

Increasing phosphorus limitation along three successional forests in southern China

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Abstract

Background and Aims Phosphorus (P) is commonly one of most limiting nutrients in tropical and subtropical forests, but whether P limitation would be exacerbated during forest succession remains unclear.

Methods Soil phosphatase activity is often used as an indicator of P limitation. Here we examined soil acid phosphatase activity (APA) underneath tree species in pine forest (PF), mixed pine and broadleaf forest (MF) and monsoon evergreen broadleaf forest (MEBF) which represented the early, middle and late successional stages of subtropical forests in China, respectively. We also analyzed other indicators of P status

(soil available P and N and P stoichiometry of the tree species).

Results APA or APA per unit soil organic carbon under tree species was relatively low in the early successional forest. Different from PF and MF, soil available P beneath the tree species was lower than in the bulk soils in MEBF. Soil APA was closely related to N:P ratios of tree species across all three forests.

Conclusions Our results imply that P limitation increases during forest succession at our site. The dominant tree species with low soil APAs in MEBF are likely more P-limited than other tree species.

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Keywords Phosphorus limitation · Acid phosphatase activity · N:P ratio · Forest succession · Subtropics.

Abbreviations

APA acid phosphatase activity
SOC soil organic carbon

Introduction

Biochemical mineralization of soil phosphorus (P) can play a significant role in soil P cycle and the amount of P available for plant uptake (McGill and Cole 1981). As plant growth becomes more limited by soil P availability, the supply of available P for plants from biochemical mineralization through the enzyme phosphatase becomes more dominant (Olander and

Vitousek 2000). Therefore, high phosphatase activity is often used as a proxy for P limitation in many studies (Krämer and Green 2000; Turner et al. 2002; Sinsabaugh et al. 2008).

Phosphatase can be produced by plant roots and soil microbes (Johnson et al. 1999). Different tree species can affect P availability and dynamics differently by influencing soil physical and chemical properties and substrate quality through litter input to soil (Binkley and Giardina 1998; Binkley et al. 2000; Chen et al. 2008). Plant roots affect soil microbial community and function, and fundamentally alter the rate and the dominant pathways of mineralization of soil organic matter (Ushio et al. 2010). When both species composition and soil biogeochemistry undergo significant changes over time, such as during forest succession, the estimates of phosphatase activity in bulk soils with roots from different tree species may not give the correct interpretation of how P limitation varies in different forests along a succession gradient. This is because different tree species may have different requirements for P (Yan et al. 2006a). The differences in soil properties, especially soil organic carbon (SOC), underneath tree species within a forest and in different forests, may also affect soil P availability and phosphatase activity (Speir and Ross 1978; Chen et al. 2000). Therefore, studies on phosphatase activity underneath different tree species within a forest or the same species in different forests along a successional gradient in a region can further improve our understanding on how tree species affect soil P dynamics during forest succession.

In this study, we chose three forests along a successional gradient at the Dinghushan Biosphere Reserve. These three forests are pine forest (PF), mixed pine and broadleaf forest (MF) and monsoon evergreen broadleaf forest (MEBF), which belong to early, middle and late succession stages, respectively. These sites have been subjected to high atmospheric N deposition of 20 to 50 kg N ha⁻¹ year⁻¹ in the last 15 years (Fang et al. 2008). Previous studies have shown that the net primary production (NPP) increased for PF, remained quite stable for MF, and declined for MEBF over the last two decades (Huang et al. 2011; Tang et al. 2011), and the fraction of NPP allocated belowground was also the highest in MEBF and the lowest in PF (Yan et al. 2006b; Tang et al. 2011). They hypothesized that MEBF was likely to be more limited by P than PF, and therefore the NPP of MEBF has declined over the last

two decades when N deposition at this site was quite high. Here, we used the measured phosphatase activity to study the responses of different dominant tree species in a forest or the same tree species in three forests to P limitation along a forest successional gradient. Increased phosphatase production, however, does not always respond to P-limiting conditions (Speir and Ross 1978). Therefore, we also used the measured soil available P and N:P ratios to study the responses of different tree species to P limitation, as the N:P ratio has been suggested as an indicator of either N or P limitation (Güsewell 2004).

As acid phosphatase is predominant in forest soils with low soil pH, we investigated soil acid phosphatase activity (APA) beneath the dominant tree species in the three forests. The related properties (SOC and soil available P, N and P stoichiometry in tree species) and their relationships to APA were then analyzed. Our objectives were to (1) examine whether APA or APA per unit SOC differs among soils underneath tree species in the three forests; (2) investigate the patterns of other indicators of P status along forest succession; (3) test the hypothesis that P limitation increases with forest succession.

