Pedosphere **22**(5): 698–706, 2012
ISSN 1002-0160/CN 32-1315/P

Published by Elsevier B.V. and Science Press

PEDOSPHERE

www.elsevier.com/locate/pedosphere

Responses of Soil Acid Phosphomonoesterase Activity to Simulated Nitrogen Deposition in Three Forests of Subtropical China[*1]

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(Received January 4, 2012; revised July 20, 2012)

ABSTRACT

Soil acid phosphomonoesterase activity (APA) plays a vital role in controlling phosphorus (P) cycling and reflecting the current degree of P limitation. Responses of soil APA to elevating nitrogen (N) deposition are important because of their potential applications in addressing the relationship between N and P in forest ecosystems. A study of responses of soil APA to simulated N deposition was conducted in three succession forests of subtropical China. The three forests include a Masson pine (*Pinus massoniana*) forest (MPF)—pioneer community, a coniferous and broad-leaved mixed forest (MF)—transition community and a monsoon evergreen broad-leaved forest (MEBF)—climax community. Four N treatments were designed for MEBF: control (without N added), low-N (50 kg N ha^{-1} $year^{-1}$), and medium-N (100 kg N ha^{-1} $year^{-1}$) and high-N (150 kg N ha^{-1} $year^{-1}$), and only three N treatments (*i.e.*, control, low-N, medium-N) were established for MPF and MF. Results showed that soil APA was highest in MEBF, followed by MPF and MF. Soil APAs in both MPF and MF were not influenced by low-N treatments but depressed in medium-N treatments. However, soil APA in MEBF exhibited negative responses to high N additions, indicating that the environment of enhanced N depositions would reduce P supply for the mature forest ecosystem. Soil APA and its responses to N additions in subtropical forests were closely related to the succession stages in the forests.

Key Words: Dinghushan Biosphere Reserve, forest ecosystems, forest succession, phosphorus limitation, subtropical region

Citation: Huang, W. J., Zhang, D. Q., Li, Y. L., Lu, X. K., Zhang, W., Huang, J., Otieno, D., Xu, Z. H., Liu, J. X., Liu, S. Z. and Chu, G. W. 2012. Responses of soil acid phosphomonoesterase activity to simulated nitrogen deposition in three forests of subtropical China. *Pedosphere*. **22**(5): 698–706.

INTRODUCTION

Tropical forests are likely to suffer from phosphorus (P) deficiency since they are established on highly weathered and intense P adsorptive soils (Attiwill and Adams, 1993). A large part of the total soil P is also bound in the detritus as organic P and remains inaccessible to plants. P supply to plants strongly relies on its mineralization, a process by which organic P is hydrolyzed into inorganic P. The process is catalyzed by soil phosphatase enzymes (Frossard *et al.*, 2000), which play a critical role in maintaining and controlling the rate of P turn-over in forest ecosystems (Vance *et al.*, 2003). Therefore, an increase in root-associated phosphatase activity is a suitable predictor of P limitation in forest soils (Gress *et al.*, 2007).

Soil phosphatase enzymes originate from fungi, bacteria and root exudates (Johnson *et al.*, 1999), and comprise a group of enzymes such as phosphomonoesterases, phosphodiesterases and phosphotriesterases (Tabatabai, 1982). Phosphomonoesterases are the dominant phosphatases in most soils and are divided into two types according to their pH optima. One is alkaline phosphomonoesterase prevailing in soils with high pH, *e.g.*, calcareous soils. The other is acid phosphomonoesterase, predominant in soils with low pH (Juma and Tabatabai, 1988).

