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# Effects of experimental nitrogen additions on plant diversity in an old-growth tropical forest

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## Abstract

Response of plant biodiversity to increased availability of nitrogen (N) has been investigated in temperate and boreal forests, which are typically N-limited, but little is known in tropical forests. We examined the effects of artificial N additions on plant diversity (species richness, density and cover) of the understory layer in an N saturated old-growth tropical forest in southern China to test the following hypothesis: N additions decrease plant diversity in N saturated tropical forests primarily from N-mediated changes in soil properties. Experimental additions of N were administered at the following levels from July 2003 to July 2008: no addition (Control); 50 kg N ha<sup>-1</sup> yr<sup>-1</sup> (Low-N); 100 kg N ha<sup>-1</sup> yr<sup>-1</sup> (Medium-N), and 150 kg N ha<sup>-1</sup> yr<sup>-1</sup> (High-N). Results showed that no understory species exhibited positive growth response to any level of N addition during the study period. Although low-to-medium levels of N addition ( $\leq 100$  kg N ha<sup>-1</sup> yr<sup>-1</sup>) generally did not alter plant diversity through time, high levels of N addition significantly reduced species diversity. This decrease was most closely related to declines within tree seedling and fern functional groups, as well as to significant increases in soil acidity and Al mobility, and decreases in Ca availability and fine-root biomass. This mechanism for loss of biodiversity provides sharp contrast to competition-based mechanisms suggested in studies of understory communities in other forests. Our results suggest that high-N additions can decrease plant diversity in tropical forests, but that this response may vary with rate of N addition.

**Keywords:** acidification, aluminum mobility, cation availability, China, functional group, N deposition, N saturation, plant diversity, tropical forest, understory layer

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## Introduction

Biodiversity is declining at an unprecedented rate and on a global scale. Indeed, loss of ecosystem functions and services associated with such declines has generated international contention (Sala *et al.*, 2000; Phoenix *et al.*, 2006; Zhou *et al.*, 2006). Several causes have been identified to explain such loss, including increased land use by an expanding human population (Lambin & Geist, 2006; Reidsma *et al.*, 2006) and global climate change (Thomas *et al.*, 2004; Thuiller, 2007). Human alteration in the global nitrogen (N) cycle has also been identified as a contributor to declines in biodiversity (Gilliam, 2006; Bobbink *et al.*, 2010). Combustion of fossil fuel, production and application of nitrogenous fertilizer, and intensive livestock production have converted considerable amounts of N from nonreactive N<sub>2</sub> to reactive forms – N<sub>r</sub> (e.g., NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub><sup>-</sup>) (Galloway *et al.*, 2003). Release of N<sub>r</sub> to the atmosphere has resulted in elevated deposition of N to terrestrial and aquatic

ecosystems, altering the N cycle at regional and global scales (Vitousek *et al.*, 1997; Galloway *et al.*, 2003). Excess N deposition is a potentially serious threat to biodiversity of many groups of organisms, including diversity of plants (Strengbom *et al.*, 2002; Nordin *et al.*, 2006; Clark & Tilman, 2008), microorganisms (Frey *et al.*, 2004; Carfrae *et al.*, 2006) and animals (Throop & Lerdau, 2004; Xu *et al.*, 2006).

Research on the responses of forest plant biodiversity to N deposition could be traced back to 1970s when European and North American ecologists began to study the effects of N deposition on the structure and function of temperate forest ecosystems (Dirkse *et al.*, 1991; Högberg *et al.*, 2006). Recent papers have focused on the effects of N deposition on forest plant biodiversity (see Gilliam, 2006 and Lu *et al.*, 2008 for recent reviews). Response of forest biodiversity to N deposition is usually one of decline, although some studies have reported no significant change (Hurd *et al.*, 1998; Strengbom *et al.*, 2003; Gilliam *et al.*, 2006; Bobbink *et al.*, 2010). Studying understory vegetation of 557 boreal forest stands along N deposition gradients throughout Sweden, Strengbom *et al.* (2003) found that increases in

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N deposition were significantly correlated with decreases in abundance for the once-dominant species *Vaccinium myrtillus*. In hardwood forests of USA, Hurd *et al.* (1998) found that high-N inputs (e.g., 28 kg N ha<sup>-1</sup> yr<sup>-1</sup>, or four times annual ambient N deposition) significantly decreased the cover of prominent herbaceous species, *Oxalis acetosella*, *Maianthemum canadense* and *Huperzia lucidula*. By contrast, 6 years of aerial applications of N (35 kg N ha<sup>-1</sup> yr<sup>-1</sup>) to a central Appalachian hardwood-dominated watershed had no significant effects on plant diversity (Gilliam *et al.*, 2006). Mechanisms underlying the declines of diversity include competitive exclusion of more N-efficient dominant species by relatively fast-growing nitrophilic species, as a result of high-N availability induced by N deposition (Gilliam, 2006; Bobbink *et al.*, 2010). Other such mechanisms include increased susceptibility to secondary stress and disturbance factors (e.g., disease and herbivory), and species invasions (Gilliam, 2006; Bobbink *et al.*, 2010). It is notable that our understanding of these mechanisms of plant response to N deposition comes from temperate/boreal forests and herb-dominated ecosystems, commonly N-limited under natural conditions (e.g., Suding *et al.*, 2005; Gilliam, 2006). To our knowledge, there has been no report to date on the effects of N deposition on forest plant diversity in tropical or subtropical areas (Lu *et al.*, 2008; Bobbink *et al.*, 2010).

There are several important reasons to address the effects of N deposition on plants diversity in tropical forest ecosystems. First, tropical forests have the highest biodiversity of all terrestrial ecosystems in the biosphere, providing habitat for over 50% of the Earth's plant and animal species, and containing 70% of the world's vascular plants (Wilson, 1988). Second, N deposition in tropical regions is projected to increase greatly in the coming decades due to the rapid expansion of industrial and agricultural activities (Lamarque *et al.*, 2005; Galloway *et al.*, 2008). Third, tropical forests have a more complex community structure and contain much more dominant tree species almost in all layers than temperate forests (Rosenzweig, 1995; Wiegand *et al.*, 2007). Finally, tropical ecosystems are typically N rich and phosphorus (P) limited compared with most temperate and boreal ecosystems (Vitousek, 1984; Matson *et al.*, 1999). Fang *et al.* (2009) found that leaching of dissolved inorganic N was substantially higher in an old-growth forest of southern China than that of most temperate forests studied in Europe and North America. Sotta *et al.* (2008) found that, although N is often in relative excess in old-growth lowland forest soils of Eastern Amazonia, N availability is greatly influenced by soil texture and, thus, often a function of soil weathering. Indeed, many tropical forest soils are highly

weathered, with low base cation concentrations and high Al concentrations (Matson *et al.*, 1999). Therefore, soil-based responses of plant diversity to N deposition in tropical forests may be different from those in temperate/boreal forests, wherein N-mediated changes in biotic interactions (e.g., competition and herbivory) are often primary mechanisms behind plant diversity response to N (Gilliam, 2006).

