna* Hance. In the pine forest, *P. massoniana* remained the dominant tree (Table 1). Thus, these forests vary both in level of human impacts as well as stages of succession, site conditions, and species assemblages (Mo et al., 2003). The mean annual litter biomass production was 8.3, 8.5 and 3.3 Mg ha^{-1} yr^{-1}, in the old-growth, mixed and pine forests respectively. Stem density, tree height and mean diameter at breast height in the three forests are given in Table 1.

The reserve has a typical monsoon and humid climate pattern (*sensu* Holdridge, 1967). The average annual precipitation of 1927 mm has a distinct seasonal pattern, where 75% of it falls from March to August (wet–warm season) and only 6% of it falls from December to February (dry–cool season) (Huang and Fan, 1982). The mean annual temperature is 21 °C, while the coldest monthly mean temperature is 12.6 °C in January and warmest is 28.0 °C in July. Annual mean relative moisture is 80%. During the study period, daily precipitation and temperature followed this long-term seasonal pattern. The N deposition measured as inorganic N in throughfall was 34, 24, and 26 kg N ha^{-1} yr^{-1} in 2004 and 2005 for the old-growth, mixed and pine forest, respectively, with an additional input as dissolved organic N at 15–20 kg N ha^{-1} yr^{-1} (Fang et al., 2008).

The soil in the reserve is lateritic red earth formed from sandstone (Mo et al., 2003). The soil depth varies with forest types. In the old-growth forest the depth is more than 60 cm to the top of the C horizon, 30–60 cm in mixed forest and less than 30 cm in the pine forest. The forest stands used in the experiment are situated on mountain slopes ranging from 15° to 35°. The old-growth forest showed significantly higher concentrations of soil organic matter (SOC), N and P, but lower pH, C/N ratio and bulk density than the pine and mixed forests. Soil properties are listed in Table 2.

2.2. Experimental treatments

Two treatments were established (each in five replicates) in the three forests: Control and P addition (15 g m^{-2} year^{-1}) since 2007. The plots of 5 m × 5 m were established (25 m^2 in each forest type each surrounded by a 5 m wide buffer strip). Control plots and treatments were laid out randomly. Solutions of NaH_2PO_4 were sprayed in two monthly portions below the canopy since Feb. 2007 and continued through October 2009. The fertilizer was mixed with 5 L of water and sprayed by a hand worked backpack sprayer. Each control plot received 5 L water with no fertilizer.

2.3. Field sampling and measurements

Soil sampling was conducted twice a year from 2007 to 2009 during February and August, representing the dry-cool season and wet-warm season, respectively. These seasonal trends (wet season and dry season) have been shown in many studies conducted in this region including studies on soil microbes (Yi et al., 2005; Wang et al., 2006). For examples, Yi et al. (2005) found that soil microbial biomass was lower in winter (January) than in summer (July). The sampling in Feb. 2007 was done prior to the first fertilizer application and thus represents pre-treatment conditions for all plots. From each plot, 5 soil cores (2.5 cm inner diameter) were collected randomly from 0 to 10 cm soil depths and combined to one composite sample. The litter layer was carefully removed before sampling. After removing stones and coarse roots, the sample was sieved to 2 mm mesh size and divided into two parts. One part of the composite sample was retained for the estimation of soil chemical parameters and the other fresh part was used for the analysis of microbial biomass and community structure.

Soil moisture content was measured gravimetrically using 10 g of field moist soil sample oven at 105 °C for 24 h. The pH of the soil sample was measured in a 1:2.5 soil/water suspension. The content of SOC was determined by dichromate oxidation and titration with ferrous ammonium sulphate. Total N concentration was determined by the semimicro-Kjeldahl digestion (Bremner and Mulvaney, 1982) followed by detection of ammonium with a Wescan ammonia analyzer, while total P concentration was analyzed colorimetrically after acidified ammonium persulfate digestion (Anderson and Ingram, 1989).

