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Original article

Nitrogen and phosphorus status and their influence on aboveground production under increasing nitrogen deposition in three successional forests[☆]

Wen-Juan Huang^{a,b}, Guo-Yi Zhou^{a,*}, Ju-Xiu Liu^a^aSouth China Botanical Garden, Chinese Academy of Sciences, Xingke Road 723, Tianhe District, Guangzhou 510650, China^bGraduate University, Chinese Academy of Sciences, Beijing 100049, China

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ABSTRACT

Compared with temperate ecosystems, tropical ecosystems will respond differently to increasing nitrogen (N) deposition due to indirect effects on the availability of phosphorus (P). Data in this study were collected from the long-term research plots and controlled experiments in a succession series, including 50-year-old pine forest (PF), 80-year-old mixed pine and broadleaved forest (MF), and more than 400-year-old monsoon evergreen broadleaved forest (MEBF), in southern China to study growth patterns and biogeochemical processes. The results showed that soil total N and available N were significantly higher in MEBF than in the other two forests. Soil available P was significantly lower in MEBF than in PF though the total P was the same. The N/P ratios in living leaves were increasing with succession stages. The standing biomass and productivity of MEBF have been declining over the last 30 years. Our findings suggest that the high N stock in soil pools through atmospheric deposition and self-accumulation in MEBF would require more available P to prevent deficiency that would limit plant growth. However, as more organic matter accumulated and thus, more P was bound in MEBF, there was much less available P in soils. These processes result in significantly higher N/P ratios in living leaves of the old-growth forest, which may be responsible for the decline.

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1. Introduction

Enhancement of nitrogen (N) deposition is a widespread global issue. It is estimated that human activities have more than doubled inputs of N to terrestrial ecosystems worldwide through fertilizer consumption, fossil-fuel emissions and biomass burning (Matson et al., 2002). Even the temperate forests that have been traditionally considered to be N limited (Vitousek and Howarth, 1991; Lu et al., 2004) are receiving excessive amounts of N deposition in some areas (Aber et al., 1998, 2003; Fenn et al., 1998; Phoenix et al., 2006). Correspondingly, tropical forests that are generally recognized as N rich but phosphorus (P) poor (Hedin, 2004; Reich and Oleksyn, 2004; Townsend et al., 2007) may become N saturated (Mo et al., 2003, 2006, 2008; Fang et al., 2007). The consequences of anthropogenic inputs of N into tropical and subtropical forests have aroused wide interest. Compared with temperate ecosystems, it is expected that tropical ecosystems will respond differently to increasing N deposition and are unlikely to increase and possibly to

decrease productivity due to indirect effects on acidity and availability of P and cations (Matson et al., 1999).

Correlations between foliar nutrient concentration and soil nutrient availability have been found in many ecosystems (Vitousek, 1982, 1984; Vitousek and Farrington, 1997; Hobbie and Gough, 2002), which implies that increasing N in the environment may eventually result in enhancement of foliar N concentration. These relationships have also led to the use of foliar nutrients as an index of nutrient status and to the prediction of broad-scale patterns in ecosystem processes (Townsend et al., 2007). When many species are examined, N and P concentrations in leaves are always correlated positively at a very significant level (Garten, 1976; Bedford et al., 1999; Güsewell and Koerselman, 2002; Güsewell, 2004). A higher foliar N concentration would require more available P to prevent deficiency that would limit plant growth. Thus, a growing focus on ecological stoichiometry has led to a broader use of the N/P ratio in leaves as a measure of potential nutrient limitation in terrestrial net primary productivity (Tessier and Raynal, 2003; Güsewell, 2004; Wassen et al., 2005; Güsewell and Verhoeven, 2006; Townsend et al., 2007). Güsewell (2004) has suggested that, at a vegetation level, N/P ratios <10 and >20 correspond to N- and P-limited biomass production, respectively. Phosphomonoesterase (PME) activity has also been

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* Corresponding author. Tel.: +86 20 37252708; fax: +86 20 37252615.

E-mail address: gyzhou@scib.ac.cn (G.-Y. Zhou).

proposed as indicators of P limitation (Johnson et al., 1998; Gress et al., 2007). Johnson et al. (1999) found that root-associated PME activity was significantly increased when plants were transplanted into soils with N additions for seven years. Gress et al. (2007) suggested that an increase in root-associated PME activities is a sufficient indicator of P limitation, although such a response is not always necessary to meet the P requirements.

Long-term monitoring on ecosystem processes of three key tropical vegetation types in Dinghushan Biosphere Reserve (DBR) has been conducted since 1978 (Zhou et al., 2006a) and experiments of N and P addition have also been implemented since 2002 and 2003 (Mo et al., 2003, 2006, 2008; Fang et al., 2007). The three vegetation types are pine forest (PF) (pioneer seral), mixed pine and broadleaved forest (MF) (successional seral) and monsoon evergreen broadleaved forest (MEBF) (climax). Preliminary results have revealed differences in N availabilities in PF, MF and MEBF (Mo et al., 2003). The soil N availability increases with vegetation succession from PF to MEBF. The response of litter decomposition rates to N additions varies in the three ecosystems (Mo et al., 2006; Fang et al., 2007). N addition to MEBF caused a reduction in soil respiration, litter decomposition and NPP (Mo et al., 2006, 2008).

Although the mechanism of direct damage on trees and forest stands by emissions of gaseous pollutants, including NO_x, has been well addressed (Blank, 1985; Blank et al., 1988; Schulze, 1989), how the gradual enhancement of N deposition influences the stability of old-growth forests has not been quantified, especially for tropical forests. Understanding the dynamics of old-growth forests and their causal factors is important for assessing the capacity for carbon sequestration and other ecological functions in the tropics.

Here, we use data from long-term measurements and controlled experiments on ecosystem processes of PF, MF and MEBF in DBR to analyze the status of N and P during the succession of vegetation development. We hypothesize that: (1) the increasing atmospheric N deposition would lead to different N and P status in the three successional forests; and (2) the imbalance of N and P status could cause the different changes in standing biomass and productivity in the three forests.

