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Phosphate addition enhanced soil inorganic nutrients to a large extent in three tropical forests

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Elevated nitrogen (N) deposition may constrain soil phosphorus (P) and base cation availability in tropical forests, for which limited evidence have yet been available. In this study, we reported responses of soil inorganic nutrients to full factorial N and P treatments in three tropical forests different in initial soil N status (N-saturated old-growth forest and two less-N-rich younger forests). Responses of microbial biomass, annual litterfall production and nutrient input were also monitored. Results showed that N treatments decreased soil inorganic nutrients (except N) in all three forests, but the underlying mechanisms varied depending on forests: through inhibition on litter decomposition in the old-growth forest and through Al³⁺ replacement of Ca²⁺ in the two younger forests. In contrast, besides great elevation in soil available P, P treatments induced 60%, 50%, 26% increases in sum of exchangeable (K⁺+Ca²⁺+Mg²⁺) in the old-growth and the two younger forests, respectively. These positive effects of P were closely related to P-stimulated microbial biomass and litter nutrient input, implying possible stimulation of nutrient return. Our results suggest that N deposition may result in decreases in soil inorganic nutrients (except N) and that P addition can enhance soil inorganic nutrients to support ecosystem processes in these tropical forests.

Soil nutrient availability commonly regulates primary production and other basic ecosystem processes in terrestrial ecosystems. Plants take up essential nutrients from the soil to support metabolism and photosynthesis and return nutrients to soil via litter decomposition¹. This cycle can be considered as the “internal nutrient cycle in soil-plant system” (Fig. S1)². External perturbations to the availabilities of essential nutrients thus will influence nutrient dynamics (pools and fluxes) among different compartments of an ecosystem^{1–7}. Nitrogen (N) deposition, as high as 40 kg ha⁻¹ yr⁻¹ in some tropical areas, may be the most prominent perturbation to some old, highly-productive tropical forest ecosystems^{8,9}. Indeed, N deposition had been shown to increase nitrogen cycling in tropical regions based on long-term record of tree leaf and ring ¹⁵N isotopic signatures⁵.

Nitrogen deposition can influence ecosystem nutrient dynamics in a variety of ways by impacting plants performance and a couple of ecosystem processes, including plant nutrient uptake, photosynthesis, microbial activities, litterfall production and decomposition (nutrient mineralization)². But the direction of N effects (positive or negative) are often related with initial ecosystem N status. For example, in N-rich or even N-saturated tropical forests, N additions had negative or no effects on photosynthesis of understory species and litterfall production^{10–13}, despite foliar N enhancement following N additions¹⁴. N additions had also suppressed microbial biomass and activities, inhibiting litter decomposition and soil respiration^{10,15,16}. These processes in combination with potential base cation leaching accompanying N-enhanced nitrate loss may have resulted in the observed decreases in exchangeable base cation nutrients in the N-rich tropical forest in southern China¹⁷. In contrast, in younger or secondary tropical forests that are low in nutrients and less N-rich due to human disturbance, N additions were shown to stimulate litter production¹⁸, litter decomposition¹⁵, and hence soil P availability¹⁸. Thus it is crucial to consider forest initial N status when predicting how ecosystem nutrient dynamics would be affected by additional N input.

The series of detrimental effects of excess N in N-rich ecosystems have been recently connected with N-induced limitation by other nutrients, notably phosphorus (P) and calcium (Ca) in some temperate forests and Mediterranean ecosystems^{7,19–21}. In tropical forests, which are usually considered to be P-limited, more attention



should be paid on the role of P and its interaction with N in ecosystem processes^{22–25}. Onset of P limitation can be expected when N induced obvious soil acidification and subsequent solubilization of aluminum and iron hydroxides which can stabilize substantial amount of P^{26,27}. Moreover, N-induced soil organic matter accumulation may also promote more inorganic P converting to organic P, and thus lower soil P availability and in turn limit plant growth^{21,28}. In contrast, P fertilization might alleviate P limitation to plants and microbes and stimulate cascade of ecosystem processes, e.g., plants growth, litterfall production and nutrient mineralization, resulting in improved soil nutrient status^{19,22,24,29}. Furthermore, given the coupling nature of biotic N and P requirements, interactions between N and P are also expected^{7,25}. Nevertheless, so far we had no direct examination of the effects of N and P availabilities and their potential interaction on soil inorganic nutrients in tropical forests with contrasting initial N status.