Materials and methods

Site description

This study was conducted at the Dinghushan Biosphere Reserve (DBR) (23°09'21" N–23°11'30" N, 112°30'39" E–112°33'41" E) in southern China, about 90 km west of Guangzhou city and with an area of 1155 ha. It has a typical subtropical monsoon humid climate with a mean annual temperature of 21 °C. The lowest monthly mean temperature is 12.6 °C in January and the highest is 28.0 °C in July. The average annual precipitation is 1927 mm, of which about 80 % falls during the wet season between April and September. The bedrocks of DBR are classified as sandstone and shale belonging to the Devonian Period. Soils are classified in the Ultisol group and Udult subgroup according to the USDA soil classification system (Buol et al. 2003).

The reserve was established in 1950 to protect a remnant of undisturbed natural monsoon evergreen broadleaf forest (MEBF) in the subtropics. The pine forest (PF) was planted after clear cut in about 60 years

ago. A permanent plot of 10,000 m² was established in 1979. *Pinus massoniana* Lamb. is the predominant species in PF and accounts for about 90 % of total standing biomass in the permanent plot. The mixed pine and broadleaf forest (MF), about 90 years old, has developed from PF through natural succession. A permanent plot of 10,000 m² was established in 1979 on a south-facing slope at an altitude of 200–300 m above sea level. The dominant species are *Castanopsis chinensis* (Spreng.) Hance, *Pinus massoniana* and *Schima superba* Gardner et Champ.. They together account for over 90 % of total standing biomass in the permanent plot. The MEBF at this site has not been disturbed for more than 400 years according to ¹⁴C dating and many other research results in vegetation communities (Wang and Ma 1982; Shen et al. 1999, 2001; Chen et al. 2002; Zhou et al. 2006, 2007). A permanent plot of 10,000 m² was established in 1978 at an altitude of 200–300 m above sea level on a south-facing slope. The dominant species are *Acmena acuminatissima* (Blume) Merr. et Perry, *Castanopsis chinensis*, *Gironniera subaequalis* Planch., *Schima superba* and *Syzygium rehderianum* Merr. et L. M. Perry. They together account for over 65 % of total standing biomass. One N₂-fixing species, *Ormosia glaberrima* Y. C. Wu, is also commonly found in MEBF. The selected tree species in this study and further information of the three forests were presented in Table 1 and Table 2, respectively.

Field sampling

Measurements of soil respiration at these three forests suggested that belowground biological activity was the largest in July within a year (Yan et al. 2006b). All samples were thus taken in July 2010. For soil samples, the top 20 cm mineral soils were collected by a soil corer with a diameter of 2.5 cm. Five trees of similar age (five replicates) per species in each forest were randomly selected. The selected individuals of tree species common to the two or three forests also had the similar tree-age. Soils were taken at four locations beneath each tree approximately 20 cm from the stem, and then homogenized into one sample for each individual tree. Soil samples were also taken from five randomly selected undisturbed locations with at least 70 cm away from any tree stem in each forest, which represented the community level and were referred to bulk soils or control soils. All soil

samples were placed in sealed plastic bags and taken to the laboratory for analysis. In the laboratory, soil samples were screened using a 2 mm sieve and refrigerated at 4 °C before APA analysis within 7 days. A subsample of each soil sample was air dried for analyzing its chemical properties.

Fresh live leaves were collected from the medium-sized twigs of each selected tree of the three forests. All visible fine roots (<2 mm) belonged to the selected tree were picked from the collected soil samples. All leaf and root samples were dried in a 70 °C oven to constant weight, and ground prior to analysis.

Laboratory analysis

Soil organic carbon (SOC) was determined by titration with a Fe²⁺ solution after dichromate oxidation. Soil available P was extracted with an acid-ammonium fluoride solution (0.03 M NH₄F and 0.025 M HCl) (Bray and Kurtz 1945; Liu et al. 1996). In brief, 5 g of air-dried soil (<2 mm) was extracted with 50 ml of the acid-ammonium fluoride solution by shaking for 5 min. Samples were filtered through filter papers. P concentration in the filtrate was determined by molybdate colorimetry (Murphy and Riley 1962). The concentrations were expressed on an oven-dry soil weight basis. Gravimetric moisture content of a soil sample was determined by drying soils in oven at 105 °C for at least 72 h. APA was measured following the method of Schneider et al. (2000), using *p*-nitrophenylphosphate (*p*-NPP) as a substrate in a modification of the original method of Tabatabai and Bremner (1969). Enzymatic activity was expressed in μmol *p*-NP g⁻¹ h⁻¹ and μmol *p*-NP g⁻¹ h⁻¹ per unit SOC.

Leaf and root samples were digested by Kjeldahl digestion. N and P concentrations in the digestion solution were determined with a Flow Injection Analysis Automated Ion Analyzer (QuickChem 8000, LACHAT, USA) (Dong 1996). Mass N:P ratios (gN/gP) were used in our study.