2. Materials and methods

2.1. Site description

The research 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, covering an area of 1155 ha. Due to its location on the periphery of the Pearl River Delta, one of the most concentrated industrialized areas in China, DBR is subject to considerable atmospheric N deposition (Zhou and Yan, 2001). DBR was established in 1950 to protect natural monsoon evergreen broadleaved forests (MEBF) in the south subtropics and was acknowledged as the first National Natural Reserve in China in 1956 (Zhou et al., 2006b). In addition to the MEBF, pine (*Pinus massoniana* Lamb) forest (PF) and mixed pine and broadleaved forest (MF) also exist in the reserve, representing the early and mid-successional stages of MEBF (Table 1). The research sites of the three forest types are less than 500 m from one another. Annual precipitation is 1680 mm, concentrated between April and September. Annual mean relative humidity and temperature are 78% and 22.3 °C. The bedrock is sandstone and shale. Soils, with pH 4.0–4.9, are classified in the ultisol group and adult subgroup according to USDA soil classification system (Buol et al., 2003).

PF occurs in the periphery of the reserve. An experimental site of 4 ha is located on a south-facing slope at an altitude of about 200 m above sea level (*a.s.l.*), in which a permanent plot of 1 ha was

Table 1
Summary of characteristics of the three forests.

| Forest types | PF | MF | MEBF |
|---|-------------------|-------------------|-------------------|
| Elevation (<i>a.s.l.</i>) (m) | 170–220 | 200–300 | 200–300 |
| Terrain | Hilly | Hilly | Hilly |
| Annual precipitation (mm) | 1670 | 1680 | 1700 |
| Mean annual temperature (°C) | 22.8 | 22.2 | 21.7 |
| Soil family | Ultisol | Ultisol | Ultisol |
| Soil pH | 3.94 | 3.81 | 3.65 |
| SOC content (0–20 cm) (g Kg ⁻¹) | 11.3 | 16.5 | 25.4 ^a |
| SOC stock (0–20 cm) (g m ⁻²) | 2806 | 3247 | 4633 ^a |
| SOC content (0–60 cm) (g Kg ⁻¹) | 7.4 ^b | 11.3 ^b | 17.5 ^b |
| SOC stock (0–60 cm) (g m ⁻²) | 4659 ^b | 6252 ^b | 8829 ^b |
| STN (0–20 cm) (g Kg ⁻¹) | 0.60 | 0.96 | 1.32 |
| STP (0–20 cm) (g Kg ⁻¹) | 0.24 | 0.31 | 0.26 |

SOC, soil organic carbon; STN, soil total N; STP, soil total P.

^a Source: Zhou et al. (2006a).

^b Source: Zhou et al. (2006b).

established in 1979. The forest, about 50 years old, was initially a single species stand of *P. massoniana* which was gradually invaded by *Schima superba* Gardn. et Champ. Nevertheless, *P. massoniana* was the predominant species in the study period.

MF is distributed between the core area and periphery of the reserve. The forest, about 80 years old, has developed naturally from PF. Three types of MF plots with different succession status were permanently set up in 1979, 1994 and 1999 on a south-facing slope at an altitude of 200–300 m *a.s.l.* Each plot is 1 ha. The upper canopy dominated by *S. superba*, *Castanopsis chinensis* (Spreng.) Hance, and *Craibiodendron kwangtungense* (S.Y.Hu) Judd. *P. massoniana* accounts for about 35% of the biomass of the community.

MEBF is distributed in the core area of the reserve, which has been strictly protected since its establishment. The forest has not been disturbed for more than 400 years (Wang and Ma, 1982; Zhou et al., 2006a). An experimental site of 8 ha is located at an altitude of 200–300 m *a.s.l.* on a south-facing slope, in which a permanent plot of 1 ha was established in 1979. The upper canopy is dominated by a small number of individuals, including *C. chinensis*, *Canarium album* (Lour.) Rauesch, *S. superba*, and *Engelhardtia roxburghiana* Wall. The sub-canopy is mainly composed of *Cryptocarya concinna* Hance and *Machilus chinensis* (Champ. ex Benth.) Hemsl.

Each permanent plot was subdivided into 100 subplots each of 100 m². All subplots in each plot were numbered.

Nitrogen addition experiments were initiated within each of the three forest types in 2002–2003. N was applied as NH₄NO₃ mixed with 20 L water. Four N addition treatments (control: without N added; low-N: 5 g N m⁻² y⁻¹; medium-N: 10 g N m⁻² y⁻¹ and high-N: 15 g N m⁻² y⁻¹) were established in MEBF, and three treatments were established in MF and PF (control, low-N and medium-N) (Mo et al., 2006, 2008; Fang et al., 2007). Each treatment had three replicates. Each plot measured 20 m × 10 m, with a 10 m wide buffer around it. All plots and treatments were randomly laid out. N applications were made monthly using a backpack sprayer.

2.2. Monitoring of rainfall and N deposition

Rainfall data have been recorded manually or automatically from a weather station located on a low grass-covered hilltop near the south-eastern corner of the reserve since DBR was established in 1956. Monthly samples for total N content in precipitation (N wet deposition) and collection of dry deposition with the instrument of APS-2B were conducted in 1989, 1994, 1998, 1999, 2002, 2004, 2005 and 2007. The collected dry deposition was dissolved in high purity water. Total N was determined using K₂S₂O₈ oxidation and UV spectrophotometry.

2.3. Foliar sampling

In 1992, 1996, 1999 and 2004, leaves of the species whose biomasses were larger than 1% of the total were collected in MEBF to measure the nutrient contents, including N and P. After finding the trend toward an increase in N/P ratios in living leaves, we initiated an intensive foliar sampling campaign in 2006 and 2008. Leaves of almost all species in the arbor, small arbor, shrub and herbaceous layers of the three communities were included. Samples were collected from at least five individuals of each species when possible. If there were less than five individuals available in a forest for a few species (*Tsoongiodendron odorum* Chun and 6 newly invaded species), more than one sample was collected from an individual, so that at least five samples were obtained to ensure sufficient replication for each species in each forest.