In Asia, anthropogenic activities have increased use and emissions of N<sub>r</sub> in from 14 Tg N yr<sup>-1</sup> in 1961 to 68 Tg N yr<sup>-1</sup> in 2000, and is expected to reach 105 Tg N yr<sup>-1</sup> in 2030 (Zheng *et al.*, 2002). This has led to rates of wet deposition of N (NH<sub>4</sub><sup>+</sup> + NO<sub>3</sub><sup>-</sup>) in some forests of southern China as high as 30–73 kg N ha<sup>-1</sup> yr<sup>-1</sup> (Ma, 1989; Ren *et al.*, 2000; Xu *et al.*, 2001). In old-growth tropical forests of the Dinghushan Biosphere Reserve (DHSBR), N deposition was 36 kg N ha<sup>-1</sup> yr<sup>-1</sup> in 1990 and 38 kg N ha<sup>-1</sup> yr<sup>-1</sup> in 1999 (Huang *et al.*, 1994; Zhou & Yan, 2001), comparable to the highest levels of N deposition occurring in Europe (MacDonald *et al.*, 2002; Aber *et al.*, 2003), where N deposition has negatively affected plant diversity (Stevens *et al.*, 2004; Bobbink *et al.*, 2010). It is therefore critical to explore the effects of increasing deposition of N on plant diversity in these forests of China, especially in southern China where industry has been developed rapidly.

The purpose of this study was to examine effects of artificial N deposition applications on plant diversity (defined as species richness, density and cover in this study) in an old-growth tropical forest in the DHSBR, one which has been demonstrated to have become N saturated due to both long-term high-N deposition in the region and its relatively undisturbed state of development (no direct human land use disturbance for more than 400 years) (Mo *et al.*, 2006, 2008b). Work in other tropical forests has shown that inputs of anthropogenic N deposition in tropical forests may alter other aspects of the N cycle without time lag, resulting in soil acidification, increased losses of base cations and Al mobilization, and decreased availability of limiting nutrients, particularly Ca<sup>2+</sup> and Mg<sup>2+</sup> (Matson *et al.*, 1999). These changes may damage fine roots, alter foliar nutrient balance and inhibit plant growth, or even reduce plant production (Watmough & Dillon, 2003; Magill *et al.*, 2004), and at last threaten plant biodiversity. It is also proposed that changes of soil properties (e.g., soil acidification, increased base cation leaching and concentrations of potentially toxic metals) induced by high-N deposition may be an important mechanism leading to the declines of diversity (Gilliam, 2006; Wallace *et al.*, 2007; Bobbink *et al.*, 2010). Given the high availability of N in soils at our study site, we hypothesize the following: N additions decrease plant diversity in N saturated tropical forests primarily from N-mediated changes in

soil properties. Because the understory layer makes an important contribution to plant diversity (Gilliam, 2007), we focus on the diversity of understory layer in this study.

## Methods

### Study site

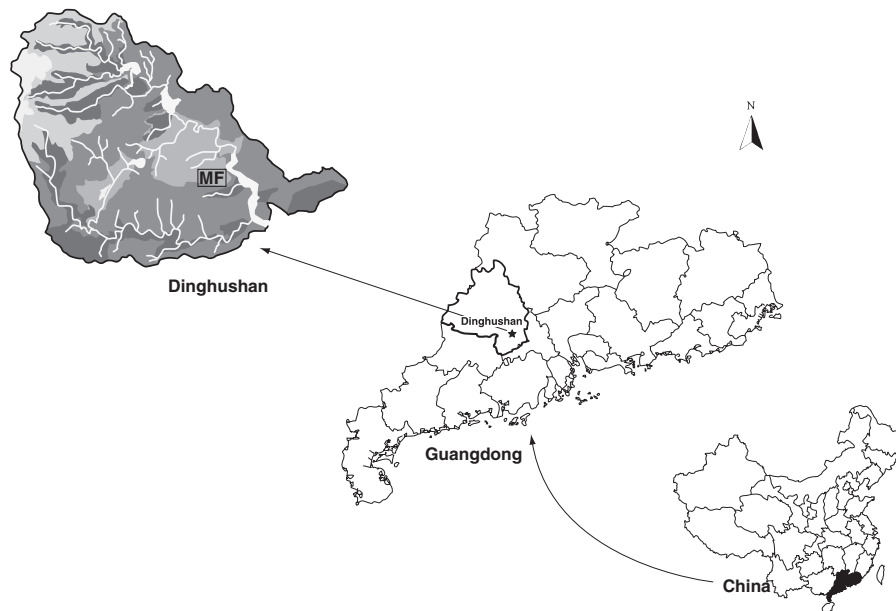
This study was conducted in the DHSBR, an UNESCO/MAB site located in the middle Guangdong Province in southern China (112°10'E, 23°10'N) (Fig. 1). The Reserve covers an area of 1155 ha, has a monsoon climate, and is located in a subtropical/tropical moist forest life zone (*sensu* Holdridge, 1967). The Reserve was established in 1950 to protect a remnant of undisturbed natural monsoon evergreen broadleaf forests in the subtropics, and was accepted as the first MAB reserve in China in 1978. The mean annual rainfall of 1927 mm is distributed seasonally, with 75% of it falling from March to August; 6% falls from December to February (Huang & Fan, 1982). Annual mean relative humidity is 80%. Mean annual temperature is 21.0 °C, with an average coldest (January) and warmest (July) temperature of 12.6 and 28.0 °C, respectively. Nitrogen deposition (inorganic N in precipitation) measured in 2004 and 2005 was 34 and 32 kg N ha<sup>-1</sup> yr<sup>-1</sup>, respectively, 60% of which was in the form of NH<sub>4</sub><sup>+</sup>-N (Fang *et al.*, 2008).

We established our research site in monsoon evergreen broadleaf forest in 2002 at elevations between 250 and 300 m above sea level. The forest has been protected from direct human land use disturbance for more than 400 years, according to <sup>14</sup>C measurement (Shen *et al.*, 1999), and contains a

complex variety of plant species (Cao *et al.*, 2002). The above-ground community can be divided into five layers: three arbor (tree) layers, one shrub layer, and one grass layer. Shrub and grass layers of these forests are commonly considered the understory layer. In addition, the aboveground community has many kinds of interlayer plants (liana and epiphytes). Predominant species in the canopy and subcanopy layers are evergreen tree plants, most of which are often natives of the tropics and subtropics, including *Castanopsis chinensis* Hance, *Schima superba* Chardn. & Champ., *Cryptocarya chinensis* (Hance) Hemsl., *Cryptocarya concinna* Hance, *Machilus chinensis* (Champ. Ex Benth.) Hemsl., and *Syzygium rehderianum* Merr. & Perry. Canopy closure is typically above 95%. Plant diversity in the understory layer is very high and most of the species diversity of the forests occurs in this layer, including tree seedlings, woody vines, shrubs, and herbaceous plants (Appendix S1). The soil in the study site is lateritic red earth (oxisols) formed from sandstone with a soil depth >60 cm (Mo *et al.*, 2003).