Atmospheric nitrogen (N) deposition in the tropical and subtropical regions is on the increase due to increasing agricultural activities and fossil fuel combu-

[*1]Supported by the "Strategic Priority Research Program" of the Chinese Academy of Sciences (No. XDA05050208), the National Natural Science Foundation of China (Nos. 31070439 and 40730102) and the Knowledge Innovation Program of the Chinese Academy of Sciences (Nos. KSCX2-EW-Q-8 and KSCX2-EW-J-28).
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stion (Matson *et al.*, 2002; Galloway *et al.*, 2003). There is strong evidence that many tropical and subtropical regions are currently suffering from frequent acid rains associated with high N deposition (Galloway *et al.*, 2003). The biological and ecological implications of high N deposition on terrestrial ecosystems have drawn a great deal of attention in recent years (Tietema *et al.*, 1998; Matson *et al.*, 1999, 2002; Magill *et al.*, 2004). Increasing N input into natural ecosystems may enhance plant demand for other nutrients, particularly for P, leading to P limitation in natural ecosystems (Aber *et al.*, 1989). For tropical forests, P limitation will result in low carbon storage (Hall and Matson, 2003; Kellogg and Bridgham, 2003).

The rapidly expanding agricultural and manufacturing sectors and the growing volume of traffic in China are associated with high emissions of N oxides and atmospheric N deposition (30–73 kg ha^{-1} $year^{-1}$) that are likely to impact natural ecosystems, particularly the tropical forests (Foell *et al.*, 1995; Busch *et al.*, 2001; Rodhe *et al.*, 2002). The functional relationship between soil acid phosphomonoesterase activity (APA) and P availability in natural ecosystems provides a suitable entry point for assessing the impact of increasing N deposition on tropical and subtropical forests of China, which are likely to experience P limitation. Previous studies have shown changes in soil APA induced by N inputs in other natural ecosystems elsewhere (Carreira *et al.*, 2000; Turner *et al.*, 2002; Pilkington *et al.*, 2005) and in China (Wang, Q. K. *et al.*, 2008). To the best of our knowledge, such investigations, except for Wang, Q. K. *et al.* (2008), have not been conducted in any of the Chinese tropical and subtropical forests.

Dinghushan Biosphere Reserve is a natural undisturbed subtropical forest located in the middle of Guangdong Province in southern China, with distinct forest types at different succession stages, from the pioneer community (Masson pine forest, MPF) *via* the transition community (coniferous and broad-leaved mixed forest, MF) to the regional climax vegetation (monsoon evergreen broad-leaved forest, MEBF) (Zhou *et al.*, 1986). These study sites have been exposed to high atmospheric N deposition of 20–50 kg N ha^{-1} $year^{-1}$ in the last 15 years (Fang *et al.*, 2008). These forest stages are associated with different P requirements, which could allow us to examine the impact of increased N deposition on forest production and stability. The study in this reserve has showed that the N/P ratios in living leaves were increasing with succession stages and that the standing biomass and productivity of MEBF have been declining over the last 30 years (Huang *et al.*, 2011). Thus, the aim of this study was to understand the response of soil APA to the increased N deposition so as to provide important information on P cycling in subtropical forests, by hypothesizing that 1) soil APA would be greater in MEBF than those in both MPF and MF, 2) soil APAs would be stimulated by N addition in both MPF and MF due to their low soil N availability, and 3) N addition would have little or even negative effects on soil APA in MEBF due to its high soil N availability.

MATERIALS AND METHODS

Site description

The study was conducted in Dinghushan Biosphere Reserve located in the middle of Guangdong Province in southern China (112° 33′ E, 23° 10′ N), about 84 km from Guangzhou City and with an area of 1 133 ha. It has a typical subtropical monsoon humid climate. The average annual precipitation is 1 927 mm, of which about 80% occurs between April and September. The annual average temperature is 21 °C, with the lowest monthly mean temperature at 12.6 °C in January and the highest at 28 °C in July. The mean annual relative humidity is 80%.