In the present study, we selected three forests with different land-use history and initial N status to full factorial N × P fertilizations from 2007 at Dinghushan Biosphere Reserve (DHSBR), southern China²⁵. The old-growth forest was suggested to be N-saturated, based on inhibition of litter decomposition and high N leaching after N additions. The other two younger forests (mixed-species dominated and pine dominated) were less N-rich. Precipitation inorganic N input of 34 kg N ha⁻¹ yr⁻¹, and organic N input of 18 kg N ha⁻¹ yr⁻¹ was recorded in the study sites in 2005³⁰. In the present study, we reported responses of soil inorganic nutrients to 2.5 years of full factorial N and P treatments (150 kg N or P ha⁻¹ yr⁻¹). We examined responses of soil pH, N and P availability, exchangeable base cations and Al³⁺ in the surface mineral soils. Concomitant microbial biomass carbon (MBC), annual litterfall production and litter nutrient input were also monitored to explore the potential mechanisms. Based on the different initial N status of the three forests, we had three hypotheses: 1) In the old-growth forest, N treatments would

reduce the availabilities of P and exchangeable base cations through inhibitive effect of N addition on litter decomposition as shown previously from this experiment¹⁶; 2) In the two younger forests, which were suggested to be N-limited based on the positive effects of N on litter decomposition therein¹⁵, N additions would increase soil inorganic nutrients by promoting microbial biomass and litter nutrient input, and thus nutrient return; 3) In all three forests, given the rather low soil P availability (< 2 mg kg⁻¹)³¹, P additions would increase soil inorganic nutrients (N, P and base cations) by stimulating microbial biomass and litter nutrient input, and hence nutrient return.

Results

Old-growth forest vs. the two younger forests control plots. In the control plots, the old-growth forest was lower in pH (Fig. 1a), but higher in soil NO₃⁻-N (Fig. 1c), soil exchangeable base cation nutrients and Al³⁺ (Table S1) than the two younger forests. Soil pH values in the control plots averaged 3.82, 4.06 and 3.98 in the old-growth, mixed, and pine forests, respectively. Consistent with the low pH values, exchangeable cation pools of all the three forests were predominated by Al³⁺ (Table S1). Calculated annual litter nutrient input (annual litterfall production × litter concentrations of N, P, K, Ca, Mg; Table 1, S2) of K and Mg were significantly higher in the old-growth forest than in the pine forest (Table 1).

Effects of N additions. In the old-growth forest, N treatments decreased soil available P by 72% (1.76 mg kg⁻¹ in control to 0.49 mg kg⁻¹ in N addition plots; Fig. 1b), had no effect on pH or any single exchangeable cation species (Fig. 1a; Table S1), but resulted in a 6% decrease in sum of exchangeable (K⁺+Ca²⁺+Mg²⁺) (One-way ANOVA, LSD test, *P* < 0.05; Fig. 2). N additions did not affect MBC, annual litterfall production, or annual litter nutrient input (Fig. 3; Table 1) in the old-growth forest.