Data analyses

All data were checked for normality of distributions and homogeneity of variances prior to analysis. ANOVA followed by LSD multiple comparison test was performed to test the effects of tree species on APA, soil available P, and N and P stoichiometry in

Table 1 Selected tree species in the three forests at Dinghushan Biosphere Reserve

Forest	Selected tree species
PF	<i>Pinus massoniana</i> (107149 kg, 89.0 %) ^a , <i>Schima superba</i> (<1204 kg, < 1.0 %)
MF	<i>Castanopsis chinensis</i> (94747 kg, 50.9 %), <i>Pinus massoniana</i> (34935 kg, 18.8 %), <i>Schima superba</i> (46471 kg, 25.0 %)
MEBF	<i>Acmena acuminatissima</i> (13304 kg, 5.2 %), <i>Castanopsis chinensis</i> (107719 kg, 41.7 %), <i>Gironniera subaequalis</i> (14587 kg, 5.6 %), <i>Ormosia glaberrima</i> (519 kg, 0.2 %), <i>Schima superba</i> (29998 kg, 11.6 %), <i>Syzygium rehderianum</i> (2841 kg, 1.1 %)

^a(the total standing biomass of the permanent plot with 10,000 m² in each forest, the ratio of the biomass of tree species to total standing biomass in the permanent plot in each forest)

PF pine forest, MF mixed pine and broadleaf forest, MEBF monsoon evergreen broadleaf forest

leaves and roots in each forest, and the significant differences in the variables between forests in the control soils or the same tree species. All relationships between variables were analyzed using Pearson correlation tests. Analysis of covariance was also used to test the fitted linear lines. For statistical tests, we chose a significance level of 0.05, above which the difference was considered to be not statistically significant. All statistical analyses were conducted using SPSS 16.0 for windows.

Results

Soil acid phosphatase activity

Soil APA in the bulk soils (or control soils) was $7.2 \pm 0.6 \mu\text{mol } p\text{-NP g}^{-1} \text{ h}^{-1}$ in PF, $13.0 \pm 2.3 \mu\text{mol } p\text{-NP g}^{-1} \text{ h}^{-1}$ in MF and $16.1 \pm 2.3 \mu\text{mol } p\text{-NP g}^{-1} \text{ h}^{-1}$ in MEBF. The differences among the three forests were statistically significant (Fig. 1a).

To analyze how species and different soils influenced APA, we compared the differences in APA underneath different species in the same forests and the same species in different forests.

The soil APA differed among tree species in each forest (Fig. 1a). In PF, the soils beneath *Schima superba* showed significantly higher APA than the soils beneath *Pinus massoniana* or in controls. In MF, APAs underneath *Pinus massoniana* or *Schima superba* were significantly higher than those under *Castanopsis chinensis* or in controls. In MEBF, the soils beneath tree species all exhibited high APAs when compared with the soils in the controls. APA was the highest under *Ormosia glaberrima*, followed by *Gironniera subaequalis* and *Schima superba*. The values under these three tree species were significantly larger than those under the other tree species and in controls. *Castanopsis chinensis*, whose biomass accounted for 42 %, showed the lowest soil APA among the tree species.

Soil APA beneath the same tree species also varied among different forests. *Schima superba*, a dominant species common to all three forests, had significantly larger APA in MEBF or MF than that in PF. The pioneering species, *Pinus massoniana*, which accounted for 89 % of total standing live biomass in PF and 19 % of total standing live biomass in MF (Table 1), showed significantly lower APA in PF than that in MF. The differences in APA under *Castanopsis chinensis* common to MF and MEBF ($13.0 \mu\text{mol } p\text{-NP g}^{-1} \text{ h}^{-1}$

Table 2 Soil chemical properties of the three forests at Dinghushan Biosphere Reserve

Forest	Soil pH	SOC (%)	Total N (mg g ⁻¹)	Total P (mg g ⁻¹)	Available P (mg kg ⁻¹)
PF	3.86±0.06	1.42±0.33 b	1.02±0.17 b	0.20±0.01 b	0.66±0.17 b
MF	3.86±0.10	2.28±0.63 ab	1.69±0.40 a	0.32±0.03 a	1.11±0.56 ab
MEBF	3.80±0.04	2.41±0.52 a	1.88±0.23 a	0.30±0.01 a	1.83±0.51 a

The mean±standard deviation followed by different lowercase letters has significant differences among the three forests by one-way ANOVA ($n=5$, $P<0.05$)

PF pine forest, MF mixed pine and broadleaf forest, MEBF monsoon evergreen broadleaf forest, SOC soil organic carbon