MPF, MF and MEBF are the main forest types in Dinghushan Biosphere Reserve, which represent a sequence of natural succession stages. The MPF, belonging to the primary stage of succession, was planted in the 1950s. It is from 50 to 200 m above sea level (a.s.l.), occupying 20% of the reserve area and the dominant species is *Pinus massoniana* (Brown *et al.*, 1995). The MF is a sub-climax forest community that was a coniferous plantation established in the 1930s, but due to colonization from natural dispersal of regional broadleaf species, its composition has greatly changed over time. It is at about 200 m a.s.l., accounting for 50% of the reserve area and the main canopy species are *Pinus massoniana*, *Schima superba*, and *Castanopsis chinensis* (Wang and Ma, 1982). If sufficient time is allowed, it may develop into a monsoon evergreen broad-leaved forest type. The MEBF, a regional climax forest community, has been well protected for more than 400 years. It is at about 200–350 a.s.l., holding 20% of the reserve area, and the main canopy species are *Castanopsis chinensis*, *Machilus chinensis*, *Schima superba*, *Cryptocarya chinensis*, and *Syzygium rehderianum*.

The elevation, aspect and slope of the three forests are similar with slopes ranging from 15° to 35°. The soils of the three forests are lateritic red earth formed from sandstone, with variable soil depths. The soil depth is less than 40 cm in MPF, ranges from 30 to 60 cm in MF and is deeper than 60 cm in MEBF (Brown *et al.*, 1995).

Experimental design

The experiment was started in each of the three forests in July 2003. Three N addition treatments were designed in MPF and MF: control (without N added), low-N (50 kg N ha^{-1} year^{-1}), and medium-N (100 kg N ha^{-1} year^{-1}), and four N addition treatments were set up in MEBF: control, low-N, medium-N, and high-N (150 kg N ha^{-1} year^{-1}). Each treatment had 3 replicates resulting into a total of 30 plots established in the three forests (9 in MPF, 9 in MF and 12 in MEBF). Each plot measured 20 m × 10 m, with a 10 m wide buffer strip around it. All plots and treatments were laid out randomly. For each N application, NH_4NO_3 fertilizer was weighed, mixed with 20 L water, and sprayed monthly onto the ground below the canopy using a backpack sprayer. The same amount of water without N added was applied to the control plots (Mo *et al.*, 2006).

Soil sampling

In July 2009, soil samples were collected from two mineral soil layers (top soil layer, 0–10 cm; lower soil layer, 10–20 cm) after removal of the litter layer. Fifteen soil cores (2.5 cm in diameter) were taken randomly and homogenized into one sample for each soil profile per plot. Thus, there were a total of six soil samples including two soil layers in each treatment. Soil samples were put in sealed plastic bags and immediately taken to the laboratory. After the removal of roots, stones, and other impurities, each soil sample was screened to pass a 2-mm sieve and stored at 4 °C until analysis of soil APA. The measurement procedures of soil APA were completed within 28 days. A subsample of each soil sample was air-dried for analysis of related soil chemical properties.

Determination of soil chemical properties and soil APA

Gravimetric soil moisture was determined from mass loss after the soil sample was dried at 105 °C for 24 h. Soil pH was measured in a deionized water suspension using a glass electrode at a ratio of 25 mL water to 10 g soil (Liu *et al.*, 1996). Soil organic matter (SOM) was determined by dichromate oxidation before titration with a Fe^{2+} solution (Liu *et al.*, 1996). Total N (TN) was analyzed by semimicro-Kjeldahl digestion followed by steam distillation and final titration of ammonium (Liu *et al.*, 1996). Total P (TP) was measured colorimetrically after digestion (Liu *et al.*, 1996). Available P was extracted with 0.03 mol L^{-1} NH_4F-0.025 mol L^{-1} HCl and determined colorimetrically (Liu *et al.*, 1996).