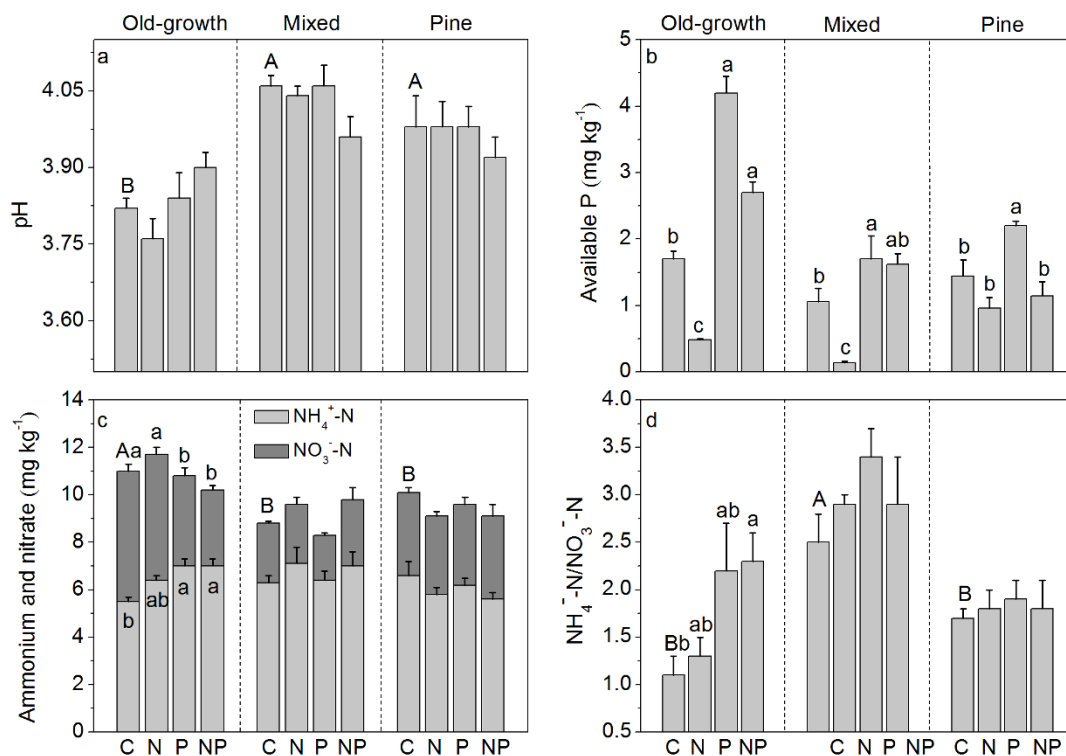


Figure 1 | Soil pH (a), concentrations of available P (b), available N (c, NH₄⁺-N and NO₃⁻-N), and ammonium to nitrate ratio (d) in the 0–10 cm soil depth in response to 2.5 years of N and P additions in the three forests. Bars indicate +1 SE (N=5). Different capital letters indicate significant differences among the three study forests (One-way ANOVA, Tukey's test, *P* < 0.05). Different lowercase letters indicate significant differences among treatments in each forest (One-way ANOVA, Tukey's test, *P* < 0.05).



Table 1 | Calculated annual litter nutrient input (ALNI, $\text{g m}^{-2} \text{yr}^{-1}$) following N and P additions in 2009. Calculations were done by timing litterfall production (Fig. 3a) with litter nutrient concentration determined by other studies (Table S2). Different capital letters indicate significant differences in control plots among forests (One-way ANOVA, Tukey's b test, $P < 0.05$). Different lowercase letters indicate significant differences among treatments in each forest (One-way ANOVA, Tukey's b test, $P < 0.05$)

Forests	Treatments	N	P	K	Ca	Mg
Old-growth	Control	8(0.6)b	0.3(0.02)b	1.7(0.1)Ab	1.7(0.1)b	0.8(0.1)Ab
	N	10(0.5)b	0.4(0.04)ab	2.0(0.1)ab	2.0(0.1)ab	1.0(0.1)ab
	P	14(1.1)a	0.5(0.02)a	2.8(0.2)a	2.9(0.2)a	1.4(0.1)a
	N*P	11(0.2)ab	0.4(0.01)ab	2.1(0.1)ab	2.2(0.1)ab	1.1(0.1)ab
Mixed	Control	6(0.5)b	0.2(0.05)b	1.4(0.2)ABb	1.5(0.3)b	0.6(0.1)ABb
	N	7(0.7)b	0.3(0.07)b	1.6(0.3)ab	1.7(0.4)b	0.7(0.2)ab
	P	10(0.9)a	0.4(0.08)a	2.2(0.4)a	2.4(0.4)a	1.0(0.2)a
	N*P	9(1)ab	0.3(0.10)ab	2.0(0.4)ab	2.2(0.5)ab	0.9(0.2)ab
Pine	Control	5(0.8)b	0.1(0.04)b	0.9(0.2)Bb	1.2(0.2)b	0.2(0.1)Bb
	N	5(0.7)b	0.1(0.04)b	1.1(0.1)ab	1.5(0.2)ab	0.2(0.1)b
	P	7(0.8)a	0.2(0.04)a	1.4(0.2)a	1.9(0.2)a	0.3(0.1)a
	N*P	8(1)a	0.2(0.11)a	1.5(0.4)a	2.0(0.6)a	0.3(0.1)a