In 2009, leaves of all species in the N addition experiment plots were also sampled in the same way as above.

Each sample was made up of about 100 g of vigorous living leaves from a moderate twig in an individual for arbor and small arbor layers, from a whole individual for the shrub layer and from several adjacent individuals for the herbaceous layer. The sampled leaves were iced at once and transported to laboratory in a day for chemical measurements.

2.4. Litterfall sampling

Fifteen litterfall traps of 1 m² were placed randomly in each plot. Litterfall has been collected once a month since 1981. Unfortunately, some data were lost (in 1992 and 1993 for MEBF, in 1981 and 1983–1991 for MF and in 1981, 1992 and 1999 for PF) (Zhou et al., 2007).

2.5. Soil sampling

To monitor the long-term dynamics of soil available N (soil water dissolvable N) and available (labile) P, soil samples were collected to a depth of 20 cm in the mineral layer using a 30 mm diameter auger. After the organic layer of 1–2.5 cm in depth was excluded, entire mineral soil in the top 20 cm layer was collected. In each permanent plot, 20 randomly located subplots were selected every sampling time. In each subplot, five cores of entire mineral soil in the top 20 cm layer were taken and mixed to make up a composite sample, resulting in 20 composite samples for each sampling time and each forest type (each permanent plot). All samplings were done in September–October when the transition from wet to dry seasons occurs. Samples were collected in 1992, 1994, 1999 and every year in 2005–2008 for MEBF, in 1994, 1996, and every year for 1999–2001, and 2003–2008 for both MF and PF. After air-drying, plant residues were carefully picked out, and the soil samples were then milled to pass a sieve with a mesh of 93,000/m².

Soil bulk densities were measured in 1992, 1996, 2001 and 2005. Soil cores were taken from five randomly selected undisturbed locations within each of 8–20 randomly selected subplots using a stainless steel corer (5.65 cm in diameter, 4 cm in depth, and 100 cm³ in volume). All of the sample cores within each subplot were pooled together and oven-dried at 105 °C to constant weights. Soil bulk density was calculated as the ratio of total dry weight to total soil volume (Zhou et al., 2006a).

2.6. Biomass inventory

The diameter at breast height (DBH), height, coordinates in the plots, and other measures of growth state for each tree with DBH >1 cm were monitored when the plots were set up. Surveys were done in 1978, 1982, 1992, 1994, 1999, 2004 and 2008 for MEBF, in

1978, 1982, 1984, 1987, 2004 and 2008 for MF and in 1982, 1990, 1995, 2002, 2004 and 2008 for PF. The biomass was estimated according to the empirical equations (Liu et al., 2009).

2.7. Estimation of aboveground net primary productivity (ANPP)

Annual ANPP was estimated using the equation:

$$\text{ANPP}_i = L_i + B_{fi} + C_i \quad (1)$$

Where, ANPP_{*i*} is annual ANPP in the year *i*, *L_i* is annual litterfall in the year *i*, *B_{fi}* is net flux of aboveground standing biomass in the year *i*, and *C_i* is annual influx of CWD in the year *i* that is calculated as follows:

$$C_i = D + \frac{\text{CWD}_k - \text{CWD}_j}{k - j} \quad (2)$$

Where, *C_i* is the CWD influx in year *i*; *D* is the yearly averaged CWD decomposition rate determined through CWD decomposition experiments (Yang et al., 2010); *CWD_k* and *CWD_j* are the standing CWDs in year *k* and *j* (*j* < *k*, *j* ≤ *i* ≤ *k*).

2.8. Chemical analysis and N/P ratio calculations

Litter and leaf samples were digested with concentrated sulfuric acid (H₂SO₄) and perchloric acid (HClO₄). N and P concentrations in the digestion solution were determined with a Flow Injection Analysis Automated Ion Analyzer (QuickChem 8000, LACHAT, USA) (Dong, 1996).

Soil available N (the sum of NH₄-N, NO₃-N, amino-N, acyl-amine-N and labile protein-N) was deoxidized and hydrolyzed into ammonia with FeSO₄. The ammonia was absorbed by H₃BO₃ solution and then titrated with sulfuric acid (Liu, 1996). Soil available P was extracted with ammonium fluoride (NH₄F, 0.03 mol L⁻¹) and hydrochloric acid (HCl, 0.025 mol L⁻¹) and measured by vis-spectrophotometer (Spectrumlab 24, Shanghai, China) (Liu, 1996).

2.9. Measurement of PME activity

In October, 2008 (dry season) and June, 2009 (wet season), rhizosphere soils were sampled in mineral layers, 0–10 cm and 10–20 cm mineral layers. The two sampling campaigns were conducted in the same 20 randomly selected subplots of each plot. Acid PME activity was measured according to the method of Schneider et al. (2000), using *para*-nitrophenylphosphate (*p*-NPP) as substrate as modified from the original method of Tabatabai and Bremner (1969). One gram of fresh soil was weighed 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) and 1 ml of 100 mM *p*-NPP substrate dissolved in buffer. After incubation, the flasks were immediately placed on ice and then 1 ml of 2 M CaCl₂ and 4 ml of 0.2 M NaOH were added to terminate the reaction and to extract the *p*-NP formed. The samples were diluted with 90 ml of deionized water, and then filtered through Whatman-42 filter paper. Further dilutions were made when acid PME activity levels were too high. The absorbance of the released *para*-nitrophenol (*p*-NP) was determined spectrophotometrically at 400 nm. Four replicates including one blank were used for each soil. For the blanks, *p*-NPP was added after (instead of before) the incubation.