Soil APA was measured according to Schneider *et al.* (2000), using *para*-nitrophenyl phosphate (*p*-NPP) as a substrate in a modification of the original method from Tabatabai and Bremner (1969). One gram of fresh soil was placed into a 100 mL Erlenmeyer flask and incubated for 30 min in a water bath at 30 °C with 4 mL of modified universal buffer (MUB, pH = 6.5) containing 1 mL of 100 mmol L^{-1} *p*-NPP substrate. After incubation, the flask was immediately placed on ice, and then 1 mL of 2 mol L^{-1} $CaCl_2$ and 4 mL of 0.2 mol L^{-1} NaOH were added to terminate the reaction and extract the formed *para*-nitrophenol (*p*-NP). The sample was diluted with 90 mL of deionized water and then filtered through Whatman-42 filter paper. Further dilution would be made if soil APA was too high. Absorbance of released *p*-NP was determined spectrophotometrically at 400 nm. Four replicates including one blank were used for each soil sample. For the blank, *p*-NPP was added after (instead of before) the incubation. Enzymatic activity was expressed in μmol *p*-NP g^{-1} h^{-1}. All soil chemical properties and soil APA were expressed on an oven-dry soil weight basis.

Statistical analysis

Homogeneity of variances and normality of distributions of data sets were checked. Data that were not homogeneous were logarithmically transformed prior to analysis. An independent-samples *t*-test was used to test difference between two soil depths. A one-way ANOVA with Tukey multiple comparison test was performed to determine statistical differences of soil chemical properties and soil APA among the forests and N treatments. All analyses were conducted with SPSS 11.5 for Windows. Statistically significant differences were accepted at $P < 0.05$ unless otherwise stated.

RESULTS

Soil chemical properties

Soil pH was the lowest in the control plots in MEBF, followed by MPF and MF (Table I). SOM and TN contents were significantly higher in MEBF than in MF and MPF in two soil layers of 0–10 and 10–20 cm. The differences between MF and MPF were however not significant. TP contents in all three forests showed no significant differences. Available P content

TABLE I

Soil chemical properties[a)] and their responses to nitrogen (N) additions in Masson pine forest (MPF), coniferous and broad-leaved mixed forest (MF) and monsoon evergreen broad-leaved forest (MEBF) of subtropical China

Soil layer	Forest	Treatment	Soil pH	SOM	TN	TP	Available P
cm				mg g^{-1}			mg kg^{-1}
0–10	MPF	Control	3.89ab[b)]	24.0b	0.95b	0.18a	0.61b
		Low-N	3.82	25.2	0.96	0.19	0.64
		Medium-N	3.78	21.7	0.85	0.16	0.54
	MF	Control	3.91a	15.0b	0.70b	0.16a	0.59b
		Low-N	3.83	17.4	0.90	0.18	0.21
		Medium-N	3.82	15.2	0.77	0.18	0.36
	MEBF	Control	3.70b	41.9a	1.85a	0.20a	1.57a
		Low-N	3.69	44.4	1.88	0.22	1.79
		Medium-N	3.59	44.7	1.80	0.23	1.55
		High-N	3.62	45.0	1.77	0.23	1.72
10–20	MPF	Control	3.99ab	13.2b	0.67b	0.20a	0.27a
		Low-N	3.90	14.2	0.68	0.17	0.46
		Medium-N	3.92	12.8	0.64	0.17	0.18
	MF	Control	4.10a	8.7b	0.59b	0.19a	0.16a
		Low-N	4.06	8.0	0.61	0.18	0.08
		Medium-N	4.03	7.9	0.61	0.16	0.16
	MEBF	Control	3.87b	23.7a	0.97a	0.18a	0.38a
		Low-N	3.92	25.4	0.99	0.19	0.51
		Medium-N	3.76	24.6	1.12	0.19	0.47
		High-N	3.80	28.0	1.14	0.20	0.57

[a)]SOM = soil organic matter; TN = total nitrogen; TP = total phosphorus.
[b)]Mean values followed by different letter(s) within a column for a given soil layer are significantly different among the controls of the three forests at $P < 0.05$.

was significantly ($P < 0.01$) higher in MEBF than those in MF and MPF only in top soil layer (0–10 cm), but not in lower soil layer (10–20 cm), while the differences between MF and MPF were not significant.

Soil pH, SOM, TN, TP and available P in the three forests were not significantly influenced by N additions. However, N addition treatments in the three forests almost lowered soil pH when compared with controls except for low-N treatments in the lower soil layer of MEBF (Table I).