In the two younger forests, N additions had no effects on soil pH, $\text{NH}_4^+\text{-N}$ or $\text{NO}_3^-\text{-N}$ concentrations, but reduced available P by 80% in the mixed forest (Fig. 1). N additions resulted in elevated soil exchangeable Al^{3+} in the mixed forest, but lower exchangeable Ca^{2+} and sum of exchangeable ($\text{K}^+ + \text{Ca}^{2+} + \text{Mg}^{2+}$) in the pine forest (Fig. 2; Table 2, S1). Neither of MBC, annual litterfall production or litter nutrient input was altered by N treatments in the two younger forests (Fig. 3; Table 1, 2). Linear model analysis showed significantly negatively correlated exchangeable Ca^{2+} and Al^{3+} in the mixed and the pine forests ($R^2 = 0.16$; $N = 40$; $P = 0.005$; Fig. 4).

Effects of P additions. In the old-growth forest, P additions increased soil pH (Table 2), soil available P (Fig. 1b), soil $\text{NH}_4^+\text{-N}$ (Fig. 1c), but decreased $\text{NO}_3^-\text{-N}$ (Fig. 1c) simultaneously, resulting in significantly higher $\text{NH}_4^+\text{-N}$ to $\text{NO}_3^-\text{-N}$ ratio (Fig. 1d). All species of soil exchangeable base cations (K^+ , Ca^{2+} , Mg^{2+}) were improved by P treatments in this forest (Table 2, S1), corresponding to a 60% increase in sum of exchangeable ($\text{K}^+ + \text{Ca}^{2+} + \text{Mg}^{2+}$) (One-way ANOVA, LSD test, $P < 0.05$; Fig. 2). P additions increased MBC (by 33.1%) and annual litterfall production (by 66.5%) in this forest (One-way ANOVA, Tukey's b test, $P < 0.05$; Fig. 3). Calculated litter input of all nutrient species (N, P, K, Ca, Mg) were stimulated by P (One-way ANOVA, Tukey's b test, $P < 0.05$; Table 1). N and P treatments interacted to influence sum of exchangeable ($\text{K}^+ + \text{Ca}^{2+} + \text{Mg}^{2+}$) and annual litterfall production (Two-way ANOVA, $P < 0.05$; Table 2).

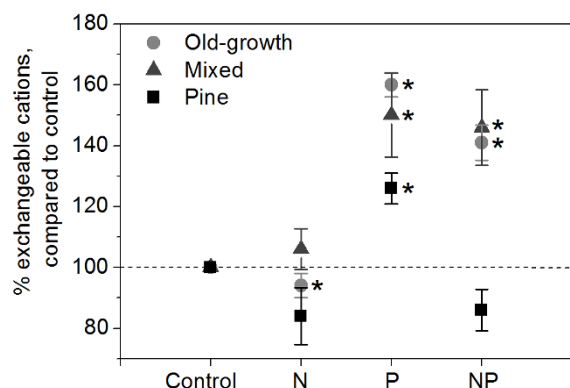


Figure 2 | Sum of exchangeable base cations (K^+ , Ca^{2+} , Mg^{2+}) in the 0–10 cm soil depth in the N, P or NP treatments relative to control (control=100%) in the three forests. Bars indicate ± 1 SE ($N=5$). “***” indicates significant differences between treatments and controls (One-way ANOVA, LSD test, $P < 0.05$).

In the two younger forests, P additions increased soil available P by 60% in the mixed forest, and by 53% in the pine forest (Fig. 1b), while having no significant impact on soil pH, $\text{NH}_4^+\text{-N}$ or $\text{NO}_3^-\text{-N}$ concentration (Fig. 1a, c) in either younger forest. In the mixed forest, P treatments increased exchangeable K^+ , Ca^{2+} , Mg^{2+} , and sum of exchangeable ($\text{K}^+ + \text{Ca}^{2+} + \text{Mg}^{2+}$) (Fig. 2; Table S1). In the pine forest, P additions increased exchangeable Mg^{2+} , Al^{3+} and sum of exchangeable ($\text{K}^+ + \text{Ca}^{2+} + \text{Mg}^{2+}$) (Fig. 2; Table S1). N, P interaction only existed on soil available P in the mixed forest (Two-way ANOVA, $P < 0.05$; Table 2). Microbial biomass carbon of the two younger forests were not affected by P (Fig. 3a). But both annual litterfall production (Two-way ANOVA, $P < 0.05$; Table 2) and litter input of N, P, K, Ca, Mg (One-way ANOVA, Tukey's b test, $P < 0.05$; Table 1) were promoted by P treatments.