2.10. Statistical analysis

We used the weighted least squares method to obtain a linear fit of trends of N deposition in DBR, N/P ratios in leaves and litterfall of MEBF, standing biomass, litterfall production and ANPP of the three

forest types. If the slopes of fitted linear lines are significantly different from zero (t -test: $P < 0.05$), the trends are considered to be statistically significant.

ANOVA was performed on N/P ratios in living leaves to determine the statistical difference between forest types at species and community levels. Repeated measures ANOVA (SAS Institute, 2003) was performed to examine the differences of soil water dissolvable N and available P in mineral soils among the three forests for the period between 1990s and 2000s.

Correlations between P and N concentrations in leaves and litter were analyzed with Pearson correlation tests using the SAS CORR procedure.

3. Results

3.1. Atmospheric N deposition

Total N deposition was at a high level (about $4.6 \text{ g N m}^{-2} \text{ yr}^{-1}$) after 1989 (Fig. 1). The dry deposition amounted to only 25% of the total; however, it was significantly ($P < 0.001$) increasing at the rate of $0.019 \text{ g N m}^{-2} \text{ yr}^{-1}$ after the initial measurement in 1989. Wet deposition amounted to 75% of the total. The N concentration per unit rainfall was significantly ($P < 0.0001$) increasing at the rate of $2.5 \times 10^{-5} \text{ g N m}^{-2} \text{ mm}^{-1}$.

3.2. N and P concentrations and N/P ratios in living leaves and litterfall

There was a significant relationship between P and N concentrations in standing leaves ($P < 0.0001$) (Fig. 2a) and litterfall ($P < 0.001$) (Fig. 2b). The fitted correlations between P and N were significantly different among the three communities, which were reflected from the slopes of the regression lines. Only the slope of the regression line of MEBF (19.2 ± 3.0) was significantly ($P < 0.05$) greater than that of PF (11.8 ± 2.9) and no significant difference existed between the slopes for MF (16.4 ± 3.5) and PF. From this, the N/P ratios of standing leaves were 21.8, 25.0 and 26.0 for the living leaves of arbor and small arbor species in PF, MF and MEBF, respectively (Fig. 2c).

Both the averaged concentration-based N/P ratios (the ratio of foliar N and P concentration) and leaf stock-based N/P ratios (the ratio of foliar N and P biomasses) were increasing with community succession (Table 2). The differences in concentration-based N/P ratios among the three communities were all significant ($P < 0.05$). When using the leaf stock-based N/P ratios, the significant differences were even larger ($P < 0.0001$).

There were 25 species common to MEBF and MF, whose biomasses amounted to 71% and 66% of the two stands, respectively.

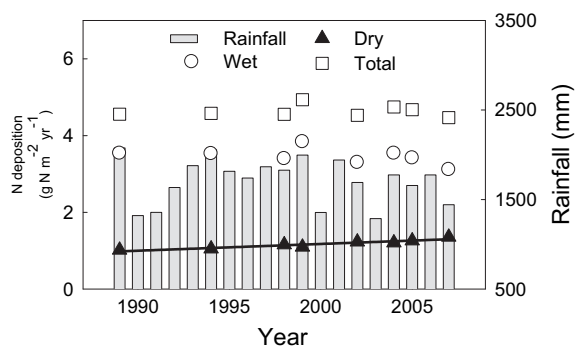


Fig. 1. Historic rainfall and N input through wet and dry deposition at Dinghushan Biosphere Reserve. Linear regressions: dry deposition: $y = 0.018x - 34.04$, $R^2 = 0.88$, $n = 8$, $P = 0.0006$.

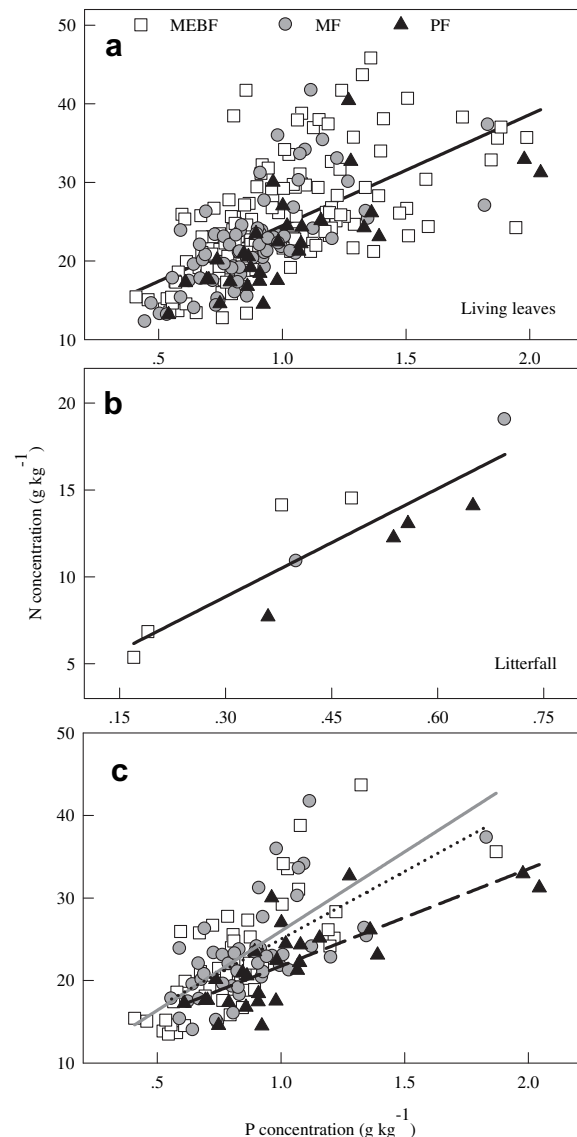


Fig. 2. Relationship between P and N concentrations in living leaves and litterfall based on measurements in 2006 and 2008. (a): in living leaves of all species of the three forests, including the common species ($y = 14.14x + 10.34$, $R^2 = 0.41$, $n = 243$, $P < 0.0001$); (b): in litterfall of most dominant species of the three forests ($y = 20.72x + 2.64$, $R^2 = 0.77$, $n = 10$, $P = 0.0008$); (c): Respective relationships between P and N concentration in the living leaves of arbor and small arbor species for the three forests (MEBF: $y = 19.20x + 6.79$, $R^2 = 0.55$, $n = 49$, $P < 0.0001$; MF: $y = 16.43x + 8.61$, $R^2 = 0.41$, $n = 47$, $P < 0.0001$; PF: $y = 11.78x + 9.98$, $R^2 = 0.57$, $n = 27$, $P < 0.0001$).