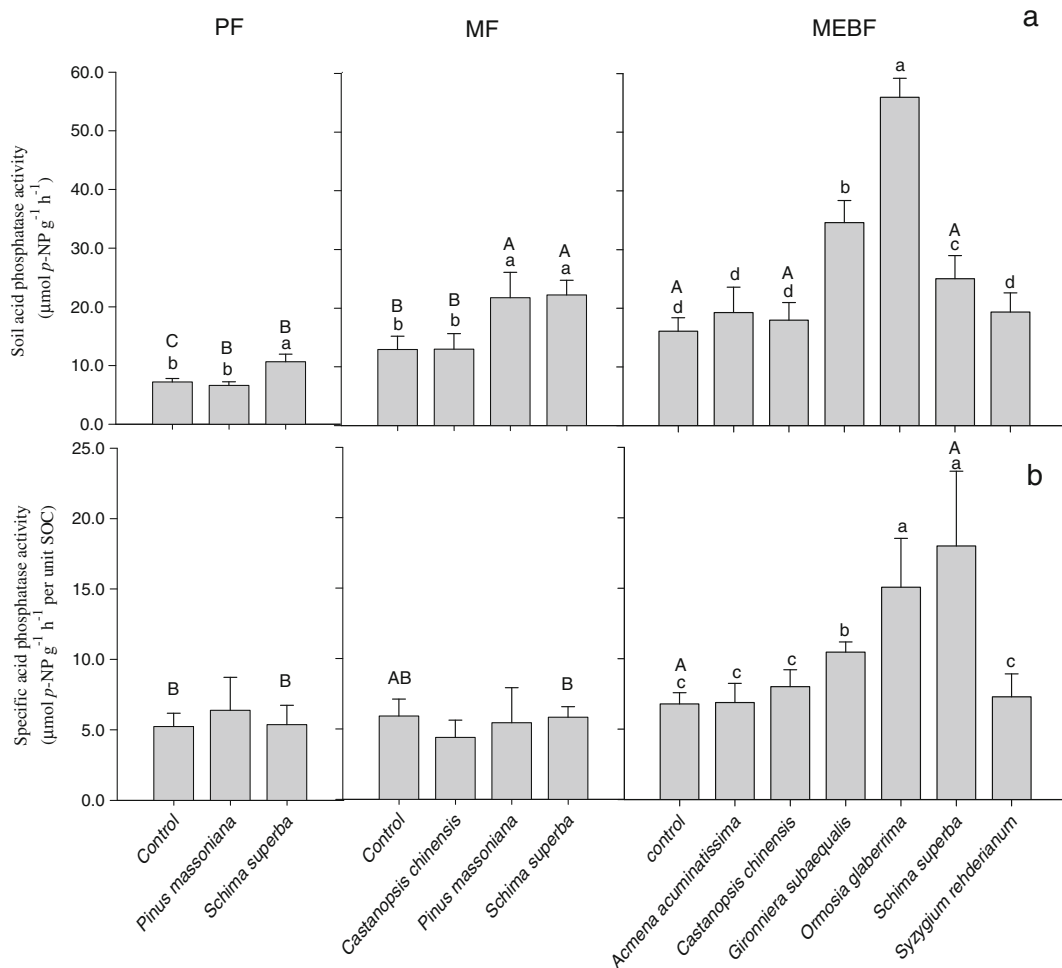


Fig. 1 Soil acid phosphatase activity (or soil acid phosphatase activity per unit soil organic carbon) in the bulk soils (controls) and under plant species in the three forests at Dinghushan Biosphere Reserve. Error bars represent standard deviation. **a** Soil acid phosphatase activity; **b** Soil acid phosphatase activity per unit soil organic carbon (SOC) (Specific acid phosphatase activity).

for MF and $18.0 \mu\text{mol } p\text{-NP g}^{-1} \text{ h}^{-1}$ for MEBF) also differed significantly.

As SOC differed among forests and plant species and was related to APA, we further calculated APA per unit SOC (specific APA) (Fig. 1b) in order to eliminate the influence of SOC on APA. Specific APA in the control soil in MEBF was significantly higher than in PF. There were no significant effects of tree species on specific APA in both PF and MF (Fig. 1b). However, specific APAs under *Schima superba*, *Ormosia glaberima* or *Gironniera subaequalis* in MEBF were significantly higher than those under other species and in the control. The species common to the three

Different lowercase letters denote significant differences among tree species and the control in each forest by one-way ANOVA ($n=5$, $P<0.05$). Different uppercase letters indicate significant differences between forests in the controls or the same species by one-way ANOVA ($n=5$, $P<0.05$). PF, pine forest; MF, mixed pine and broadleaf forest; MEBF, monsoon evergreen broadleaf forest

forests, *Schima superba*, exhibited the significantly higher specific APA in MEBF than in MF or PF.

Soil available P

Soil available P increased with forest succession, but only the difference between PF and MEBF was statistically significant (Table 2). In both PF and MF, soil available P in the controls was lower than those beneath tree species. However, in MEBF, soil available P in the control was significantly higher than those beneath tree species except for *Castanopsis chinensis* (Fig. 2).