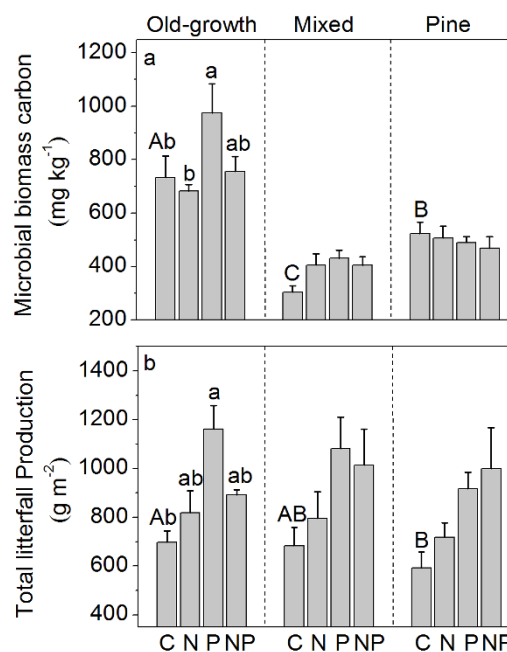


Figure 3 | Soil microbial biomass carbon (a) and annual total litterfall production (b) in surface mineral soil from the three forests after 2.5 years of N and P additions. Bars indicate ± 1 SE ($N=5$). Different capital letters indicate significant differences among the three study forests (One-way ANOVA, Tukey's b test, $P < 0.05$). Different lowercase letters indicate significant differences among treatments in each forest (One-way ANOVA, Tukey's b test, $P < 0.05$).



Table 2 | Fertilization effects on soil chemistry, litterfall production and microbial biomass in the three forests. Values are (treatment mean/control mean) from N, P or NP treatment plots. Two-way ANOVA results are given by *, $P < 0.05$; **, $P < 0.01$; *, $P < 0.001$**

Forests	Old-growth			Mixed			Pine		
	N	P	N*P	N	P	N*P	N	P	N*P
pH (H ₂ O)	0.98	1.02*	1.00	0.99	1.00	0.97	1.00	1.00	0.98
Available P	0.28*	2.47***	1.59	0.13**	1.60**	1.53*	0.67	1.53**	0.79
NH ₄ ⁺ -N	1.16	1.27*	1.27	1.13	1.01	1.11	0.91	0.96	0.89
NO ₃ ⁻ -N	0.96	0.68**	0.59	0.98	0.76	1.12	0.86	0.90	0.91
(NH ₄ ⁺ -N) + (NO ₃ ⁻ -N)	1.06	0.98	0.93	1.09	0.94	1.11	0.89	0.95	0.90
(NH ₄ ⁺ -N)/(NO ₃ ⁻ -N)	1.22	2.11**	2.16	1.17	1.36	1.14	1.07	1.11	1.06
Exchangeable K ⁺	1.00	1.31**	1.23	1.13	2.40***	3.26	0.75	0.98	0.82
Exchangeable Ca ²⁺	0.97	1.26**	1.07	1.05	1.40*	1.31	0.85*	1.27	0.84
Exchangeable Mg ²⁺	0.95	1.19*	1.14	1.03	1.43*	1.19	0.99	1.51*	1.03
Exchangeable Al ³⁺	1.11	1.05	1.00	1.15*	1.09	1.21	0.99	1.13*	1.13
Sum of (K ⁺ +Ca ²⁺ +Mg ²⁺)	0.94*	1.60**	1.40**	1.04	1.48**	1.45	0.85**	1.26*	0.86
Total Litterfall Production	1.17	1.66*	1.28***	1.17	1.58*	1.48	1.21	1.55**	1.69
Microbial Biomass Carbon	0.93	1.33*	1.03	1.33	1.32	1.33	0.97	0.93	0.90