Soil microbial biomass C (MBC) was estimated by chloroform fumigation-extraction (Vance et al., 1987). The last set of soil samples from August 2009 were analysed for Phospholipid Fatty Acids (PLFAs) using the method described by Bossio and Scow (1998) in three replicates per treatment. The abundance of individual fatty acids was determined as nmol per g of dry soil and standard nomenclature was used (Tunlid et al., 1989). Concentrations of each PLFA were calculated based on the 19:0 internal standard concentrations. Frostergård and Bååth (1996) chose a set of fatty acids to represent bacterial PLFAs, out of which i14:0, 15:0, i15:0, a15:0, i16:0, 16:1ω7c, 17:0, a17:0, i17:0, cy17:0, 18:1ω7 and cy19:0 were present in our samples. We calculated the sum of i14:0, i15:0, a15:0, i16:0, a17:0 and i17:0 as an indicator of gram-positive bacteria. In our study, Gram-negative bacteria were identified by the PLFAs: 16:1ω7c, cy17:0, 18:1ω7 and cy19:0 (Zelles, 1999). The fungi were identified by the PLFAs 18:2ω6,9c (Frostergård et al., 1993), and PLFAs 16:1ω5c were used as a marker for arbuscular mycorrhizal fungi (AMF) (Olsson, 1999). Other PLFAs such as 14:0, 16:0, 16:1 2OH, 16:1ω9c, 17:1ω8c, 18:1ω9c and 18:3ω6c were also used to analyze the composition of microbial community. The ratio of 18:2ω6,9c to total bacterial PLFAs was used to estimate the ratio of fungal to bacterial biomass (F:B) in soils (Bardgett et al., 1996; Frostergård and Bååth, 1996). Taken together, all of the PLFAs indicated above were considered to be representative of the total PLFAs of soil microbial community.

Soil respiration was measured using the static chamber and gas chromatography techniques. The static chambers were as a 25-cm-diameter PVC rings made from of a 16-cm-height PVC pipe permanently anchored 8 cm into the soil. To avoided effect from disturbed cutting fine roots, they were installed two months before the initial sampling measurement. During flux measurements, a 30-cm-height removable cover chamber was attached to the anchor ring tightly with adhesive tape on the outside. Gas samples were collected from each chamber from 9:00 to 10:00 local time. Diurnal studies found that greenhouse gas fluxes measured during mid-morning (9:00–10:00) are close to daily means in the adjacent forests (Tang et al., 2006). Gas samples were taken with a 60 ml plastic syringe at 0 and 30 min after chamber closure and were analyzed within 12 h by using gas chromatography (Agilent 4890D, Agilent Co., Santa Clara, CA, USA).

Table 2
General characteristics of the 0–10 cm mineral soils from the studied forest sites.

Forest type	Old-growth forest	Mixed forest	Pine forest
pH value (H_2O)	3.98 (0.02) ^{a}	4.15 (0.06) ^{b}	4.12 (0.05) ^{b}
Total N (mg g^{-1})	1.99 (0.18) ^{a}	0.93 (0.08) ^{b}	1.15 (0.16) ^{b}
Organic matter (%)	7.3 (0.8) ^{a}	3.7 (0.2) ^{b}	5.2 (0.2) ^{b}
C/N ratio	21.0 (0.6)	23.8 (2.2)	28.1 (3.9)
Total P (mg g^{-1})	0.49 (0.03) ^{a}	0.38 (0.01) ^{b}	0.44 (0.01) ^{ab}
Available P (mg kg^{-1})	2.2 (0.5) ^{ab}	1.5 (0.5) ^{a}	2.9 (0.2) ^{b}
Soil moisture (%)	22.6 (1.1) ^{a}	16.4 (1.9) ^{b}	15.3 (1.1) ^{b}
Bulk density (g soil cm^{-3})	1.0 (0.1)	1.2 (0.1)	1.2 (0.1)

Values are means. Standard error in parentheses, $n = 5$, means not sharing the same superscript letter were statistically different at p-value of 0.05, measured in August 2007 in Control plots.

One litter trap (0.5 m × 0.5 m) with a mesh size of 1 mm was placed randomly in each plot about 0.5 m above the ground surface. The traps were emptied once every month during the year.