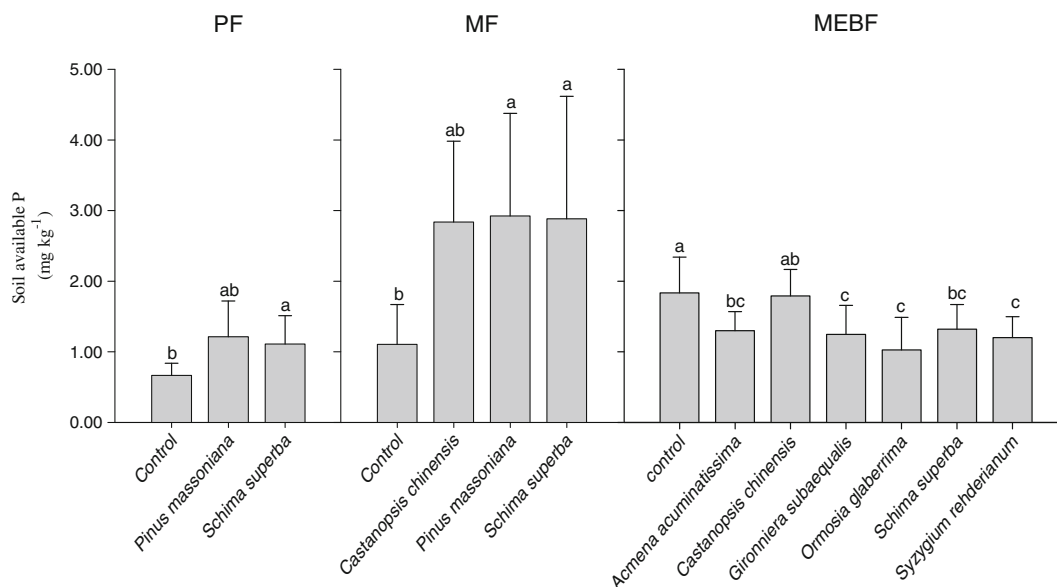


Fig. 2 Soil available phosphorus (P) in the bulk soils (controls) and under tree species in the three forests at Dinghushan Biosphere Reserve. Error bars represent standard deviation. Different lowercase letters denote significant differences among tree

species and the control in each forest by one-way ANOVA ($n=5$, $P<0.05$). PF, pine forest; MF, mixed pine and broadleaf forest; MEBF, monsoon evergreen broadleaf forest

N and P stoichiometry

N and P concentrations were relatively higher in leaves than in roots (Table 3). There were significant differences in N, P or N:P ratios in leaves and roots among the tree species in each forest. In PF, *Schima superba* had significantly lower foliar P and higher N:P ratios in leaves and roots than *Pinus massoniana*. When compared with *Castanopsis chinensis* and *Pinus massoniana* in MF, *Schima superba* also showed significantly higher N:P ratios in the leaves due to its higher N and lower P. In MEBF, the mean values of N:P ratios in leaves and roots were generally greater than 20. *Girroniera subaequalis* and *Ormosia glaberrima* showed relatively high N:P ratios in the leaves and roots because of their high N.

We also analyzed N, P or N:P ratios of leaves or roots of the same tree species in different forests, *Schima superba* in MEBF exhibited relatively lower P but higher N:P ratios in leaves and roots than that in PF or MF, although only the difference of P in roots between MEBF and PF was significant. N:P ratios in the roots of *Castanopsis chinensis* due to its high N in MEBF were significantly higher than those in MF. Foliar N:P ratios of *Pinus massoniana* in MF were significantly higher than those in PF, as a result of the much lower foliar P in MF.

Major drivers of APA

As showed in Fig. 3, *Ormosia glaberrima* had significantly higher APA for a given SOC or available P, and thus the relationships between APA and SOC or available P were examined by excluding *Ormosia glaberrima*. Significant correlations were found between APA and SOC ($R^2=0.27$, $P<0.01$), but not between APA and available P. Regression analysis showed that the intercepts of the linear regressions between APA and SOC were significantly different but the slopes were not among the three forests (Fig. 3a). The intercept of the regression line was for $4.3 \mu\text{mol } p\text{-NP g}^{-1} \text{ h}^{-1}$ for PF, $6.9 \mu\text{mol } p\text{-NP g}^{-1} \text{ h}^{-1}$ for MF and $12.9 \mu\text{mol } p\text{-NP g}^{-1} \text{ h}^{-1}$ for MEBF.

When data from all trees sampled from the three forests were pooled together, we found that soil APA was significantly and positively correlated with N or N:P ratios in the leaves and roots. Because of the very high N content in the leaves or roots of *Girroniera subaequalis* and *Ormosia glaberrima* (Fig. 4), the dataset excluding these two tree species were further examined, and we found that soil APA was significantly associated with P or N:P ratios in the leaves and roots.