### Experimental treatments

Nitrogen-addition experiments were initiated in July 2003 (Mo *et al.*, 2006). Four N-addition treatments (in three replicates) were established: Control (without N added), Low-N (50 kg N ha<sup>-1</sup> yr<sup>-1</sup>), Medium-N (100 kg N ha<sup>-1</sup> yr<sup>-1</sup>), and High-N (150 kg N ha<sup>-1</sup> yr<sup>-1</sup>). Twelve 20 m × 10 m plots were established with each plot surrounded by a 10 m wide buffer strip. All plots and treatments were laid out randomly. In addition, two 1 m × 1 m subplots were permanently established in each plot, for a total of 24 subplots. Monthly applications of NH<sub>4</sub>NO<sub>3</sub> solution were administered by hand to the



**Fig. 1** Map of China with the other Provinces indicated by lines and with Guangdong Province shaded in black. Guangdong Province is expanded with Dinghushan Biosphere Reserve is marked with asterisk. Dinghushan Biosphere Reserve is also expanded, and shows the location of the old-growth monsoon evergreen broadleaf forest (MF, mature forest).

forest floor of these plots as 12 equal applications over the whole year. During each application, fertilizer was weighed, mixed with 20 L of water, and applied to each of the 20 m × 10 m plots using a backpack sprayer below the canopy. Two passes were made across each plot to ensure an even distribution of fertilizer. Control plots received 20 L of deionized water.

### Field sampling

The understory layer, defined here as all vascular plants  $\leq 1$  m in height (similar to the herbaceous layer *sensu* Gilliam & Roberts, 2003), was monitored within the two permanent 1 m<sup>2</sup> subplots in each plot. We chose this layer and definition because (1) it is widely used in the literature, (2) this stratum is sensitive to changes in nutrient availability, and (3) most of the plant biodiversity of forests is typically found there (see Gilliam, 2007 for a review). For the sake of including all plants tallied during the pretreatment sampling, any individual plants within this stratum that eventually grew above 1 m in height were included in further sampling and analysis. We performed a field survey of each subplot in July every year, and recorded all the vascular plants in the understory layer. The cover (percentage) of the individual plant species was estimated visually using a square grid method. Field tests were carried out to check the between-observer assessment level, and to calibrate it when necessary.

To explore possible mechanisms for changes of understory in diversity, we carried out two-time collections of soil samples in September 2004 and 2005, respectively, for determining soil pH, inorganic N (NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N), and extractable soil Ca and Al (Lu *et al.*, 2009). Soil inorganic N was extracted with 2 mol L<sup>-1</sup> KCl. Exchangeable Ca was extracted with 1 mol L<sup>-1</sup> NH<sub>4</sub>OAc and exchangeable Al was extracted with 1 mol L<sup>-1</sup> KCl (10:1, solution: soil). We determined fine root biomass (diameter  $\leq 2$  mm) in September 2005 (Mo *et al.*, 2008b). In addition, we also used Plant Canopy Analyzer LAI-2000 (LICOR Inc., Lincoln, NE, USA) to estimate tree canopy closure in August 2008 (Machado & Reich, 1999; Li *et al.*, 2008).

### Data analysis

All understory plants were classified into one of five functional groups on the basis of intrinsic morphological differences: (1) tree seedlings (height  $\leq 1$  m, not including germinated seedlings of current year), (2) woody vines (height  $\leq 1$  m), (3) shrubs (height  $\leq 1$  m), (4) herbaceous plants, and (5) ferns. Although ferns are usually grouped with herbaceous plants in studies of the understory layer, we distinguish the two groups in our study because of differences in reproduction (nonflowering vs. flowering) and because of the demonstrated differences in how ferns can alter competitive outcomes in forest herb communities (George & Bazzaz, 2003). To simultaneously test for overall N treatment effects over time for the study period from 2003 to 2008, we subjected data to two way repeated-measures analysis of variance (ANOVA) with Tukey's honestly significantly different (HSD) test (using STATISTIX 9, ANALYTICAL SOFTWARE 2008) on the following variables: density

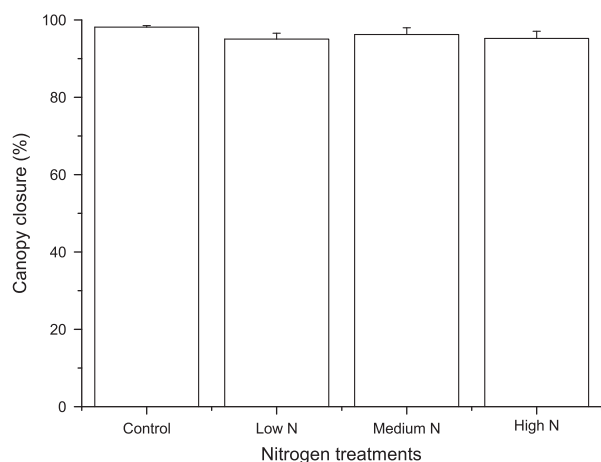
(mean number of plants m<sup>-2</sup> in each replication), cover (mean percent cover of plants in each replication) and richness (mean number of species m<sup>-2</sup> in each replication). One-way ANOVA with Tukey's HSD test was performed to test the differences of the above variables among treatments for the same year and among years for the same treatment. We also conducted the planned contrast analysis to test the differences between Control plots against the N-treatment plots. Relative measures of richness, density, and cover were calculated as the plot average for the specified period divided by the average diversity of species in the Control in that field over the same period. The proportional loss/gain of species relative to Control was calculated using the method of Clark & Tilman (2008): one minus the ratio of relative species diversity. In addition, we used a general linear model to analyze the relationships between density and richness, cover and richness, and cover and density in all plots in 2008. For fine root biomass, soil chemical properties (soil pH, inorganic N, and extractable Al and Ca) and canopy closure, one-way ANOVA with Tukey's HSD test was also performed to test the differences among treatments. One-way ANOVA, planned contrast analysis and linear analyses were conducted using SPSS 14.0 for Windows® (SPSS, Chicago, IL, USA). Statistically significant differences were set at  $P < 0.05$  unless otherwise stated.