2.4. Statistical analysis

To account for plot differences before the start of the experiment, pre-treatment MBC data for all the plots were obtained in Feb. 2007. The data obtained after P addition were divide by the pre-treatment data to eliminate plot differences. One-way ANOVA was used to test the effects of treatments on microbial biomass, the ratio of fungal to bacterial and the mass of litterfall. To determine differences of CO_2 between treatments, for each forest type and in each of the sample months, we performed a repeated measures ANOVA analysis as well as to identify the overall P treatment effects.

Twenty-two individual PLFAs (mol %) from the PLFA analysis of soil samples were subjected to principal component analysis (PCA) after standardisation for equal unit variance. Differences in individual soil PLFAs between the three forests and treatments were tested with one-way analysis of variances (ANOVA). All analyses were conducted using SPSS 13.0 for Windows. Statistically significant differences were identified when *P*-values <0.05 unless otherwise stated.

3. Results

3.1. Microbial biomass

3.1.1. Comparison of the control plots between forests

The MBC in the control plots was significantly higher in the old-growth forest than in the mixed and pine forests in both wet and dry seasons ($p < 0.05$), with averages in the wet season at 566 (±42), 327 (±10) and 450 (±29) mg kg^{-1} in the old-growth, mixed and pine forest, respectively. Seasonally, MBC values were significantly higher in the wet season than in the dry season across all forests (Fig. 1). The difference between forests could be due to significant differences in SOC (Table 2). However, when corrected for C content the forest differences in MBC still remained. For example, MBC in the wet season in 2009 was 16.9 (±0.6), 12.9 (±0.9), 9.7(±0.9) mg g^{-1} organic C for the old-growth, mixed and pine forest.

3.1.2. Effects of P addition

In the old-growth forest, MBC was increased significantly by P addition both in dry and wet seasons (when adjusted for pre-treatment differences). However, there were no significant differences in MBC between the control and P addition in the mixed and pine forests (Fig. 2). These results were also confirmed by the values of total PLFAs, bacterial PLFAs and fungal PLFAs (indicator of total microbial biomass, bacterial biomass and fungal biomass, respectively) that were all significantly increased after P addition in the old-growth forest but not in the two disturbed forests (Fig. 3).

3.2. Microbial community composition

3.2.1. Comparison of the control plots between forests

The first principal component (PC1) using 22 PLFAs accounted for 32.7% and the second component (PC2) for 24.8% of the variation in the dataset of the control plots (Fig. 4a, b). The old-growth forest was clearly separated from the two disturbed forests on the PC2 axis ($p < 0.001$) (Fig. 4a). This difference was mainly caused by the relatively higher abundances of the Gram-negative bacteria and the relative lower abundances of AM fungi in the old-growth forest. There was, however, no significant difference between the old-growth forest and the disturbed forests in Gram-positive bacteria and fungi (Fig. 5).

3.2.2. Responses to P addition

P addition changed the microbial community composition significantly ($p < 0.05$) in the old-growth forest, as is indicated by the significant shift along the PC1 axis of the P addition plots compared to the control plots (Fig. 4a). The relative abundances of gram-positive bacterial PLFAs were significantly decreased and fungal PLFAs were significantly increased after P addition in the old-growth forest (Fig. 5). As a result, the ratio of fungal-to-bacterial PLFAs was also increased significantly (Fig. 3). In the mixed forest, P addition also shifted the PC1 scores to the right (Fig. 4a) but the change was not significant. However, a significant increase in the relative abundances of fungal PLFAs as well as of the ratio of fungal-to-bacterial PLFAs was found (Figs. 3 and 5).