Table 3 N and P stoichiometry in leaves and roots of tree species in the three forests at Dinghushan Biosphere Reserve

Organs	Forests	Tree species	N (mg g ⁻¹)	P (mg g ⁻¹)	N:P ratios
Leaves	PF	<i>Pinus massoniana</i>	17.8±3.0	1.30±0.21 a	13.8±1.8 b
		<i>Schima superba</i>	18.9±1.4	0.76±0.07 b	24.8±1.3 a
	MF	<i>Castanopsis chinensis</i>	18.6±0.8 a	0.93±0.06	20.1±1.6 b
		<i>Pinus massoniana</i>	16.3±1.7 b	0.92±0.07	17.8±2.2 b
		<i>Schima superba</i>	19.9±1.6 a	0.82±0.13	24.4±2.2 a
	MEBF	<i>Acmena acuminatissima</i>	22.7±3.6 c	1.05±0.14 a	21.9±4.0 bc
		<i>Castanopsis chinensis</i>	19.2±1.5 d	0.96±0.21 a	20.6±3.7 c
		<i>Gironniera subaequalis</i>	41.3±0.9 a	1.16±0.05 a	35.8±2.0 a
		<i>Ormosia glaberrima</i>	27.1±1.3 b	0.75±0.10 b	36.8±5.2 a
		<i>Schima superba</i>	18.4±2.3 d	0.70±0.09 b	26.2±1.6 b
		<i>Syzygium rehderianum</i>	14.8±1.0 e	0.59±0.06 c	25.1±0.9 b
Roots	PF	<i>Pinus massoniana</i>	8.1±2.4	0.43±0.12	19.2±3.6 b
		<i>Schima superba</i>	9.8±2.3	0.40±0.07	24.2±2.4 a
	MF	<i>Castanopsis chinensis</i>	8.1±0.9	0.41±0.15	21.4±6.2
		<i>Pinus massoniana</i>	7.1±1.1	0.32±0.04	22.3±1.0
		<i>Schima superba</i>	8.2±1.2	0.34±0.10	25.7±6.9
	MEBF	<i>Acmena acuminatissima</i>	9.3±1.6 cd	0.47±0.11 a	20.1±2.5 d
		<i>Castanopsis chinensis</i>	10.3±1.9 c	0.34±0.05 bc	30.0±2.4 c
		<i>Gironniera subaequalis</i>	29.3±7.1 a	0.42±0.09 ab	69.8±3.5 a
		<i>Ormosia glaberrima</i>	13.9±2.3 b	0.39±0.07 bc	36.0±4.7 b
		<i>Schima superba</i>	8.1±1.0 de	0.30±0.04 c	27.7±4.8 c
		<i>Syzygium rehderianum</i>	7.2±0.6 e	0.36±0.04 bc	20.0±2.2 d

The mean±standard deviation followed by different lowercase letters has significant differences among the tree species in each forest by one-way ANOVA ($n=5$, $P<0.05$)

PF pine forest, MF mixed pine and broadleaf forest, MEBF monsoon evergreen broadleaf forest

Discussion

Changes in soil acid phosphatase activity beneath tree species in the successional forests

Previous studies suggest that NPP of many tropical forests will peak at an age of 30 to 120 years, then decline (Pregitzer and Euskirchen 2004; Wardle et al. 2004). This decline is also observed in the mature forest, MEBF, over the last three decades (Tang et al. 2011). The exact causes for the decline in MEBF, however, remain elusive. Studies in the reserve have showed that MEBF was more likely limited by P rather than by N through the experiment of litter decomposition in response to N addition and the observation of foliar N and P of the most dominant species in the three forests (Mo et al. 2006; Huang et al. 2011). However, the results from previous studies in these three forests cannot be considered as being

conclusive due to the response of plants to nutrient limitation differing from that of the litter decomposer and the changing species composition during forest succession (Read 1993; Aerts and Chapin 2000).

Our study has overcome the above-mentioned shortcomings of previous studies by directly measuring the soil APA beneath the tree species. Phosphatase is an inducible enzyme and can vary with the demand for P by plant roots and soil microbes (Juma and Tabatabai 1978; Spiers and McGill 1979; Chen et al. 2008). Our results showed that APA in the bulk soils increased from early to late successional forests and the values underneath tree species in the early successional forest were relatively low. This indicates that the requirement for P by plants and/or microbes increased during forest succession. Species replacement is a characteristic of forest succession. Within the three forests, a few early successional species are able to persist through to middle or late succession (Odum