Soil APA in control plots

The top soil layer (0–10 cm) in the control plots showed a significantly ($P < 0.01$) higher soil APA level than the lower soil (10–20 cm) (Fig. 1). The differences of soil APA between the two soil layers varied with forest types. In MEBF, the soil APA in the top soil layer was approximately twice (1.92) as high as that in the lower soil layer, while in MF and MPF, the ratios of soil APA in the top soil layer to that in the lower soil layer were 1.67 and 1.63, respectively. In the top soil layer, soil APA was significantly higher ($P < 0.01$) in MEBF (21.40 μmol p-NP g^{-1} h^{-1}) than those in both MPF (11.25 μmol p-NP g^{-1} h^{-1}) and MF (9.03 μmol$p$-NP g^{-1} h^{-1}), but there was no significant difference between MPF and MF. The same results occurred in the lower soil layer, with 11.14 μmol p-NP g^{-1} h^{-1} in MEBF, 6.72 μmol p-NP g^{-1} h^{-1} in MPF and 5.55 μmol p-NP g^{-1} h^{-1} in MF. Soil APA in MEBF was significantly higher than that in MF or MPF ($P < 0.01$).

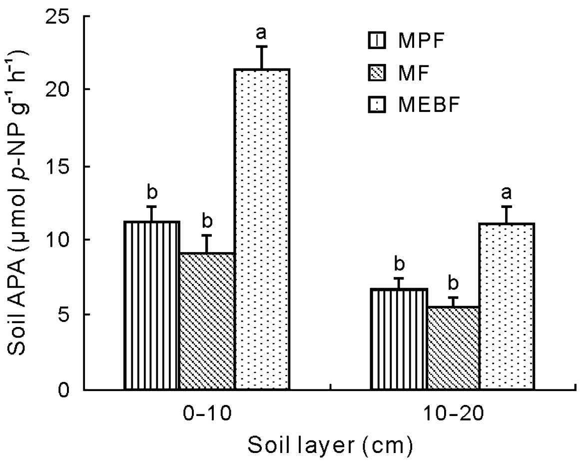

Fig. 1 Soil acid phosphomonoesterase activity (APA) in control plots of Masson pine forest (MPF), coniferous and broad-leaved mixed forest (MF) and monsoon evergreen broad-leaved forest (MEBF) of subtropical China. Vertical bars represent the standard errors of the means ($n = 3$). Bars with the same letter for a given soil layer are not significantly different among the three forests at $P < 0.05$.

Effects of N addition on soil APA

Responses of soil APA to N addition varied with forest type, soil depth and N treatment level (Fig. 2). In the top soil layer of MPF, soil APA was a little higher (no statistical differences) in low-N treatment (11.88 µmol *p*-NP g^{-1} h^{-1}) than that in the control (11.25 µmol *p*-NP g^{-1} h^{-1}) (Fig. 2a), but it significantly decreased by 29% in the medium-N treatment (7.97 µmol *p*-NP g^{-1} h^{-1}) compared with the control. In the lower soil layer, soil APA declined with increasing N addition but without significant differences among the three treatments (Fig. 2b). It ranked as medium-N (5.04 µmol *p*-NP g^{-1} h^{-1}) $<$ low-N (6.03 µmol *p*-NP g^{-1} h^{-1}) $<$ control (6.72 µmol *p*-NP g^{-1} h^{-1}).