3.3. Soil respiration

Soil respiration was not significantly different among the forest, with averages in control plots at 78 (±8), 70 (±5), 69 (±12) mg

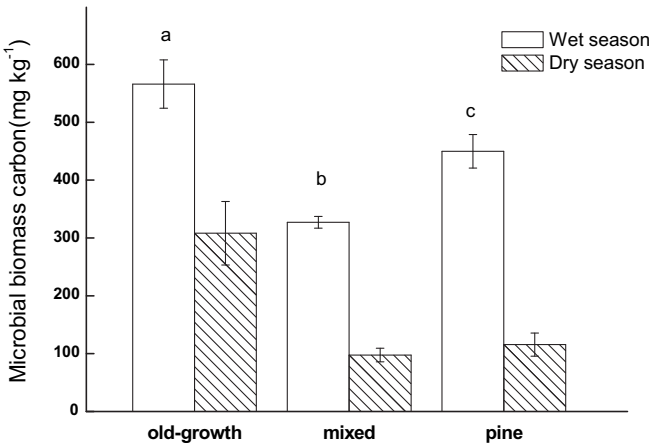

Fig. 1. Seasonal variation of microbial biomass C in control plot soils in the three forest types, averages for 2007 to 2009, error bars show SE ($n = 15$). Significant differences ($p < 0.05$) among forests, when present, are indicated by different letters, season effects were significant for all forests.

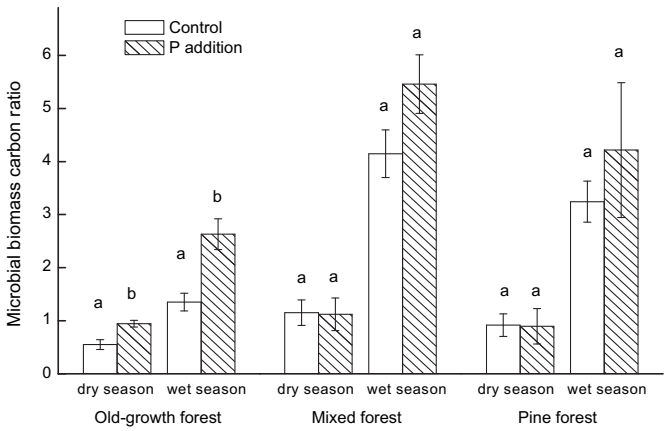

Fig. 2. Microbial biomass C in soils from the three forest types after P addition (standardized to levels in each plot before the experiment started). P addition = phosphorus addition since 2007. Dry season: Feb. 2009, Wet season: Agu. 2009. Significant differences ($p < 0.05$) among treatments are indicated by different letters. Error bars show SE ($n = 5$).

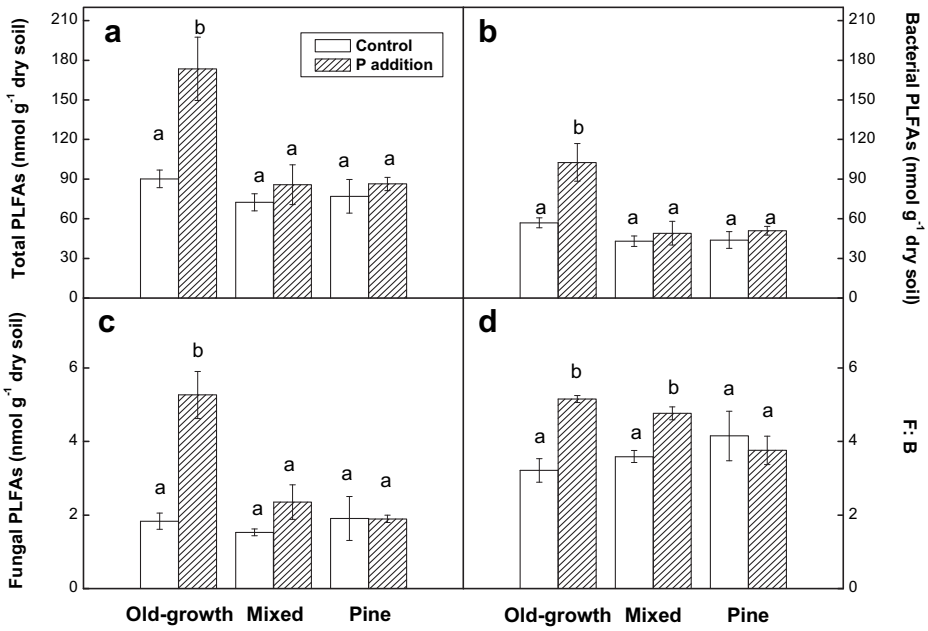

Fig. 3. a–d. Soil microbial PLFAs in the three forest types. P addition = phosphorus addition since 2007. F: B indicates the ratio of fungal to bacterial PLFAs. Significant differences ($p < 0.05$) among treatments are indicated by different letters. Error bars show SE ($n = 3$).