### Results

Sampling within our subplots captured a total of 48 plant species belonging to five functional groups (i.e., tree seedlings, woody vines, shrubs, herbaceous plants, and ferns, Appendix S1). Before treatments in 2003, the understory vegetation of the experimental site was homogeneous and there were no significant ( $P > 0.05$ , Tukey's HSD test) differences between the Control and N treatment plots for any measured variables (richness, density and cover). Among the five functional groups, tree seedlings were dominant in terms of species richness, density, and cover, making up a major proportion (about 50%) of total plant diversity in all plots in the first year. Of the total of 107 individual tree seedlings tallied in our study, only four grew higher than 1 m (1.05, 1.05, 1.1, and 1.2 m, respectively) in 2008. Furthermore, there were no significant differences among N treatments in mean overstory cover over the course of the study (Fig. 2).

#### *Response of total plant diversity to N additions*

Repeated measures ANOVA showed significant effects of N treatment and time on total plant richness, density and cover, and significant interaction between treatment and time for cover and richness (Table 1A–C). Although none of the N treatments significantly altered plant richness and density in 2003–2004, the High-N treatment significantly decreased these relative to the Control beginning



**Fig. 2** Mean canopy closure for the forest overstory for all N treatments: control, low N, medium N, and high N. Means were not significantly different among N treatments by Tukey's honestly significantly different test (Tukey's HSD test). Bars indicate  $\pm$  SE.

in 2005. A similar response occurred for plant cover beginning in 2006. N-treatment effects on plant richness, density, and cover became significant in Low-N plots in 2008, compared with the Control (Fig. 3a–c). During the study period from 2003 to 2008, plant richness, density, and cover showed minor variations between years in the Control and Low-N plots, but decreased slightly with time in the Medium-N plots (Fig. 3a–c). This decreasing trend was more pronounced in the High-N plots, where plant richness, density, and cover decreased significantly with time ( $P = 0.008, 0.03, \text{ and } 0.055$ , respectively). In the high-N treatment, plant richness significantly decreased after 2004 ( $P = 0.043$ ), as did plant density from 2006 ( $P = 0.043$ ), compared with 2003. Plant richness, density, and cover all decreased by approximately 74% from 2003 to 2008 following 5 years of treatment in the High-N plots (Fig. 3a–c). In general, N-addition treatments significantly decreased total plant diversity with years, and this response pattern was mainly due to the response of tree seedling group because of its dominance in understory.

Relative measures of all plant diversity parameters showed a slightly increased trend (e.g., about 22% per year for richness compared with 2003) in the first 4 years in the Low-N plots, and then decreased in 2008, especially for plant cover, which decreased by around 54% relative to that of the Control plots (Fig. 3d–f). These parameters decreased greatly with time in the Medium-N and High-N plots (Fig. 3d–f). After 5 years of continuous N additions, plant richness, density, and cover decreased by  $\sim 50\%$  in the Medium-N plots relative to the Controls. In the High-N plots, plant richness, density, and cover decreased approximately by 80% relative to Controls.

**Table 1** Results of repeated measures analysis of variance for understory layer species richness, density and cover for Dinghushan Forest, 2003–2008

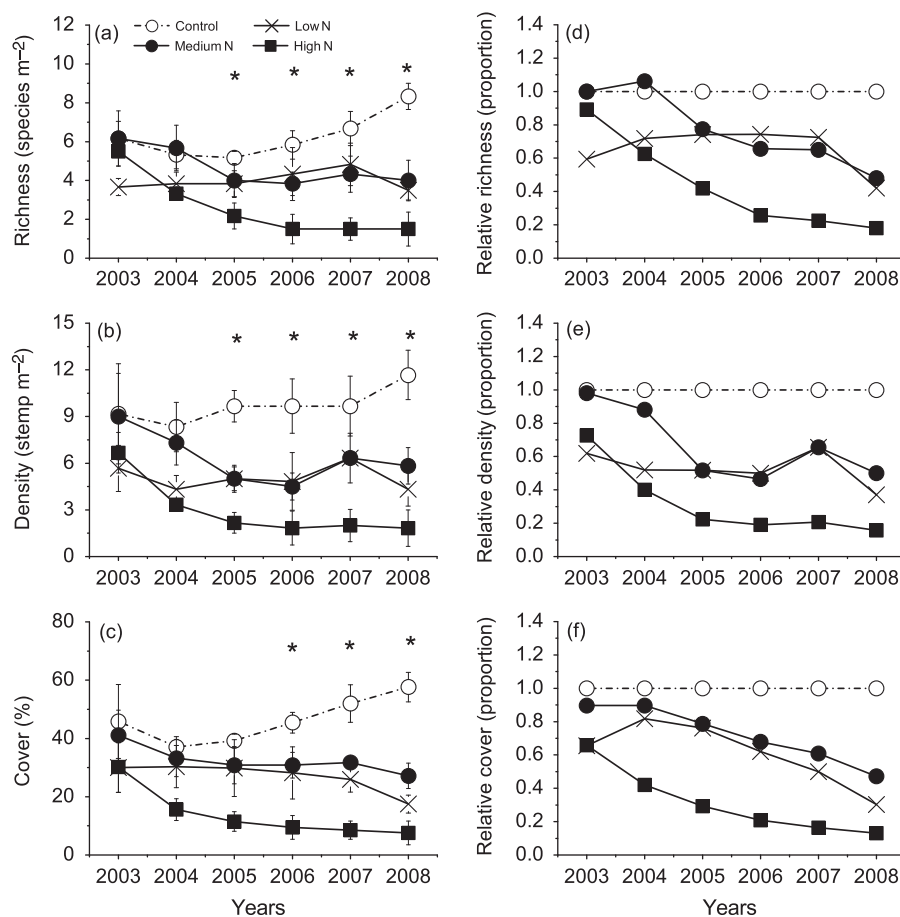
Source	DF	SS	MS	F	P
<i>(A) Richness (species/m<sup>2</sup>)</i>					
Treatment	3	124.6	41.5	5.71	<b>0.023</b>
Error: treatment $\times$ replication	8	58.2	7.3		
Year	5	19.4	3.9	3.97	<b>0.005</b>
Treatment $\times$ year	15	57.0	3.8	3.90	<b>0.000</b>
Error: treatment $\times$ replication $\times$ year	40	39.0	1.0		
Total	71				
<i>(B) Density (stems/m<sup>2</sup>)</i>					
Treatment	3	426.7	142.2	6.61	<b>0.015</b>
Error: treatment $\times$ replication	8	172.2	21.5		
Year	5	42.1	8.4	2.56	<b>0.042</b>
Treatment $\times$ year	15	80.4	5.4	1.63	0.108
Error: treatment $\times$ replication $\times$ year	40	131.3	3.3		
Total	71				
<i>(C) Cover (%)</i>					
Treatment	3	9598.7	3199.6	7.22	<b>0.012</b>
Error: treatment $\times$ replication	8	3543.9	443.0		
Year	5	804.9	160.8	3.01	<b>0.021</b>
Treatment $\times$ year	15	1970.1	131.3	1.46	<b>0.012</b>
Error: treatment $\times$ replication $\times$ year	40	2137.0	53.4		
Total	71				

Significant sources of variation indicated in bold font.