The averaged concentration-based N/P ratio was 26.9 ± 0.18 in MEBF, significantly ($P < 0.05$) higher than 25.2 ± 0.17 in MF. The leaf stock-based N/P ratio was 28.4 ± 0.17 in MEBF, very significantly higher than 24.8 ± 0.16 in MF ($P < 0.0001$). There were 11 species common to MF and PF, whose biomasses amounted to 55% and 99% of the two stands, respectively. The concentration-based N/P ratio was 23.2 ± 0.12 in MF, significantly ($P < 0.05$) higher than 22.5 ± 0.17 in PF. The leaf stock-based N/P ratios were 22.2 ± 0.13 and 19.2 ± 0.19 in MF and PF, also very significantly different ($P < 0.0001$) from each other.

Our data demonstrated that the N/P ratios in living leaves and litterfall of MEBF had been significantly ($P < 0.001$ for both) increasing recently (Fig. 3). The N/P ratios in living leaves of species whose biomass was more than 1% of MEBF community had increased from 21.0 to 26.2 in the past 16 years, and the N/P ratios in litterfall from 21.7 to 27.8 in the last 26 years.

3.3. Influences of N addition on N/P ratio in living leaves

The N addition experiment demonstrated that, compared with the control, N/P ratios in living leaves of MEBF were increased significantly ($P < 0.05$) with medium and high nitrogen addition, but those of MF and PF were not statistically different from each other (Fig. 4).

3.4. Concentrations and stocks of soil available N and P

Soil bulk densities in top 20 cm for MEBF, MF and PF were 0.933 g cm^{-3} , 1.155 g cm^{-3} and 1.314 g cm^{-3} . There were no significant differences during the sampling years.

For comparison, we considered two temporal scales. One temporal scale was decades and the other was years (Table 3). Available N concentration and stocks in MEBF were very stable temporally. Those in MF increased significantly ($P < 0.01$) from the 1990s to the 2000s, but the values were not significantly different if compared only between 1994 and 2005. The downward trends in available N concentration and stocks in PF, possibly because of the rapid accumulation of aboveground biomass, were not significant in either temporal scale. Soil available N concentrations in MEBF were significantly ($P < 0.05$) higher than those in the other two forests in the 1990s, and the soil available N concentrations in both MEBF and MF were significantly ($P < 0.05$) higher than those in PF in the 2000s. The available N stocks in the top 20 cm of soil showed similar patterns.

Contrary to available N, available P concentrations in the top 20 cm of soil were decreasing with the succession sequence of PF→MF→MEBF and temporal sequences of 1990s→2000s and 1994→2005, but the differences among succession stages and temporal sequences were not significant. Available P stocks in the top 20 cm of soil showed the same decreasing trend as the available P concentrations in succession and temporal sequences: only the difference between PF and MEBF was statistically significant ($P < 0.05$) in the 2000s and 2005 (Table 3).

3.5. Phosphomonoesterase activities with forest succession stages

In the top 10 cm of soil, PME activities were $9.42 \pm 3.54 \mu\text{mol h}^{-1} \text{g}^{-1}$, $15.45 \pm 8.79 \mu\text{mol h}^{-1} \text{g}^{-1}$ and $17.90 \pm 3.64 \mu\text{mol h}^{-1} \text{g}^{-1}$ for PF, MF and MEBF, respectively. And in the 10–20 cm layer, they were $5.09 \pm 1.83 \mu\text{mol h}^{-1} \text{g}^{-1}$, $8.29 \pm 4.92 \mu\text{mol h}^{-1} \text{g}^{-1}$ and $9.43 \pm 2.36 \mu\text{mol h}^{-1} \text{g}^{-1}$. PME activities in MEBF were significantly higher ($P < 0.05$) than those in PF in both depths, and MF had intermediate values.

3.6. Long-term trends of standing biomass, litterfall and ANPP of the three forest types

The standing biomass of MEBF had declined significantly ($P < 0.001$) since monitoring started in 1978. In contrast, those of MF and PF had significantly ($P < 0.001$ for both) increased since 1978 or 1982 (Fig. 5a). The average rate of change of standing biomasses for MEBF, MF and PF during the period of from 1978 to 2008 were

Table 2
Average N/P ratios in living leaves for 2006 and 2008 for the three communities.

| Stands | Species number | Biomass percentage | N/P ratios (concentration-based) | N/P ratios (leaf stock-based) |
|--------|----------------|--------------------|----------------------------------|-------------------------------|
| PF | 15 | >98.7% | $22.5 \pm 0.16a$ | $19.2 \pm 0.18a$ |
| MF | 36 | >99.4% | $24.7 \pm 0.16b$ | $22.7 \pm 0.16b$ |
| MEBF | 71 | >97.9% | $25.8 \pm 0.19c$ | $26.5 \pm 0.17c$ |

Values followed by means are standard deviation. Different lower case letters represent N/P ratios that are significantly different at $P < 0.05$.