CO_2–C m^{-2} h^{-1}, in the old-growth, mixed and pine forests. In the old-growth and mixed forests, soil respiration was increased significantly after P addition ($p < 0.05$). However, there were no significant differences in soil respiration between the control and P addition plots in the pine forest (Fig. 6).

3.4. Litterfall

The mass of total litterfall in all forests showed a strong seasonal pattern, with a peak flux observed in September and the lowest input in the dry season (Fig. 7a–c). The annual total litterfalls in the control plots were 713 ± 68, 614 ± 85 and 613 ± 47 g m^{-2} yr^{-1} in the old-growth forest, mixed forest and pine forest, respectively, and were not significantly different among forests ($P = 0.51$). In the old-growth forest and the pine forest, the mass of total litterfall significantly increased during wet season (Jul–Sep.) after P addition. The mass of total litterfall also significantly increased in Oct. and Nov. after P addition in the old-growth forest. In the mixed forest, however, significant increase was found only in May and Nov. after P addition (Fig. 7a–c). In summary, the annual total litterfall fluxes were significantly increased after P addition in three forests, and were 1160 ± 97, 1082 ± 129 and 917 ± 68 g m^{-2} yr^{-1} in the old-growth forest, mixed forest and pine forest (Fig. 7a–c).

4. Discussion

4.1. Microbial biomass and community composition in three forest types

The MBC obtained in the present study (86–733 mg kg^{-1}, Fig. 1) falls well within the ranges (61–2000 mg kg^{-1}) reported for various temperate and tropical forest soils (Vance et al., 1987; Henrot and Robertson, 1994). The percentage of microbial biomass C to organic C in the three forest soils varied from 1% to 1.7% under wet seasons, which is within the normal MBC concentration of 1–5% of the total soil organic C (Sparing, 1992). The significantly higher soil MBC in the old-growth forest compared to the two disturbed forests (Fig. 1) still remained when corrected for C content, and was consistent with previous findings in the studied forests (Yi et al., 2007). The higher soil microbial biomass found in the old-growth forest than in the mixed and pine forests (Fig. 1) is corresponding to earlier observations of significantly higher decomposition rates in the old-growth forest (Mo et al., 2006; Fang et al., 2007). This result indicates that successional stage, and land-use history, can have a significant effect on the soil microbial biomass.

Disturbances from harvesting of understory and collection of litter and woody debris were previously quantified for the pine plantation and amounted to 3.3 Mg DW/ha (Brown et al., 1995). Furthermore, this harvest practice removed44 to 73 percent of the nutrients (N, P, K, Ca, and Mg) in the annual production of litterfall and understory biomass, and also reduced the active soil organic matter pool, thus resulting in lower levels of organic substrate for microbial activity and nutrient mineralization (Mo et al., 1995). These harvest activities may also have promoted erosion on the mountain slopes. The degradation of the disturbed stands are clearly reflected in the soil characteristics (Table 2) with significantly lower organic matter, N and P concentrations in the top soil compared to that of the old-growth forest but higher pH and bulk density compared to the undisturbed old-growth soil (Table 2). All this may indicate that the acidified humus rich top layer has been eroded and lower soil layers have been exposed in the disturbed forests. Although the mixed forest was released from the human pressure earlier than the pine forest, soil chemical and microbial characteristics did not yet differ for the two forest types.