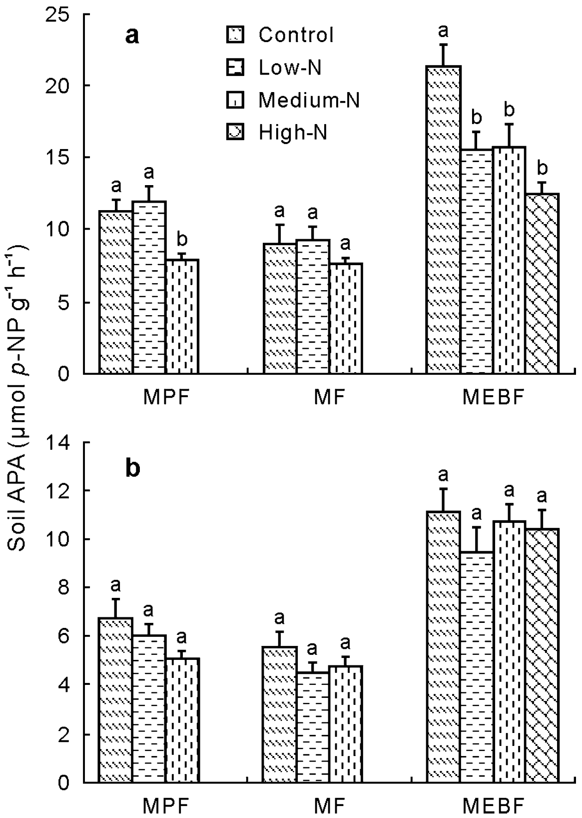

Fig. 2 Effects of nitrogen (N) addition on soil acid phosphomonoesterase activity (APA) in the (a) top soil layer (0–10 cm) and (b) lower soil layer (10–20 cm) in Masson pine forest (MPF), coniferous and broad-leaved mixed forest (MF) and monsoon evergreen broad-leaved forest (MEBF) of subtropical China. Vertical bars represent the standard errors of the means ($n = 3$). Bars with the same letter for a given forest are not significantly different among the N treatments at $P < 0.05$.

In the top soil layer of MF, the highest level (9.31 µmol *p*-NP g^{-1} h^{-1}) of soil APA was recorded in the low-N treatment plots, followed by the control (9.03 µmol *p*-NP g^{-1} h^{-1}) and medium-N treatment (7.66 µmol *p*-NP g^{-1} h^{-1}), but there were no significant differences among them (Fig. 2a). In the lower soil layer of MF, soil APA in the control plots (5.55 µmol *p*-NP g^{-1} h^{-1}) was higher than those in both low-N (4.55 µmol *p*-NP g^{-1} h^{-1}) and medium-N (4.75 µmol *p*-NP g^{-1} h^{-1}) treatments, with no significant differences among them (Fig. 2b). As a whole, N addition had no significant effects on the soil APA in either soil layer of MF.

In MEBF, N addition depressed soil APAs in both soil depths compared with those of the control. For the top soil layer, soil APA was significantly lower ($P < 0.01$) in each N addition treatment than in the control (Fig. 2a). Compared with the control (21.40 µmol *p*-NP g^{-1} h^{-1}), soil APA of the top soil layer decreased by 28%, 26% and 41% in low-N (15.49 µmol *p*-NP g^{-1} h^{-1}), medium-N (15.73 µmol *p*-NP g^{-1} h^{-1}), and high-N (12.56 µmol *p*-NP g^{-1} h^{-1}) treatments, respectively. Soil APA had no significant difference among all N addition treatments. For the lower soil layer, soil APA in each N addition treatments was lower than that in the control, but the differences were not significant among them (Fig. 2b). The mean soil APA was 11.14, 9.49, 10.71, and 10.34 µmol *p*-NP g^{-1} h^{-1} in the control, low-N, medium-N and high-N treatments, respectively.

DISCUSSION

Broad implications by soil APA values

Soil APAs in the three forests of Dinghushan Biosphere Reserve were relatively high compared with the values measured in other acidic forest soils (Pang and Kolenko, 1986; Schneider *et al.*, 2000; Trasar-Cepeda *et al.*, 2000; Dinesh *et al.*, 2004), and they were much higher than other subtropical forest soils (Chen, 2003; Wang, Q. K. *et al.*, 2008). Low available P content in the area could be responsible for high soil APA. The production of phosphatases by plant roots and microbes would be enhanced when soil available P is in shortage. Moreover, another reason for high soil APA could be the long-term high atmospheric N deposition in this region (Huang *et al.*, 1994; Zhou and Yan, 2001; Fang *et al.*, 2008). High N stock in soil pools through atmospheric N deposition would require more available P to prevent P deficiency that would limit plant growth. Thus, the high soil APA in this studied area reflected a regional deficiency in soil available P. This also partly explained the result of a significantly positive (rather than negative) relationship between soil APA and available P (Table II).