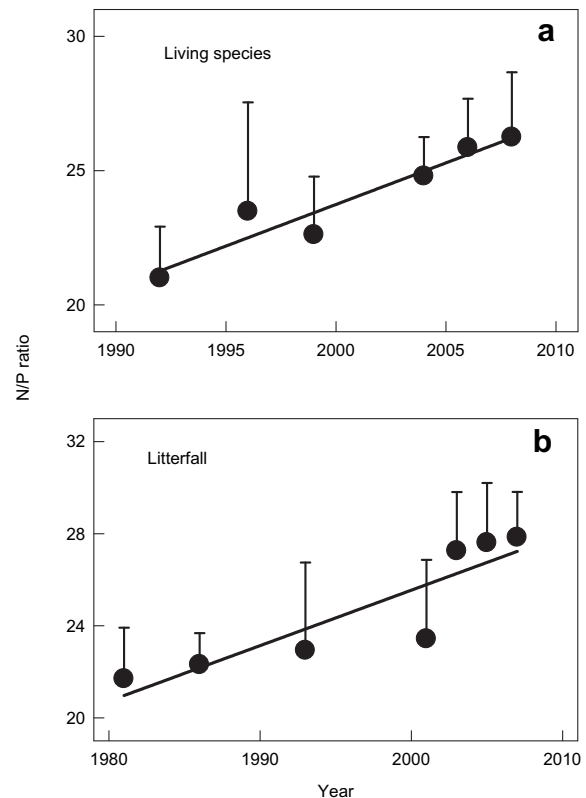


Fig. 3. Long-term trends of N/P ratios in litterfall and living leaves of species with biomass more than 1% of MEBF community. Error bars indicate the standard deviation of N/P ratios among the species. (a): in living leaves ($y = 0.31x - 595.4$, $R^2 = 0.91$, $n = 6$, $P = 0.0007$); (b): in litterfall ($y = 0.24x - 456.2$, $R^2 = 0.80$, $n = 7$, $P = 0.0008$).

$-222 \pm 56 \text{ g m}^{-2} \text{ year}^{-1}$, $+512 \pm 78 \text{ g m}^{-2} \text{ year}^{-1}$ and $+320 \pm 62 \text{ g m}^{-2} \text{ year}^{-1}$, all significantly different from 0 (t -test, $P < 0.05$).

In accordance with the biomass trends, annual litterfalls were significantly decreased ($-11.40 \pm 2.89 \text{ g m}^{-2} \text{ year}^{-1}$ for MEBF) ($P < 0.001$) and increased ($19.50 \pm 2.15 \text{ g m}^{-2} \text{ year}^{-1}$ for PF) ($P < 0.0001$) during the period of from 1981 to 2008 (Fig. 5b), both significantly different from zero (t -test, $P < 0.05$). There was no significant trend for MF over the same period.

The average ANPP of MEBF, MF and PF was $927 \text{ g m}^{-2} \text{ yr}^{-1}$, $1376 \text{ g m}^{-2} \text{ yr}^{-1}$ and $651 \text{ g m}^{-2} \text{ yr}^{-1}$, respectively, during the period of

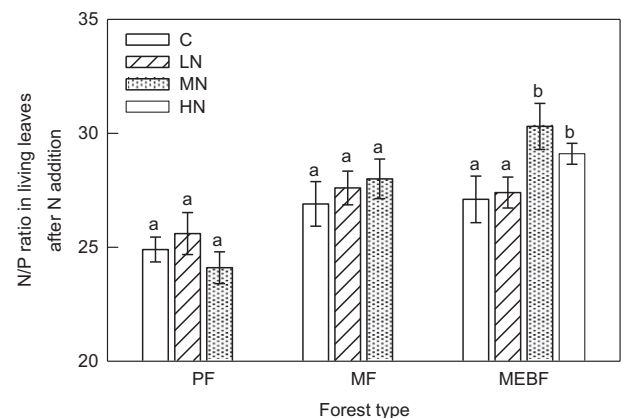


Fig. 4. N/P ratio in living leaves after five years of different N additions. Error bars indicate standard deviation; different letters indicate differences in significance at $P < 0.01$; C-control; LN-treatment with $5 \text{ g N m}^{-2} \text{ yr}^{-1}$; MN-treatment with $10 \text{ g N m}^{-2} \text{ yr}^{-1}$; HN-treatment with $15 \text{ g N m}^{-2} \text{ yr}^{-1}$.

Table 3
Contents and stocks of available N and P in the top 20 cm of mineral soils of the three forests in DBR.

| Nutrients | Forest types | 1994 | 1990s | 2005 | 2000s |
|--|--------------|---------------|---------------|---------------|---------------|
| N contents (mg N kg ⁻¹) | PF | 66.1 ± 10.7a | 70.0 ± 11.7a | 58.9 ± 7.5a | 64.0 ± 8.5a |
| | MF | 81.4 ± 14.7a | 71.7 ± 15.8a | 108.3 ± 13.4b | 115.5 ± 14.5b |
| | MEBF | 142.6 ± 19.8b | 141.8 ± 28.1b | 142.0 ± 11.5c | 141.2 ± 12.2b |
| N stocks (g N m ⁻²) | PF | 17.4 ± 2.8a | 18.4 ± 3.1ab | 15.5 ± 2.0a | 16.8 ± 2.2a |
| | MF | 18.8 ± 3.4a | 16.6 ± 3.7a | 25.0 ± 3.1b | 26.7 ± 3.3b |
| | MEBF | 26.6 ± 3.7b | 26.5 ± 5.2b | 26.5 ± 2.1b | 26.3 ± 2.3b |
| P contents (mg P kg ⁻¹) | PF | 1.75 ± 0.47a | 1.78 ± 0.53a | 1.48 ± 0.22a | 1.54 ± 0.24a |
| | MF | 1.68 ± 0.46a | 1.61 ± 0.48a | 1.34 ± 0.26a | 1.38 ± 0.33a |
| | MEBF | 1.53 ± 0.27a | 1.49 ± 0.44a | 1.28 ± 0.12a | 1.27 ± 0.17a |
| P stocks (g P m ⁻²) | PF | 0.46 ± 0.12a | 0.47 ± 0.14a | 0.39 ± 0.06a | 0.40 ± 0.06a |
| | MF | 0.39 ± 0.11a | 0.37 ± 0.11a | 0.31 ± 0.06ab | 0.32 ± 0.08ab |
| | MEBF | 0.29 ± 0.05a | 0.28 ± 0.08a | 0.24 ± 0.02b | 0.24 ± 0.03b |