Seasonally, MBC values were significantly higher during the rainy–warm season than during the dry–cool season (Fig. 1). We presume that lack of water and low winter temperatures reduce the activity and biomass of soil microorganisms, resulting in a lower rate of litter decomposition during the dry and cool period. Mo et al. (2008) observed that in the mixed and the pine forests (the same forests as studied here), the average soil respiration rates in the wet and warm season was higher compared to the winter period. Cleveland et al. (2003) and Devi and Yadava (2006) has also found a rainy season maximum for microbial biomass in tropical forest. However, Maithani et al. (1996) and Arunachalam and Arunachalam

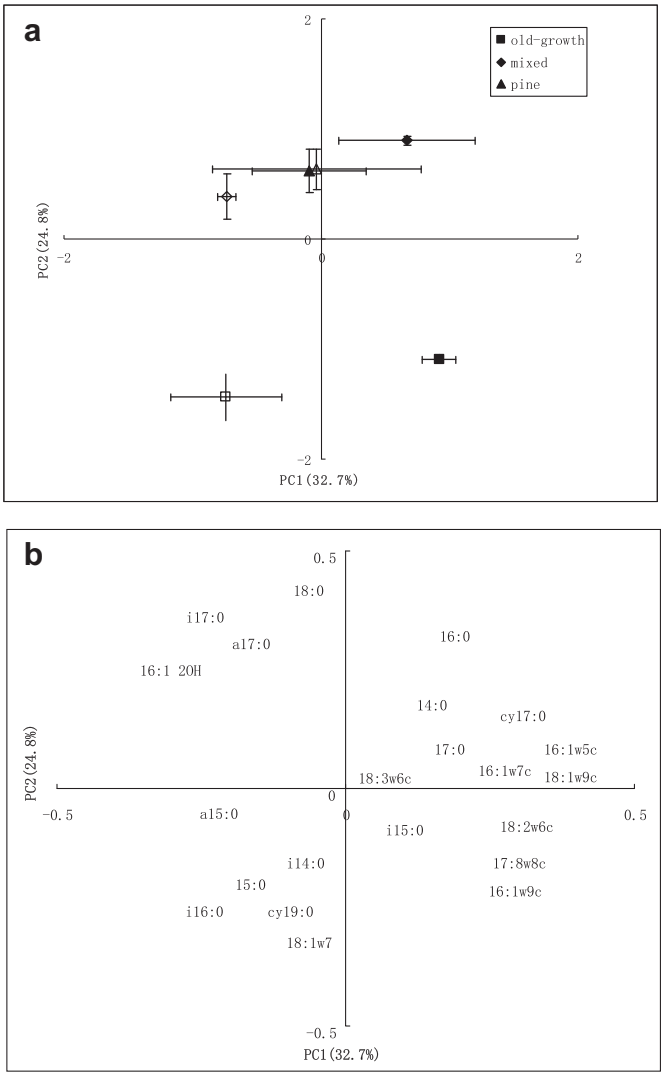

Fig. 4. a–b. The phospholipid fatty acid (PLFA) pattern in soil samples from three forest types (Aug. 2009). PLFA data were subjected to principal component analysis. a PCA scores from treatment were separated along PC1, while PCA scores from forests were separated along PC2. Control: open symbols, P addition: filled symbols. b PC loadings of the individual PLFAs.