Discussion

In this study, we examined soil inorganic nutrients (N, P, base cations) in response to N and P treatments in three tropical forests with different initial soil N status. The old-growth forest was suggested to be N-saturated, while the two younger forests were less-N-rich. Thus we expected N treatments to have opposite effects on examined parameters in the two types of forests (N-saturated vs. less-N-rich). On the other hand, since all three forests had very low soil available P, we expected P additions to increase soil inorganic nutrients in all three forests through stimulating litter nutrient input and return. It turned out that N additions resulted in decreases in soil inorganic nutrients in all three forests, with varied underlying mechanisms related to forest initial N status. P additions, on the other hand, elevated soil inorganic nutrients in all three forests as expected. We will give discussion on effects of N or P on soil inorganic nutrients separately, in the old-growth forest first, and then in the two younger forests. We also related these response patterns with the different initial N status of the two forest types (N-saturated vs. less-N-rich).

Negative effects of N on soil inorganic nutrients in the N-saturated old-growth forest. Consistent with our first hypothesis, N treatments resulted in decreases in soil available P and exchangeable base cation nutrients in the old-growth forest. N treatments decreased soil available P from 1.7 mg kg⁻¹ to 0.45 mg kg⁻¹; An N-induced 6% loss of sum of exchangeable (K⁺+Ca²⁺+Mg²⁺) were also observed.

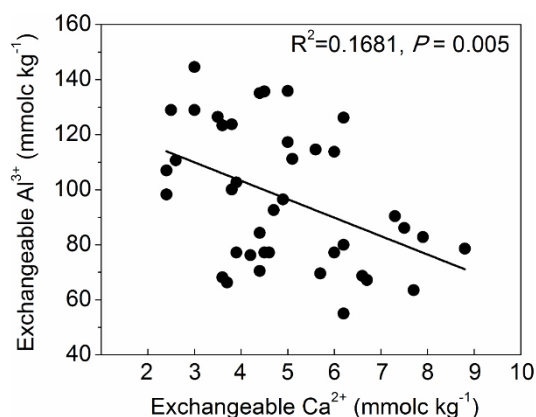


Figure 4 | Relationship between soil exchangeable Ca²⁺ and Al³⁺ with data from the mixed and the pine forests pooled together. $Y = -6.70 \cdot X + 130.06$, $R^2 = 0.1681$, $P = 0.005$.

Because we failed to observe decreases in soil pH or mobilization of Al³⁺, thus the modest, but significant decrease in sum of exchangeable (K⁺+Ca²⁺+Mg²⁺) were unlikely to result from enhanced base cation leaching in this forest. Instead, we focused on the “internal nutrient cycle in soil-plant systems” in this study (Fig. S1) and assumed that atmospheric cation deposition and chemical weathering were identical to all the plots. This enabled us to relate changes in soil exchangeable nutrients to three specified ecosystem processes, including plant nutrient uptake (output), litter nutrient return (input), and nutrient mineralization (input). Plant uptake of nutrients were not likely stimulated by N additions, given the N-saturated status of the old-growth forest¹⁵. Indeed, neither tree growth³² (adult trees, seedlings or understory species) nor foliar nutrient concentrations³³ were stimulated by N additions in the old-growth forest (results from another ongoing N fertilization experiment in the same forests). On the contrary, declines in foliar Ca, Mg concentrations after 3 years of N treatments were observed for dominant species in the old-growth forest³³ (50, 100, 150 kg N ha⁻¹ yr⁻¹). Litter nutrient input were not reduced by N additions, according to the calculated annual litter nutrient input. Thus, this led us to relate the decreases in soil available P and exchangeable (K⁺+Ca²⁺+Mg²⁺) in the old-growth forest with inhibited nutrient mineralization (reduced input), since negative effects of N addition on litter decomposition and soil respiration were previously observed in the old-growth forest from the other ongoing N fertilization experiment in the same forests^{10,15}, and were further confirmed in this N × P interaction experiment¹⁶.