In addition, when compared across all plots and treatments, both plant density and cover were significantly and positively correlated with species richness ( $r^2 = 0.97, P < 0.0001$ , and  $r^2 = 0.86, P < 0.0001$ , respectively). Also, plant cover was significantly correlated with density ( $r^2 = 0.86, P < 0.0001$ ; Fig. 4).

#### *Response of diversity within functional groups to N additions*

During the study period, tree seedling diversity decreased with time in all N treatment plots, especially in High-N treatment plots, where tree seedling richness, density and cover decreased significantly since 2005 (Fig. 5a–c; Table 2). Repeated measures ANOVA also showed significant effects of N treatment and time on tree seedling diversity, but the interactions between treatment and time were not significant for them (data not shown). In High-N treatment plots, tree seedling richness, density, and cover decreased by 88%, 90%, and 76%, respectively, from 2003 to 2008 following 5 years of



**Fig. 3** Temporal patterns of responses of plant diversity (a, richness; b, density; and c, cover), and their relative diversity (d, richness; e, density; and f, cover) to N treatment levels in understory layers for years 2003–2008. Bars indicate  $\pm$  SE. Notes: open circles, Control; furcations, Low-N treatment; solid circles, Medium-N treatment; solid square, High-N treatment. \*Significant difference between Control plots against N-treatment plots at  $P < 0.05$  using planned contrast analysis.

treatment. Relative measures of tree seedling diversity parameters also showed decreased trends with years in all N treatment plots (Fig. 5d–f). After 5 years of continuous N additions, tree seedling richness, density, and cover decreased by 40%–55% in the Medium-N plots relative to the Controls. In the High-N plots, these parameters decreased by 80%–90% relative to Controls.

In the tree seedling group, seedlings of *Machilus chinensis*, *Crypocorya concinna*, *Randia canthioides* were dominant, based on average relative abundance over the entire experimental period. Density of dominant and nondominant species showed decreasing trends in Medium-N and High-N plots; density of nondominant species also decreased in Low-N plots.

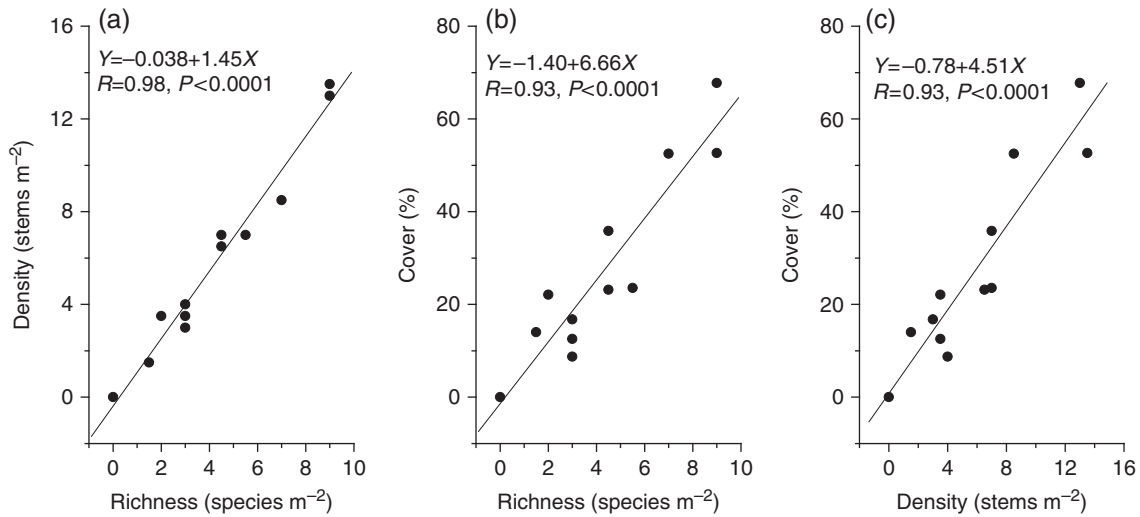
N-treatment effects were not significant on other functional groups (e.g., woody vines, shrubs, herbaceous plants, and ferns) (Table 2). In addition, only density of fern groups decreased greatly with time, especially in High-N plots ( $P < 0.001$ ). Repeated mea-

sures analysis also showed the significant time effects for fern groups ( $F = 4.78$ ,  $P = 0.017$ ).

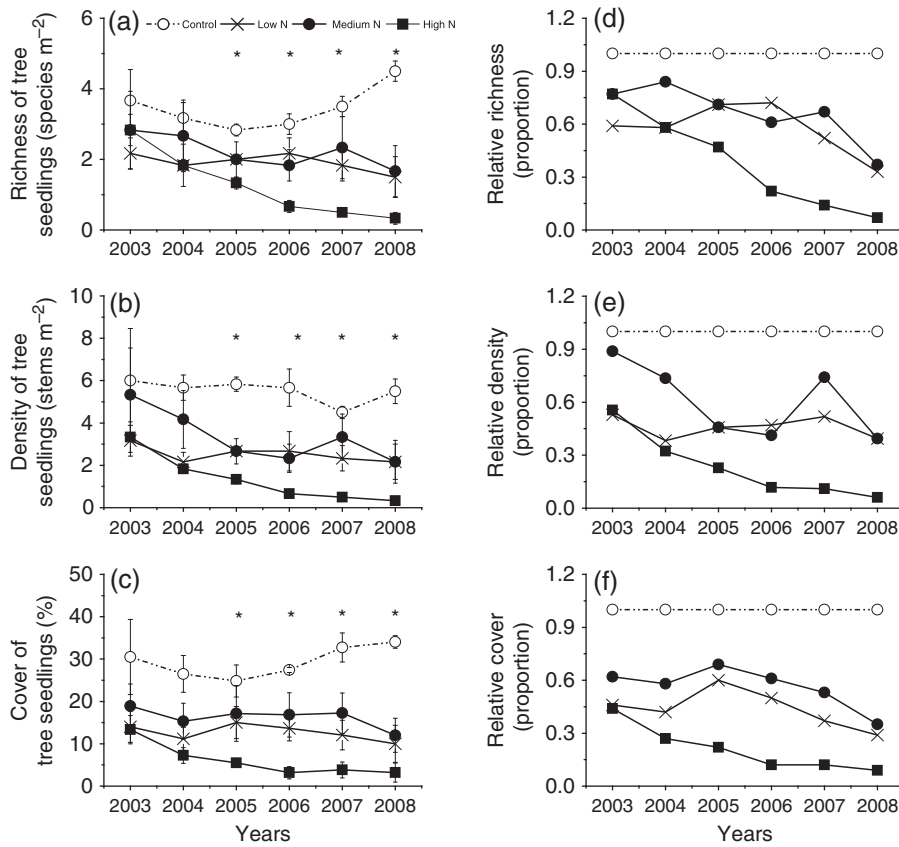
#### *Responses of soil properties and fine root biomass to N additions*