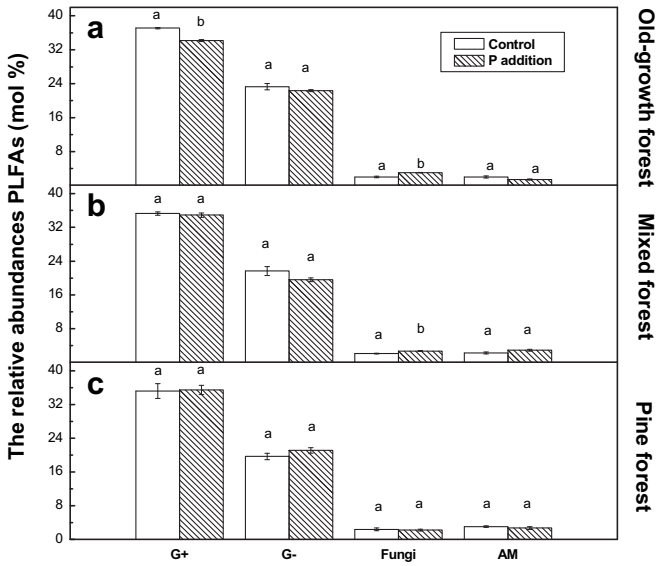

Fig. 5. a–c. The relative abundances of the individual PLFAs (mol %) in soil samples from three forest types. G^+: the proportion of gram-positive bacterial PLFAs; G^-: the proportion of gram-negative bacterial PLFAs; Fungi: the proportion of fungal PLFAs; AM: the proportion of AM fungal PLFAs. P addition = phosphorus addition since 2007. Significant differences ($p < 0.05$) among treatments are indicated by different letters. Error bars show SE ($n = 3$).

(2000) found maximum biomass values in the winter season in subtropical humid forests, which may be due to contrasting litter quality and rainfall patterns. A lower microbial biomass during the winter period may also be the result of reduced root growth, since substrates released from the roots were found to enhance microbial activity (Bottner, 1985).

We have shown that the composition of the microbial communities in the soils was significantly different between the studied forest types. However, this difference was only found in Gram-negative bacteria and AM fungi but not in Gram-positive bacteria and fungi (Fig. 5). The reasons are not clear. One of the possible explanations could be related to lower soil pH values in the old-growth forest. Soil pH is one of the most important soil properties related to the composition of microbial communities (Bååth and Anderson, 2003; Nilsson et al., 2007; Wu et al., 2009). Increased AM biomass in soil (PLFA 16:1ω5c) is often found with increasing soil pH (Bååth and Anderson, 2003; Rousk et al., 2010). Previous reports have shown that the old-growth forest is N-saturated which can lead to soil acidification (e.g. Mo et al., 2006, 2008; Zhang et al., 2008). As a result, soil pH was lower in the old-growth forest than in the mixed and pine forest. Similarly, Nilsson et al. (2007) found that N deposition had a negative effect on PLFA 16:1ω5c when a narrower pH interval was analysed. Another explanation could be related to higher soil moisture in the old-growth forest. Soil microbial communities are likely to be altered with the variation of soil temperature and moisture (Myers et al., 2001). Higher moisture would be propitious to plant growth but might restrict soil microbes through competition for resources (Singh et al., 2007).

4.2. The effect of P addition on soil microbial biomass and community composition in three forests

It is generally believed that the biomass of microbes depends directly on inputs of reduced carbon to the soil. Direct additions of readily available carbon sources such as glucose or sucrose to soil

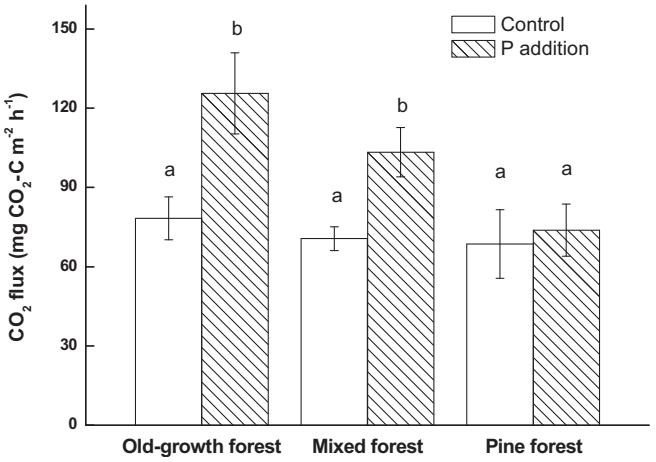

Fig. 6. Soil respiration response to P addition in the three forest types. Values are means for three months. (Data from July–Sep.2009). P addition = phosphorus addition since 2007. Significant differences ($p < 0.05$) among treatments are indicated by different letters. Error bars show SE ($n = 5$).