Negative effects of N on soil inorganic nutrients in the two less-N-rich younger forests. Contrary to our second hypothesis, in the two younger forests, no increases in inorganic nutrients, but decreases in exchangeable Ca²⁺ and sum of (K⁺+Ca²⁺+Mg²⁺) in the pine forest, and increases in exchangeable Al³⁺ in the mixed forest were observed following N treatments, all of which can be considered as certain symptoms of soil acidification. In addition, soil exchangeable Ca²⁺ negatively correlated with exchangeable Al³⁺ in the two forests combined, suggesting that decreases in exchangeable Ca²⁺ might be a result of mobilization of Al³⁺, which subsequently replace Ca²⁺ from exchangeable sites to be leached³⁴. These N-induced signs of soil acidification for the pine and the mixed forests were not observed from the other ongoing long-term N addition experiment at DHSBR, showing that 26 months of N treatments (50–150 kg N ha⁻¹yr⁻¹) failed to elicit decreases in pH or exchangeable Ca²⁺, Mg²⁺, or Al³⁺ mobilization in either younger forests¹⁷, which was attributed to the rather N-limited status of the two younger forests¹⁷. The difference between our results and the



previous study implied the possibility that base cation leaching might have started to emerge in the two younger forests under the high ambient N deposition³⁰ (34 kg N ha⁻¹ yr⁻¹, wet deposition, inorganic N). The pine forest in our study showed particularly sensitive responses of Ca²⁺ nutrient to N treatments. This is consistent with other studies in red spruce dominated ecosystems demonstrating pine species to be more prone to Ca deficiencies under high N deposition^{34,35}.

Alleviation of P-limitation increases soil inorganic nutrients in all three forests. In line with our third hypothesis, P addition induced great increases in soil available P and exchangeable base cation nutrients (sum of K⁺, Ca²⁺, Mg²⁺, or individual base cation species) in all the three study forests. For soil inorganic N, P additions caused net increase in NH₄⁺-N, but decrease in NO₃⁻-N only in the old-growth forest. These results highlights the role of P on regulating soil nutrient availabilities in these tropical forests. The potential mechanisms were that the positive effects of P on soil inorganic nutrients were manifested via stimulation on microbial biomass and annual litter nutrient input, and hence nutrient return.

In all three forests, we observed up to 147% increases in Bray-I P after P treatments, implying that P-limitation was relieved, and biotic P demand may have been saturated^{21,24}. In addition, we determined Bray-I P after 5 years of N and P treatments in July 2011, and found much more increases (up to 31 folds in Bray-I P comparing to the control)³¹. These demonstrated that continuous P addition (NaH₂PO₄, 150 kg P ha⁻¹ yr⁻¹) can saturate sorption sites in these highly weathered soils⁶. Similar gradual increases in soil available P after years of P additions (CaHPO₄, 50 kg P ha⁻¹ yr⁻¹) were also detected from an ongoing long-term NPK factorial experiment in lowland tropical forests in Panama (4 years, 50% increase in Bray-I P³⁶; 9 years, 29 folds increases in Mehlich P⁶).

In the old-growth forest, we suggested that nutrient mineralization and return should have been stimulated by P addition, since MBC, annual litterfall production and litter nutrient input were found to increase following P treatments in this forest. P treatments increased decomposition rates in a Hawaii tropical forests³⁷ and in a Panama lowland tropical forests³. In this study, P-increased soil microbial biomass indicated that P availability was a limiting factor for microbial growth in the old-growth forest, consistent with previous study from the same experiment showing stimulation of P on microbial performance in this forest²². In addition, higher fresh fallen litter may temporarily accelerate soil organic carbon mineralization (priming effect)³⁸, due to the fact that microbial activities are frequently limited by labile C availability. Thus we suggest that microbial biomass and activities may be increased by both alleviation of P limitation and improved substrate C availability²⁴ in the old-growth forest.