In September 2004, there were no significant ( $P > 0.05$ ) differences for soil properties (soil pH, inorganic N, and extractable Al and Ca) among treatment plots. Soil pH and extractable Ca showed a slight decreasing trend with elevated N treatment levels. Soil pH was  $3.92 \pm 0.03$  ( $\pm 1$  standard error of mean, and hereafter),  $3.80 \pm 0.08$ ,  $3.78 \pm 0.01$  and  $3.78 \pm 0.01$  in Control, Low-N, Medium-N, and High-N plots, respectively; and the contents of soil extractable Ca were  $380 \pm 55$ ,  $351 \pm 16$ ,  $343 \pm 38$ , and  $347 \pm 33$  mg kg<sup>-1</sup> in these treatment plots, respectively. Soil extractable Al showed a slight increasing trend with elevated N treatment levels, and the contents were  $407 \pm 24$ ,  $433 \pm 28$ ,  $457 \pm 19$ , and





**Fig. 4** Relationships between density and richness (a), cover and richness (b) and cover and density (c) in all plots of understory layers in 2008 at Dinghushan Forest. Each point represents a replication for four N treatment levels.



**Fig. 5** Temporal patterns of responses of tree seedling diversity (a, richness; b, density; and c, cover), and their relative diversity (d, richness; e, density; and f, cover) to N treatment levels in understory layers for years 2003–2008. Bars indicate  $\pm$  SE. Notes: open circles, Control; furcations, Low-N treatment; solid circles, Medium-N treatment; solid square, High-N treatment. \*Significant difference between Control plots against N-treatment plots at  $P < 0.05$  using planned contrast analysis.



**Table 2** Density (stems/m<sup>2</sup>) responses of five functional groups to experimental additions of N with years

Functional groups	Treatments	Years					
		2003	2004	2005	2006	2007	2008
Tree seedlings	Control	6.00 (2.47)Aa	5.67 (0.60)Aa	5.83 (0.33)Aa	5.67 (0.88)Aa	4.50 (0.29)Aa	5.50 (0.58)Aa
	Low N	3.17 (0.73)Aa	2.17 (0.44)Aab	2.67 (0.17)Ab	2.67 (0.93)Aab	2.33 (0.60)Aab	2.17 (0.83)Ab
	Medium N	5.33 (2.20)Aa	4.17 (1.36)Aab	2.67 (0.60)Ab	2.33 (0.67)Ab	3.33 (1.09)Aab	2.17 (1.01)Ab
	High N	3.33 (0.73)Aa	1.83 (0.17)ABb	1.33 (0.17)Bb	0.67 (0.17)Bb	0.50 (0.00)Bb	0.33 (0.17)Bb
Woody vines	Control	1.50 (0.58)ABa	0.83 (0.17)Ba	1.50 (0.29)ABa	1.33 (0.44)ABa	2.33 (0.17)ABa	3.33 (0.67)Aa
	Low N	1.00 (0.50)ABa	0.67 (0.44)Ba	0.83 (0.17)Ba	0.67 (0.17)Ba	2.67 (0.60)Aa	0.83 (0.17)ABb
	Medium N	1.33 (0.44)Aa	1.00 (0.50)Aa	1.17 (0.33)Aa	1.00 (0.29)Aa	2.00 (0.87)Aa	2.17 (0.33)Aa
	High N	0.83 (0.17)Aa	0.50 (0.50)Aa	0.33 (0.33)Aa	0.67 (0.67)Aa	0.83 (0.60)Aa	0.67 (0.44)Ab
Shrubs	Control	0.67 (0.44)Aa	0.67 (0.33)Aa	0.50 (0.29)Aa	0.50 (0.29)Aa	0.33 (0.17)Aa	0.67 (0.44)Aa
	Low N	0.67 (0.44)Aa	0.67 (0.44)Aa	0.50 (0.29)Aa	0.50 (0.29)Aa	0.17 (0.17)Aa	0.33 (0.17)Aa
	Medium N	0.67 (0.44)Aa	0.67 (0.44)Aa	0.33 (0.17)Aa	0.50 (0.29)Aa	0.33 (0.17)Aa	0.67 (0.17)Aa
	High N	0.33 (0.17)Aa	0.17 (0.17)Aa	0.17 (0.17)Aa	0.33 (0.33)Aa	0.50 (0.50)Aa	0.67 (0.67)Aa
Herbaceous plants	Control	0.17 (0.17)Aa	0.17 (0.17)Aa	0.67 (0.33)Aa	0.67 (0.17)Aa	1.33 (0.88)Aa	1.00 (0.58)Aa
	Low N	0.33 (0.33)Aa	0.17 (0.17)Aa	0.33 (0.33)Aa	0.17 (0.17)Aa	0.17 (0.17)Aa	0.17 (0.17)Aa
	Medium N	0.67 (0.33)Aa	0.67 (0.33)Aa	0.33 (0.17)Aa	0.33 (0.17)Aa	0.17 (0.17)Aa	0.50 (0.50)Aa
	High N	0.17 (0.17)Aa	0.33 (0.33)Aa	0.17 (0.17)Aa	0.17 (0.17)Aa	0.17 (0.17)Aa	0.17 (0.17)Aa
Ferns	Control	0.83 (0.44)Aa	1.00 (0.76)Aa	1.17 (0.73)Aa	1.50 (1.04)Aa	1.17 (0.60)Aa	1.17 (0.93)Aa
	Low N	0.50 (0.50)Aa	0.67 (0.44)Aa	0.67 (0.44)Aa	0.83 (0.60)Aa	1.00 (0.76)Aa	0.83 (0.60)Aa
	Medium N	1.00 (0.29)Aa	0.83 (0.33)Aa	0.50 (0.29)Aa	0.33 (0.17)Aa	0.50 (0.29)Aa	0.33 (0.17)Aa
	High N	2.00 (0.29)Aa	0.50 (0.29)Ba	0.17 (0.17)Ba	0.00 (0.00)Ba	0.00 (0.00)Ba	0.00 (0.00)Ba

The different capital letters indicate significant differences at  $P < 0.05$  level among years for the same treatment, and the different lowercase letters indicate significant differences at  $P < 0.05$  level among N treatment levels for the same year (Tukey's HSD test). Values are mean with SE in parentheses.

$465 \pm 18 \text{ mg kg}^{-1}$  in Control, Low-N, Medium-N, and High-N plots, respectively.