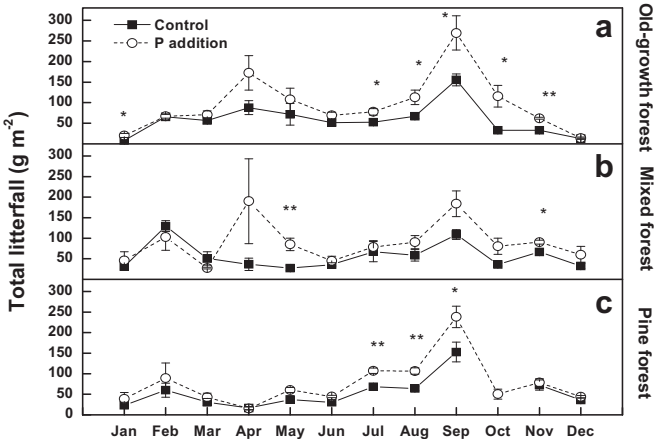

Fig. 7. a–c. Seasonal variations of total litterfall in the three forest types from January 2009 to December 2009. P addition = phosphorus addition since 2007. Error bars show SE ($n = 5$), asterisk (*) and (**) indicates significant difference between control and P addition at $p < 0.05$ and $p < 0.01$.

usually result in increases of microbial activity or biomass (Gallardo and Schlesinger, 1994; Joergensen and Scheu, 1999; Allen and Schlesinger, 2004). However, microbial biomass may also be limited by the availability of N or P (Wardle, 1992). Several studies reported that apatite addition had a significant positive influence on fungal growth in P-poor forests, but not in forests with sufficient P (Hagerberg et al., 2003; Nilsson and Wallander, 2003). Cleveland et al. (2002) reported that in a Ferralsol under a tropical rainforest microbial carbon degradation was strongly constrained by P availability. Microbial P limitation has been observed when C and N are given in ample amounts to tropical agroforestry parkland soils (Gnankambary et al., 2008) or to a native soil from western Kenya (Ehlers et al., 2010).

In this study, we found that effects of P addition on soil microbial growth varied depending on land-use history of the forest. P addition increased the microbial biomass in the old-growth forest. This was also reflected by the significant increases in soil respiration and the significant changes in the microbial community composition after P addition. These results indicated that P availability is one of the limiting factors for microbial growth in the old-growth forest. The annual total litterfalls significantly increased after P addition across all the forests, however. Our results thus suggest that P is a possible limiting factor for plant growth; therefore, P addition may increase C input to the microbes in the studied forests. However, addition of P had no effect on microbial biomass and microbial community composition in the pine forests. In the mixed forest, no significant response of microbial biomass to P addition was also noted, but soil respiration and the microbial community composition were significantly affected by P additions. These results imply that P availability may have no or little effects on soil microbial growth in both pine and mixed forests.

Zhou et al. (2006) reported an accumulation of soil C (0–20 cm depth) at about 54 g C m^{-2} yr^{-1} over two decades in the old-growth forest. The reason for this accumulation is unclear, but one suggestion is that the elevated N deposition (>30 kg N ha^{-1} yr^{-1}) over recent decades has decreased litter decomposition, as reflected by reduced soil respiration (Mo et al., 2006, 2008). Based on our result, another possible reason is that the P-limited microbial communities may increase the rates of belowground C storage in tropical systems. Cleveland et al. (2002) speculated that under high N availability significant increases in labile photosynthate production and transport to the rhizosphere are possible in these tropical forests. They suggested that if such increases occur, the more P-poor forests (with lower microbial activity) may display longer-term soil storage of new carbon inputs.

4.3. Conclusions

The old-growth forest, which saturated of N due to long-term high N deposition, showed a significant positive response in microbial biomass and composition after P addition, indicating P availability is one of limiting factors for microbial growth. Our results also indicated P deficiency of the plants. P addition alleviated this limitation and increased C input to the microbes. Two disturbed forests, still N limited due to previous land-use history, exhibited a lower soil microbial biomass but showed few responses to P additions. Our results suggest that P effect on microbial communities may display a key role in the increased C accumulation observed in these tropical forests.

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