Values followed by means are standard deviation. Different lower case letters indicate differences in significance at $P < 0.05$.

from 1978 to 2008 (Fig. 5c). The corresponding average forest debris (litterfall + coarse woody debris) amounts were 1166 g m⁻² yr⁻¹, 946 g m⁻² yr⁻¹ and 420 g m⁻² yr⁻¹. In MEBF, forest debris exceeded ANPP thus resulting in the standing biomass decrease, in contrast to the other two succession communities. ANPP in both MEBF and MF experienced a highly variable but significant ($P < 0.05$ for MEBF and $P < 0.0001$ for MF) decreased from 1978 to 2008 (Fig. 5c). ANPP in PF had been increasing significantly ($P < 0.0001$), especially since the 1990s, due to the invasion of broadleaved trees (Fig. 5c), which showed that the PF community had entered a period of rapid biomass accumulation.

The three variables of standing biomass, litterfall and ANPP in MEBF had fluctuated greatly in the study period, though with a declining trend.

4. Discussion

In this paper, we selected the MEBF and its succession series MF and PF in the subtropics of south China to address the issue of how tropical ecosystems respond to increasing N deposition. Here, we try to infer the possible relationships between the long-term high N deposition in the region and differences in N and P status of the three forest ecosystems, and the decline in standing biomass and productivity of an old-growth monsoon evergreen broadleaved forest.

4.1. Decline of the old-growth forest

Long-term monitoring at DBR has shown that the standing biomass stock, annual litterfall and ANPP of MEBF have been declining, through with great fluctuations, for at least 30 years. In contrast, PF is in the midst of an aggressive development, and the transient forest MF is approaching its maximal biomass phase (Fig. 5a).

It is a universal phenomenon that in mature forests the maximal biomass phase reached during succession cannot be maintained in the long-term absence of major disturbances (Schulze et al., 1995; Gower et al., 1996; Wardle et al., 2004). In Europe (Blank, 1985; Blank et al., 1988) direct damage by pollutants produces effects in spruce ranging from needle yellowing and loss to tree and stand mortality (Schulze, 1989). In comparison, although our MEBF has degraded rapidly over the last 30 years, no abnormal symptoms have appeared.

4.2. P shortage and high N/P ratios responsible for the decline of MEBF

Both N and P are indispensable major elements for plant physiological functions and are components of organic matter. It can be anticipated that there is an optimum N/P ratio in plant organs above or below which P or N becomes limiting for biological processes.

Wardle et al. (2004) assessed this at N/P = 16, but more recent studies have shown that the ratio can vary. Binkley et al. (2000) showed that P supply was about twice as high under *Eucalyptus saligna* (Sm) as under N-fixing species *Albizia falcataria* (L), indicating that the N/P ratio is strongly affected by N supply. However, it has been often reckoned that, for vegetation, N/P ratios <10 and >20 correspond to N- and P-limited biomass production (Güsewell, 2004). In comparable conditions such as vegetation originating from common pedogenesis, a higher N/P ratio would reflect a greater P limitation.

Where there is no alternate input (e.g., fertilization), P must be supplied almost entirely from the weathering of parent material in soils, which occurs at a very low rate (Walker and Syers, 1976). Although P comes ultimately from this source, the plant-absorbable portion in natural ecosystems depends mainly on recycling of the element. The decomposition of organic P can be an important source of plant available P in natural tropical forests (Zou et al., 1992; Meason et al., 2009; Kritzler and Johnson, 2010), although mycorrhizal fungi can gain access to P sources that are not otherwise available to plants (Smith and Read, 2008; Taylor et al., 2009). Compared with PF and MF, MEBF has a much bigger organic matter stock (Table 1) and therefore more of the P in the system is in that organic matter stock. Under the same pedogenesis and same soil total P (Table 1), the available P stocks in the top 20 cm of soils were thus in the sequence of MEBF < MF < PF (Table 3). PME activities, as an indicator of P limitation, demonstrate that increased P demand occurs in MEBF compared with PF and MF.

As pointed out by many studies (Garten, 1976; Bedford et al., 1999; Güsewell and Koerselman, 2002; Güsewell, 2004), our measurements demonstrated that although very significant correlations between N and P concentrations occur in both living leaves and litterfall (Fig. 2a and b), under the same N concentration, P concentrations in leaves were in the order MEBF < MF < PF (Fig. 2c), the same as the sequence of available P stocks in the top 20 cm of soil. Long-term monitoring data shows that the N/P ratios in the leaves and litterfall of MEBF have been significantly increasing (Fig. 3a and b). Table 2 also demonstrates that the N/P ratios in leaves of the three communities were significantly different from each other. Furthermore, when considering the common species, the differences became even greater. With forest succession from PF to MF to MEBF, N/P ratios of common species are gradually and significantly increasing, indicating that the vegetation requirements for P have been gradually becoming more difficult to obtain. Because of the bigger percentage in biomasses, the decline in growth of the 25 common species due to more serious P limits will result in a considerable decline in standing biomass and ANPP of the whole MEBF community.