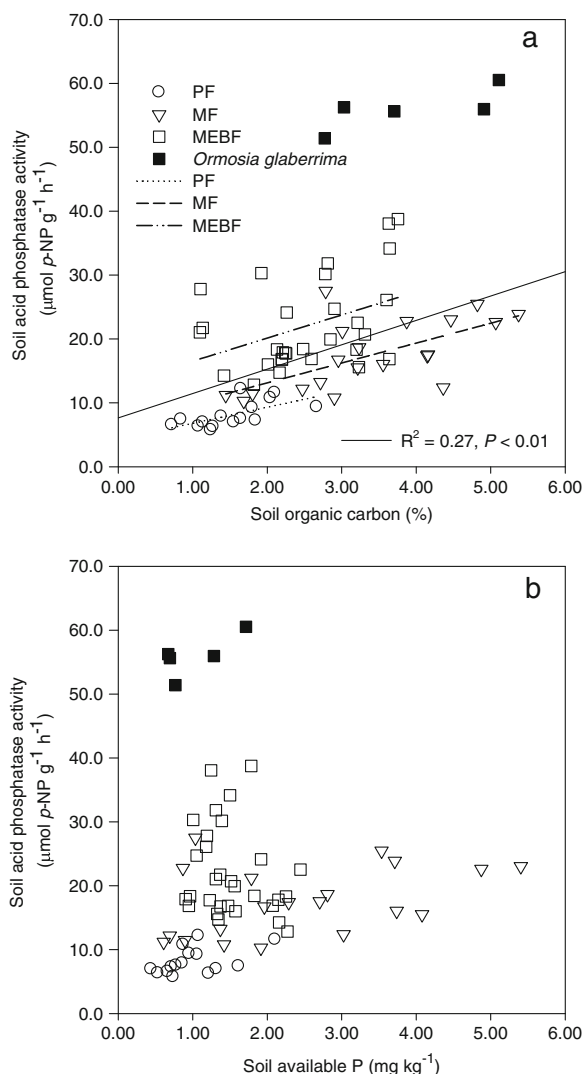


Fig. 3 The correlations between soil acid phosphatase activity and soil organic carbon **a** or available P **b** in the three forests at Dinghushan Biosphere Reserve. PF, pine forest; MF, mixed pine and broadleaf forest; MEBF, monsoon evergreen broadleaf forest. Linear regressions were estimated using the data excluding *Ormosia glaberrima*. (Respective relationships between soil acid phosphatase activity and soil organic carbon for the three forests: PF, $y=2.51x+4.3$; $R^2=0.41$; $P<0.01$; MF, $y=3.11x+6.9$; $R^2=0.41$; $P<0.01$; MEBF: $y=3.62x+12.9$; $R^2=0.16$; $P<0.05$)

1969), such as *Pinus massoniana*, *Schima superba* and *Castanopsis chinensis*. Although different species may have different responses to P limitation, the same species are supposed to consistently respond to P limitation across the three forests. Our results showed that all these tree species had higher APA in the later successional forest than in the earlier one, indicating

that competition for P by plants was greater in the later stages of forest succession. This mechanism of causing high flux of available P is also a compensation for P availability in soils where the competition for P is great.

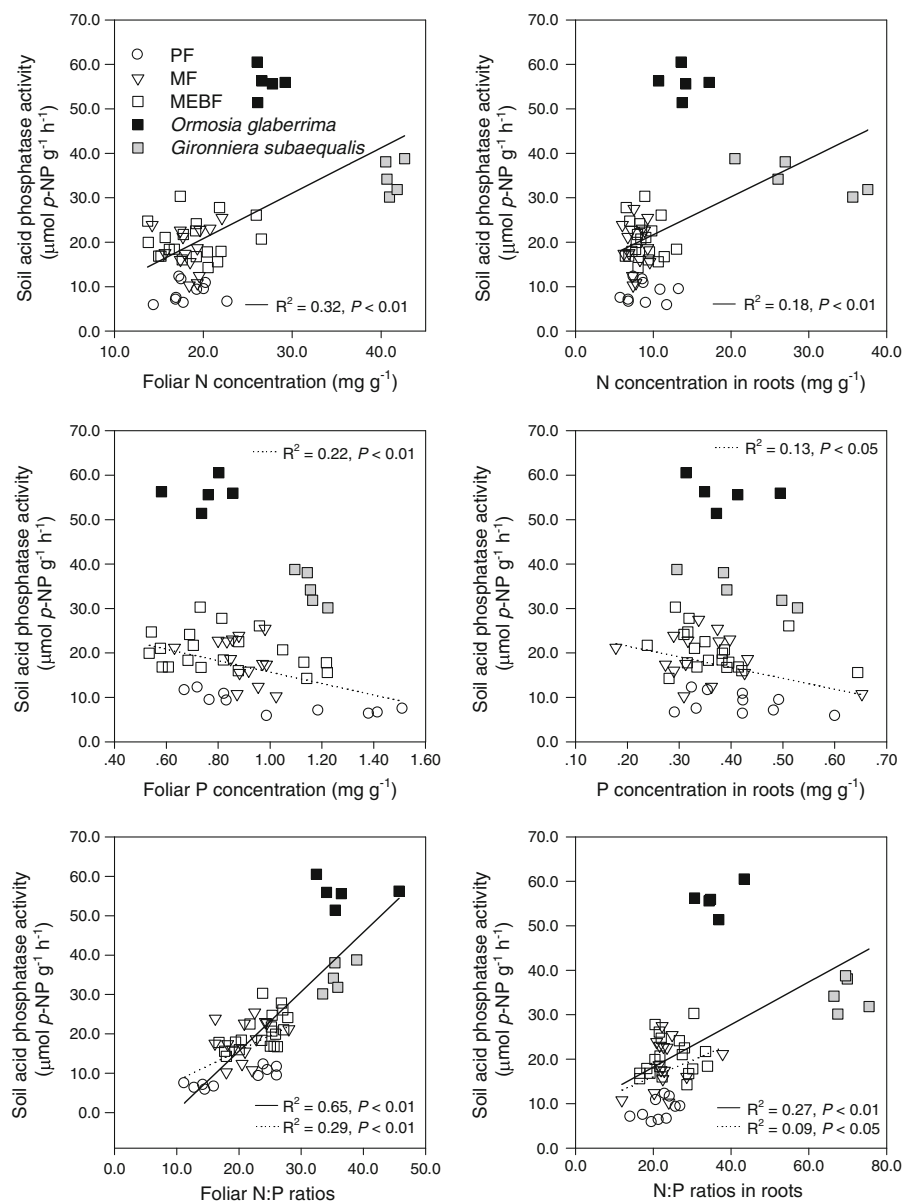
As APA is largely dependent on soil properties, such as SOC (Speir and Ross 1978), it appears that the great SOC in MEBF partly resulted in high APA. However, our results showed that soil APAs per unit SOC were relatively high in MEBF and the influences of tree species on them were obvious. To some extent, this was consistent with the pattern of soil APA in the three forests. Moreover, the comparison among the intercept values of the regression lines by fitting correlations between APA and SOC in the three forests can also provide some information on how much of the total APA measured is independent of SOC. The greater intercept of the regression line in MEBF further demonstrates that the requirement for P by organisms in MEBF is the greatest among the three forests.