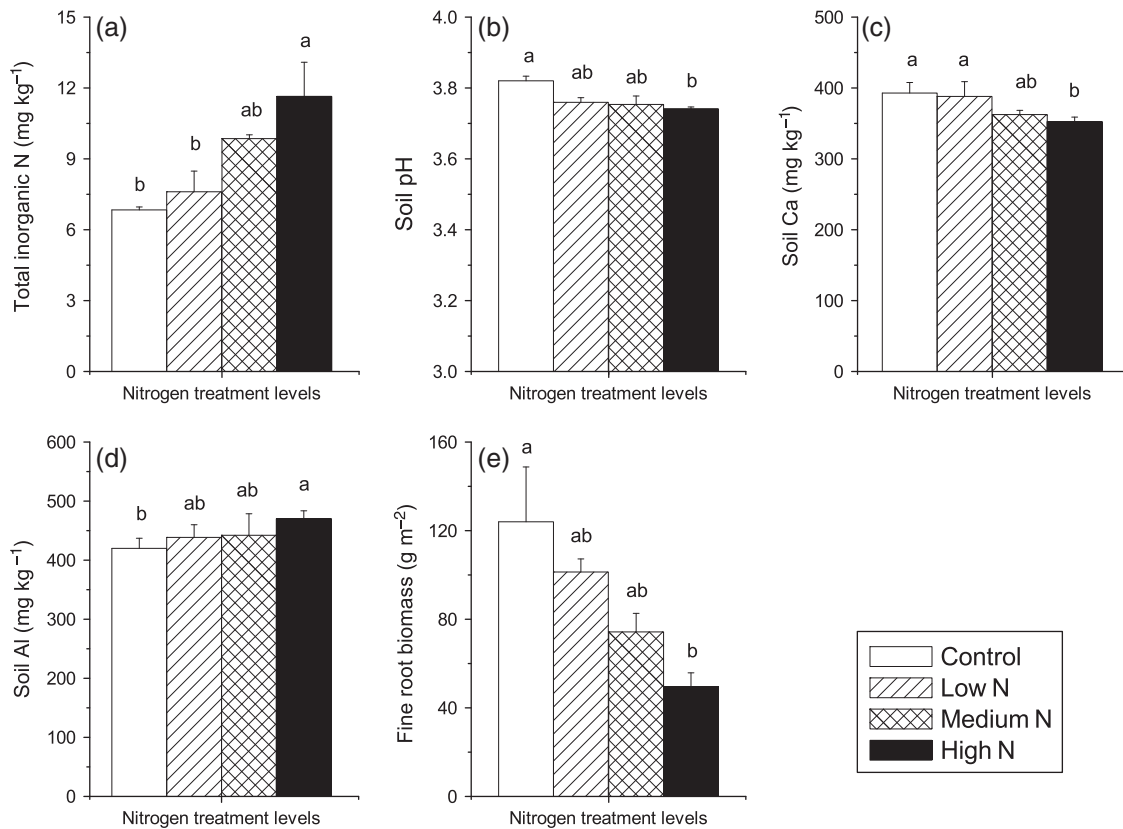
In September 2005, there were significant responses of these soil properties and fine root biomass to the High-N treatment. Total inorganic N (sum of  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N) increased significantly ( $P < 0.05$ ) in response to N treatment and  $\text{NO}_3^-$ -N accounted for 80%–90% of total inorganic N for all treatments (Fig. 6a). Soil pH and extractable Ca decreased greatly with increasing level of N additions, and the difference between the Control and High-N plots was significant ( $P < 0.05$ ) (Fig. 6b and c). Extractable soil Al, however, exhibited opposite pattern in response to N additions, with high-N additions significantly increasing extractable soil Al concentrations (Fig. 6d). In addition, biomass of fine roots decreased significantly with increasing levels of N addition (Fig. 6e).

## Discussion

We have demonstrated that N additions decreased understory plant diversity of an old-growth tropical forest, especially at the highest level of N additions, a result consistent with many reported for temperate and boreal forests (Hurd *et al.*, 1998; Rainey *et al.*, 1999;

Strengbom *et al.*, 2002; Gilliam, 2006). On the other hand, there are notable differences between our forests and those of temperate and boreal regions regarding the response to N additions for plant diversity. We found that N-mediated declines in understory diversity were more related to the reductions of plant species in tree seedling and fern functional groups (Table 2). This contrasts sharply with studies in temperate and boreal forests which have shown that forest diversity responses to N deposition were primarily a function of changes in forb and grass species (Brunet *et al.*, 1998; van Dobben *et al.*, 1999). Such a difference is especially pronounced in the low prevalence of ferns, which can be highly sensitive to N inputs, in altering the outcome of competitive interactions in the understory layer of our study site compared with other forest types wherein ferns species play a prominent role (George & Bazzaz, 2003; Coomes *et al.*, 2005).

In temperate and boreal forest ecosystems, which are commonly N limited (Vitousek & Howarth, 1991), belowground competition for N nutrient can be intense; thus, availability of N is an important factor determining species composition of vegetation. Also, most plant species from such habitats are adapted to low-N con-



**Fig. 6** Effects of different N treatment levels on soil chemical properties (a, total inorganic N, the sum of  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N; b, soil pH; c, soil Ca; d, soil Al) and fine root biomass (e). Bars indicate  $\pm$  SE. Bars with the same letter(s) are not significantly different at  $P < 0.05$  level among each N treatment by Tukey's honestly significantly different (HSD) test.

ditions, competing successfully growing in soil with low-N availability (Aerts & Chapin, 2000). The shift from N limitation to N saturation in those ecosystems experiencing elevated N deposition, however, is accompanied both by increases in the availability of N in the soil and by decreases in spatial heterogeneity of N processing, potentially reducing coexistence of greater numbers of species through enhancing competitive exclusion by fast-growing nitrophilous plants (Aerts & Chapin, 2000; Gilliam, 2006; Bobbink *et al.*, 2010), and eventually decreasing plant diversity. Bobbink *et al.* (1998) summarized the effects of N deposition on plant communities throughout Europe and North America. They found that N deposition may have shifted plant communities towards species composition typical of high-N availability. This shift has often been associated with a loss in diversity of plant species, particularly in areas with high deposition rates. In a boreal forest of Sweden, the dominant species, *Vaccinium myrtillus* (an N-efficient, ericaceous dwarf shrub), decreased significantly in density from increased N input, but *Deschampsia flexuosa* (a high N-requiring grass) showed a positive

response to increased N input (Strengbom *et al.*, 2002, 2003).

Increases in N availability simultaneously minimize belowground competition for N and intensify aboveground competition for light, conferring a competitive advantage to functional groups with high leaf area (Suding *et al.*, 2005, and references therein). Working in hardwood forests of North America, Hurd *et al.* (1998) found that cover of prominent herbaceous species declined significantly after only 3 years of N treatment; ferns species increased in cover, which likely lead to the decline in herb cover by increasing shading (George & Bazzaz, 2003).