If we accept the proposals by either Wardle et al. (2004) or Güsewell (2004) on the relationship between N/P ratios and N or P

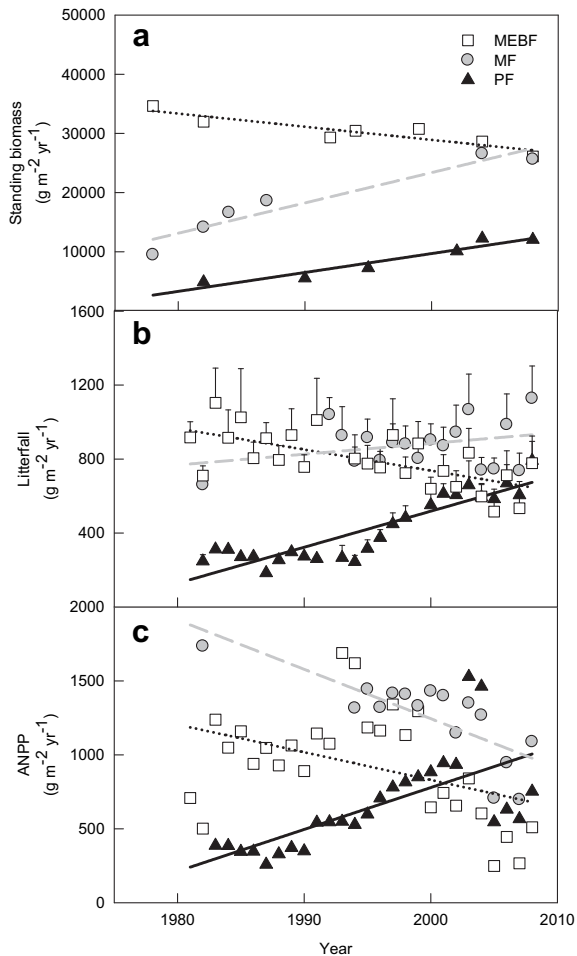


Fig. 5. Long-term trends of parameters in community production of the three forest types at Dinghushan Biosphere Reserve. (a): standing biomass (MEBF: $y = -222x + 472498$, $R^2 = 0.83$, $n = 7$, $P = 0.0003$; MF: $y = 512x - 1001410$, $R^2 = 0.92$, $n = 6$, $P = 0.0002$; PF: $y = 319x - 629240$, $R^2 = 0.92$, $n = 6$, $P = 0.0001$); (b): litterfall (MEBF: $y = -11.40x + 23543$, $R^2 = 0.44$, $n = 26$, $P = 0.0002$; MF: $y = 5.84x - 10802$, $R^2 = 0.09$, $n = 18$, $P = 0.06$; PF: $y = 19.50x - 38474$, $R^2 = 0.81$, $n = 25$, $P < 0.0001$; Error bars indicate standard deviation among the measurements of 15 traps); (c): ANPP (MEBF: $y = -18.73x + 38295$, $R^2 = 0.18$, $n = 28$, $P = 0.03$; MF: $y = -33.38x + 68011$, $R^2 = 0.61$, $n = 16$, $P < 0.0001$; PF: $y = 28.32x - 55861$, $R^2 = 0.46$, $n = 26$, $P < 0.0001$).

limitation, growth of the three forests in this study are all limited by P. This phenomenon is often observed in tropical regions, and has been described by many studies (Oberson et al., 1999; Güsewell, 2004; Cleveland et al., 2006; Cleveland and Townsend, 2006). However, a significant difference in N/P ratio of leaves and litterfall among the three communities of different succession stages with the same pedogenesis and altitude and with similar topographical conditions should reflect a difference in P limitation. The old-growth forest MEBF is more P-limited than the transient forest MF and pioneer forest PF. The limitation gradually increases with ecosystem development. Our historic N/P ratio data for leaves and litterfall of MEBF supports this conclusion. Thus, we believe that a high N/P ratio is the key factor causing the decline of productivity and growth in the old-growth forest.

The hypothesis was attested by our controlled experiment of N addition. When we added N in different amounts, N/P ratios in living leaves of MEBF were significantly increased with moderate to high nitrogen addition, but those of MF and PF did not differ from the control (Fig. 4), indicating that the supply of available P from the soils of MF and PF was richer than that from the soil of MEBF.

4.3. What drives high N/P ratios in old-growth forests?

Apart from the limited P sources, there may be other two key mechanisms for N accumulation contributing to high N/P ratios in leaves, litter and soil pools in MEBF. The first one is high N availability from self-accumulation in mature forests. As forests reach climax through succession, many ecosystem structures and processes including nutrient cycling, food webs, microbial communities become fully established. As a result, more N can be sequestered from the atmosphere through N-fixers and released to soil through decomposition processes, particularly in subtropical and tropical forests, which can lead to high N availability in old-growth forests (Vitousek, 1982, 1984; Vitousek and Farrington, 1997; Hobbie and Gough, 2002). Our results support this generalization. This mechanism would lead to more available N fixed in MEBF than in MF and PF.

The second mechanism is N deposition. As shown in Fig. 1, total N input through wet and dry deposition has been around $4.6 \text{ g N m}^{-2} \text{ yr}^{-1}$ for more than 20 years. Although this mechanism also contributes to high N deposition in MF and PF, compared with MEBF, the deposited N in PF from the atmosphere was rapidly assimilated in accumulating biomass and did not increase N concentrations and stocks in soils (Table 3).

We suggest that N self-accumulation and high N deposition are two key mechanisms causing high N levels in soil pools, in leaves and litter in MEBF, which in turn increases N/P ratios because there is no concomitant increase in P, and consequently leads to a decline in productivity.

5. Conclusion

In this study, we showed that the decline of an old-growth, monsoon evergreen broadleaved forest in terms of aboveground biomass and productivity were related to the high N/P ratio in vegetative leaves. The N self-accumulation mechanism in mature forests and high N deposition greatly increased the soil N stock and N concentration in leaves, which would, in turn, require more available P to match the increases in N to prevent the decline. However, because P must be supplied almost entirely from the weathering of parent material in soils at a very low rate, the plant-absorbable portion depends mainly on recycling of the element. With more and more available P bound up in organisms, these results in increasing N/P ratios in the soils and living leaves and litterfall of the old-growth forest. We suggest that excessive nitrogen supply may accelerate the decline of old-growth forest by actuating phosphorus limitation. In comparison, the transient and pioneer communities are still accumulating biomass, and are suffering much less P deficiency.

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