Other indicators of P status along the successional forests

Soil available P is often used as a direct indicator of P-supply limitations to plant growth (Smethurst 2000). Our study found no significant correlation between APA and soil available P (Fig. 3b). As Olander and Vitousek (2000) pointed out, the range of P supply would never be high enough to trigger inhibition if the levels of P supply were below the threshold level of demand for P by plants. Furthermore, unlike PF and MF, MEBF showed significantly higher soil available P in the bulk soils than those beneath tree species except for *Castanopsis chinensis*. This pattern suggests that the supply of available P is intensively depleted by plants in MEBF.

Apart from APA, the N:P ratio is found to be a good indicator of whether N or P is more limiting to plant productivity (Güsewell 2004). Our results showed a pattern of higher N:P ratios of tree species in the later successional forests. The N:P ratios in MEBF were generally more than 20. If we accepted the proposal by Güsewell (2004) that N:P ratios <10 and >20 often correspond to N- and P-limited biomass production for vegetation, the growths of tree species in MEBF were more limited by P than by N. We found significant correlations between APA and N:P ratios in leaves and roots whether *Gironniera subaequalis* and

Fig. 4 The correlations between soil acid phosphatase activity and N and P stoichiometry of tree species. PF, pine forest; MF, mixed pine and broadleaf forest; MEBF, monsoon evergreen broadleaf forest. Linear regressions were estimated using the whole dataset (solid lines) or the dataset excluding *Ormosia glaberrima* and *Girroniera subaequalis* (dotted lines)



Ormosia glaberrima were excluded or not. The results support the generalization that either N:P ratios or APA can indicate P limitation.

Intensified competition for P among tree species in the old-growth forest

Our results indicate that the competition for P among tree species was intense in the old-growth forest. *Girroniera subaequalis* and *Ormosia glaberrima*, which are small trees and not as productive as the dominant tree species, can have the capability to invest

N into P acquisition and result in relatively high APA because of high N content in leaves or roots (Treseder and Vitousek 2001; Houlton et al. 2008; Marklein and Houlton 2012). However, our results showed that soil available P was relatively low under the two species, suggesting that soil available P was aggressively scavenged from soils after the mineralization. On the other hand, *Castanopsis chinensis*, whose biomass accounts for more than 40 % of total standing biomass, was less capable for acquiring soil P as those N-rich tree species in the P-limited environment, therefore became more P-limited. The imbalance of N and P in *Castanopsis*

chinensis may be responsible for the observed increase in its mortality, which can largely explain the observed decline in NPP of MEBF over the last two decades (Tang et al. 2011).

Conclusions

In conclusion, P requirement by plants increased from early to late successional forests, which was reflected from increasing APA (or APA per unit SOC) in bulk soils from PF to MEBF and relatively low APAs (or APAs per unit SOC) under tree species in the early successional forest. APAs under tree species of later successional stages were higher than those in the same species of earlier successional stages. These reflect that competition for P among tree species becomes greater with forest succession. The results from other related properties in soils and plants, such as soil available P and N:P ratios in plant organs, and their relationships with APA also support an increasing limitation by P from early to late successional forests. In MEBF, the most abundant tree species with less efficient P acquisition strategy suffered more from P limitation than other N-rich small tree species, and eventually resulted in a decline in the total net primary production of the forest.

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