Soil APA was higher in the top soil layer than in the lower soil layer, showing a decreasing trend with mineral soil depths. The result was consistent with other reports, which demonstrated that the APAs in different soil depths reduced with the decrease of SOM, TN and TP (Pang and Kolenko, 1986; Chen *et al.*, 2000,

TABLE II

Pearson correlation coefficients between soil acid phosphomonoesterase activity (APA) and some soil chemical properties[a)] in Dinghushan Biosphere Reserve

	SOM	TN	TP	Available P
Pearson correlation	0.831	0.83	0.489	0.677
P value	< 0.01	< 0.01	< 0.01	< 0.01

[a)]SOM = soil organic matter; TN = total nitrogen; TP = total phosphorus.

2008; Turner *et al.*, 2002). Significantly positive relationships ($P < 0.01$) were observed between soil chemical properties (SOM, TN, TP) and soil APA (Table II), indicating that the better the soil nutrient status, the higher the soil APA would be. In addition, microbes, the producers of soil acid phosphomonoesterase, use carbon and N as their energy and nutrient sources and, consequently, the microbial biomass was depressed with soil depths (Taylor *et al.*, 2002; Yi *et al.*, 2006). Thus, the decline of soil APA with soil depths was driven by soil nutrient and microbes allocation.

Effects of forest succession on soil APA

The soil APA in control plots was significantly higher in MEBF than those in both MF and MPF, which indicated that soil APA was enhanced by the stage of forest succession (MPF→MF→MEBF). Consequently, the increase in soil APA should reflect an enhanced requirement for P by plants and/or microbes in the late succession forest. This result is supported by the suggestion that P is possibly one of the factors limiting the plant productivity in the mature forest of Dinghushan Biosphere Reserve (Mo *et al.*, 2000). Among the three forests, the highest soil APA in MEBF can be ascribed to the improved vegetation conditions (Huang *et al.*, 1998) and soil nutrient status (Table I) in the process of forest succession (Harrison, 1983; Grierson and Adams, 2000). Former studies also reported that root biomass and soil microbial biomass were higher in MEBF than those in MPF and MF (Fu *et al.*, 1995; Yi *et al.*, 2003). Therefore, the explanations for the highest soil APA in MEBF among the three forests included the advantaged nutrient status and the greater quantity of enzyme producers in mature forest.

Effects of N addition on soil APA

In the top soil layer of MPF and MF, soil APAs had no significantly positive responses to the low-N treatments. Litter decomposition experiments demonstrated that soil N was a limiting factor in both MPF and MF (Mo *et al.*, 1995, 2006). No responses of soil APA to N addition suggest that there is no shift from N to P acquisition as a result of the release of N limitation in the two forests. The results are inconsistent with the other studies showing that N fertilization increased soil APA since moderate N addition may mitigate the N limitation and stimulate the need for P in the ecosystems (Saiya-Cork *et al.*, 2002; Sinsabaugh *et al.*, 2002, 2005; Allison *et al.*, 2006; Wang, Q. K. *et al.*, 2008).

Different from the effect of low-N treatment on soil APA, the medium-N treatment induced a mild reduction of soil APA in MPF and MF. This is partially supported by the result of Wang, H. *et al.* (2008), which showed that soil microbial biomass had been decreased by the medium-N treatments in MPF and MF. In this case, the reduction of soil APA could be attributed to the depressed microbial population. Aber *et al.* (1989) noted that chronic N addition to N-limited forest soils would initially enhance soil microbial activity, but over time would cause a carbon-limited state after microbial demand for N was satisfied. Thus, the reduction of soil APA with medium-N treatments in MPF and MF may be due to the N satisfied; or perhaps N addition would induce a significant reduction of soil APA in MPF and MF over a long period of time.