It is notable that these mechanisms were based largely on observations in N-limited ecosystems to explain plant diversity changes in temperate and boreal forests and herb-dominated ecosystems, as found by Suding *et al.* (2005) in a meta-analysis of the responses of over 900 plant species in 34 N-fertilization experiments carried out in herb-dominated ecosystems throughout the USA. In contrast, our study site was an undisturbed lowland tropical forest, protected from direct human

land use disturbance for more than 400 years (Shen *et al.*, 1999) which has become N saturated from both chronic high-N deposition in the region and the age of the ecosystem (Mo *et al.*, 2006, 2008b). Accordingly, plant species of this forest may be adapted to this kind of N-rich environment, and plant competition for N should be minimal. Thus, the competitive exclusion by rapidly growing, nitrophilous plants under elevated N deposition may not develop. Our results are consistent with this notion in that no plant grew rapidly in response to any level of N addition in all measured variables (Figs 3–5, and Table 2; and plant height, data not shown), despite that fact that inorganic N (extractable  $\text{NH}_4^+ + \text{NO}_3^-$ ) essentially doubled from Control to High-N treatments (Fig. 6a). In fact, we observed two patterns of response among the five functional groups: plant diversity either showed no significant change, or showed significant declines (Table 2). We also found no significant differences among treatment plots in canopy closure (Fig. 2). Thus, it is unlikely that aboveground competition between understory plants for light was a factor to decrease plant diversity.

We suggest that reductions of plant diversity after high-N additions in our studied forest are largely related to changes in soil properties. It has been shown that elevated N inputs can lead to soil acidification (Matson *et al.*, 1999), which will negatively affect plant growth. Our results support this observation, with soil pH decreasing greatly with increasing N additions (Fig. 6b). Low pH values in the higher N-treated plots suggests a possible negative effect of N addition on plant growth, as Nakaji *et al.* (2001) reported that dry matter production of seedlings can be significantly reduced by low soil pH value. In a forest nursery experiment near our site, Mo *et al.* (2008a) found similar negative effects, as well as a reduction in seedling survival rate when soil pH declined to 4.7.

High-N additions can also lead to Al mobilization, which may be toxic to plant roots. Results from many studies in forest ecosystems have related Al mobility to toxic effects on plants, particularly in decreasing fine root biomass (Cronan *et al.*, 1989; Nygaard & de Wit, 2004). Our results showed that high-N addition simultaneously increased Al mobility and decreased fine root biomass (Fig. 6d and e), which suggests that decreased fine root biomass at high-N addition may be related to the increased Al mobility. In a seedling experiment in an adjacent region, Zhou & Norio (1995) found that fine roots were very sensitive to high Al concentration, and fine root biomass was lowest when soil soluble Al concentration was highest.

N-mediated leaching of nutrient cations (e.g., Ca or Mg) may limit plant growth. Our results suggest a negative response of soil Ca to increasing level of N

addition (Fig. 6c). Leaching of essential nutrients could lead to element imbalance in plants, negatively impacting plant growth (Cronan & Grigal, 1995; Watmough & Dillon, 2003; Bauer *et al.*, 2004; May *et al.*, 2005; Bowman *et al.*, 2008).

Finally, results from samples collected in 2004 showed that there were no significant ( $P > 0.05$ ) differences for soil properties (soil pH, inorganic N, extractable Ca and Al) among treatment plots; at the same time, total plant diversity did not show significant responses to N additions either. However, in 2005 (after 2 years of N applications), there appeared significant effects for fine root biomass (decrease), soil pH (decrease), inorganic N (increase), extractable Ca (decrease), and extractable Al (increase) under N-treatment plots; meanwhile, total plant diversity decreased significantly in N-treatment plots (especially in high-N addition plots). It has been shown that soil acidification can develop progressively during the N treatments, accompanied by an increase in exchangeable  $\text{Al}^{3+}$  and leaching losses of base cations (e.g.,  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ ) (Matson *et al.*, 1999; Jönsson *et al.*, 2003; Högberg *et al.*, 2006). Considering no significant difference in canopy closure between treatments (see Fig. 2), we believe that the decrease of diversity may be caused by N-mediated chemical changes in soil.

These three mechanisms – increased acidification, increased Al mobility, and decreased base cation fertility – can also explain why the low-N addition ( $50 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ ) treatment did not significantly change the plant diversity after 5 years of N additions. It is likely that the low-N additions in our study site did not significantly alter soil properties during the study period, despite that our rate of low-N additions was higher than the  $20 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  threshold for established for N-deposition effects on understory plant diversity in temperate and boreal forests (Bobbink *et al.*, 2010).

In conclusion, we have three important findings from this study: (1) high levels of N additions decreased plant diversity in this forest, a response seen primarily in tree seedling and fern functional groups; (2) the declines of diversity appeared to arise from N-related changes in soil properties (e.g., significant decreases in pH and extractable Ca, and increases in extractable Al); (3) N additions did not lead to any increase in plant growth (no increase in all measured variables) in this N-saturated tropical forest. Our first finding demonstrates the effect of excess N on the structure and function of tropical forest ecosystems. Community structure of tropical forests is dominated by trees in canopy and subcanopy layers. Seedlings of these tree species represented the dominant functional group in understory layer, comprising  $\sim 50\%$  of total species

diversity. Accordingly, changes in species composition and richness of the tree seedling functional group may affect forest development (including community structure and composition) and affect ecosystem productivity and carbon sequestration, under elevated N deposition in the future. The second finding provides sharp contrast to competition-based mechanisms suggested in studies of understory communities in temperate and boreal forests (Bobbink *et al.*, 1998, 2010; Strengbom *et al.*, 2001; Gilliam, 2006, 2007). It may also suggest that N-mediated declines in plant diversity of tropical forests may not be due to competition-based mechanisms, but rather to soil-related changes that are less conducive to plant growth and survival. Our third finding suggests that other nutrients (likely P) may be limiting to plant growth under N-saturated conditions. It is well established that plant growth and production in tropical ecosystems is limited by P rather than N (e.g., Vitousek, 1984). Phosphorus limitation also can explain well the lack of positive response of plant biomass to N additions. This phenomenon was demonstrated by Bobbink & Lamers (2002) in chalk grasslands of England and by Gress *et al.* (2007) in a mixed hardwood forest of eastern United States. Tian *et al.* (2009) synthesized C, N, and P data for over 5000 soils throughout China, finding that soils of the tropical and subtropical regions, which includes Dinghushan Forest, had the highest C:P and N:P ratios of all five climatic zones. Thus, P limitation may represent an additional factor explaining the response of the understory stratum to excess N in tropical forests. In addition, we intend to pursue further work in this area where we do look at plant nutrient elements (especially for N and P).

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### Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Appendix S1.** A complete species list of the understory layer during the whole studied period from year 2003 to 2008, including Latin name, common name, family, and functional group to which plants belong.

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