The results of N addition in MEBF showed a different trend from those of MPF and MF. N addition treatments had negative effects on soil APA compared with the control in MEBF. The inhibition of soil APA was even more pronounced by the high-N treatment. The results were similar to the study in southern Edinburgh (Carreira *et al.*, 2000), where soil APA was significantly lower under N- and S (sulfur)-containing acid-mist treated trees than that under control trees. These results are also supported by the experiment carried out in a spruce forest stand in Solling (Enowashu *et al.*, 2009), central Germany, which showed that soil phosphatase activities increased in the clean rain treatment (reduced N input).

There are some possible reasons responsible for the above results. First, soil pH was the lowest in MEBF among the three forests, and it was further declined upon the N addition treatments although the differences were not significant (Table I). The relatively low soil pH value can cause a decrease in soil microbial biomass in MEBF (Wang, H. *et al.*, 2008). This was supported by other studies (Smolander *et al.*, 1994; Joergensen *et al.*, 1995), which demonstrated that N addition induced the reduction of microbes. This was also partially supported by the results of significantly negative effects of N addition on litter decomposition in MEBF (Mo *et al.*, 2006). Furthermore, there was a considerable risk of aluminium (Al) toxicity in MEBF with enhancing Al content in N treatments (Lu *et al.*, 2009), which was adverse to soil conditions and indirectly inhibited the growth of plants and microbes (Liu, 2000; Wang, H. *et al.*, 2008). The depressed plants and microbes would necessarily lead to the reducing production of soil APA. Finally, MEBF is likely to be N saturated due to both long-term high atmospheric N deposition (Zhou and Yan, 2001) in this region and self-accumulation in mature forests (Vitousek, 1982, 1984). Soil available N was significantly higher in MEBF than that in MPF and MF. The proportions of NO_3^--N were also the highest in MEBF, followed by MPF and MF (Huang *et al.*, 2009; Lu *et al.*, 2009). Higher NO_3^--N proportion in soil available N has been regarded as a sign of N saturation (Aber *et al.*, 1998). A nutrient imbalance is likely to happen in MEBF with the N saturation status, and, hence, this condition is disadvantageous to microbial growth that requires balanced nutrient proportions (Kuperman, 1999). Moreover, N addition greatly elevated NO_3^--N and resulted in a relatively low percentage of NH_4^+-N in soil inorganic N (Fang *et al.*, 2004). This may lead to the reduction of soil APA in N addition treatments because ammonium, rather than nitrate, is important to determine the increase of soil APA (Johnson *et al.*, 1999). Therefore, the combined effects of soil acidification and N saturation on soil APA are likely to induce a decline in the mineralization of organic P and eventually exacerbate P-deficient stress in the subtropical mature forest.

CONCLUSIONS

With the process of forest succession (MPF→MF →MEBF), it was evident that the changes in vegetation conditions, which may affect soil properties, such as soil pH, organic carbon contents, N availability and microbial biomass, resulted in the greatest value of soil APA in the mature forest (MEBF). This was further supported by the different responses of soil APA to N addition treatments. In both MPF and MF, which are still N limited, soil APAs were slightly elevated in low-N treatments but depressed in medium-N treatments. In contrast, soil APA in MEBF exhibited negative responses to N addition, which was probably due to both long-term high atmospheric N deposition in this region and self-accumulation in mature forests. In addition, the relative high values of soil APA found in the studied region may reflect the intensity of P limitation in subtropical forests. Especially in MEBF, the significant decreases of soil APA in response to the N addition indicate that the enhancement of N content may aggravate P deficiency in the mature forest.

ACKNOWLEDGEMENT

The authors thank Mr. Mo Ding-Sheng, Mr. He Hai-Qing, Miss Li Yin, Miss Li Yan-Hong and others for the field sample collection and analysis.

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