In the old-growth forest, both annual litterfall production and litter nutrient input were elevated by P additions, implying the close relationship between P-induced higher litter nutrient input and increases in soil inorganic nutrients. We examined this potential relationship by doing rough comparison between P-enhanced litter nutrient input (N, P, K, Ca, Mg) and changes in soil inorganic nutrients (Table S3), and found that P-induced higher litter input of P, K, Mg approximated the increases in available P and exchangeable K⁺, Mg²⁺ (Table S3); However, litter Ca input quantified about one third to half of increases of exchangeable Ca²⁺ following P additions (Table S3), implying that this speculated P-enhanced nutrient input can't fully explain the observed changes in exchangeable Ca²⁺. Litter function as substrate for microbial mineralization, apart from root debris¹⁵. Litterfall and underlying forests floor in acid forests is a major source of dissolved organic carbon, nutrients and metals³⁹. The release of nutrient cations from these two layers was suggested to be crucial for tree growth⁴⁰, since these layers can contain as much as 80% of available Ca, Mg and K in soil⁴¹. In our old-growth forest, nutrients stored in the litter layer were released within one year¹⁵, and

litterfall standing biomass approximated that of annual litterfall production⁴², both resulting from relative fast decomposition. In sum, in the old-growth forest, P-induced higher microbial biomass, litterfall production and litter nutrient input may have jointly contributed to more efficient nutrient mineralization and return, resulting in increases in soil available P and base cation nutrients. For soil inorganic N, we expected it to show changes in concert with other base cations, since they are all released from nutrient mineralization. Consistent with this expectation, there was P-induced increase of soil NH₄⁺-N level in the old-growth forest.

In the two younger forests, P additions did not affect microbial biomass, consistent with previous study showing that P additions affected neither microbial biomass nor community composition²². P was suggested to have a less important role in the two younger forests than in the old-growth forest to influence microbial growth and performance²². Nevertheless, we did observe P-stimulated litterfall production, litter nutrient input. This may imply differential P limitation to plants and microbes in the two younger forests. Comparison between P-driven increases in litter nutrient input (N, P, K, Ca, Mg) and in soil inorganic nutrients (Table S3) showed that in the mixed forest, P-induced higher litter Mg input approximated increases in exchangeable Mg²⁺, with litter Ca and K input being less than one third of increases in exchangeable pool of each. In the pine forest, P input via litter roughly equaled the increase of soil available P following P additions, suggesting the importance of litter return on soil P nutrient (Table S3). This implied that some unknown processes contributed to the observed increases in exchangeable pools of other base cations. However, we failed to observe similar increases in inorganic N (NH₄⁺-N or NO₃⁻-N) after P additions in the two younger forests as in the old-growth forest. One reason might be P-enhanced biotic utilization of NH₄⁺-N^{21,43}, which may have counteracted the potential higher available N (higher NH₄⁺-N resulting from P-stimulated nutrient mineralization) after P additions. The nutrient dynamics following N and P additions in the three study forests need further studies using isotope tracer technique.

Conclusions

Our results demonstrated detrimental effects of excess N on soil inorganic nutrients (mainly inorganic P and exchangeable base cation nutrients) of different forest types (N-saturated vs. less-N-rich) with different mechanisms, i.e., likely through N-inhibited litter decomposition in the N-saturated old-growth forest, and with Al³⁺ mobilization and replacement of Ca²⁺ in the two younger, less-N-rich tropical forests. Consistent with the low soil P, P additions alleviated P limitation, and increased soil available P and base cation nutrients in all three forests to a large extent, but increased inorganic N (NH₄⁺-N) only in the old-growth forest. These positive effects of P were closely related with P-stimulation on microbial biomass, litterfall production and litter nutrient input. Our results highlights the perturbation role of N deposition on inorganic nutrient availabilities (except N) and cycling, and more importantly, how they can be reversed by P additions in some N-saturated and less-N-rich tropical forests. These results highlight the potential of P on influencing productivities of tropical forests under high N deposition.

Methods

Study site. This study was conducted in Dinghushan Biosphere Reserve (DHSBR, 112°33'E and 23°10'N), which occupies an area of approximately 1200 ha. The reserve is located in the middle part of Guangdong province, southern China. The climate is warm and humid, with annual precipitation of 1927 mm and mean annual temperature of 21.0°C. The soil is lateritic red earth formed from sandstone¹⁵. Three forests in this reserve were included in the present study: an old-growth forest as the regional climax type (more than 400 years old) and another two younger forests, a mixed pine/broadleaf forest and a pine forest. These two younger forests both originated from the 1930s clear-cut and subsequent pine plantation with sites badly eroded and degraded¹⁵. Widely different intensity and frequency of litter and vegetation harvesting between 1930s to 1998 on these two younger forests determined their different tree species composition, with *Pinus massoniana* dominated in the



pine forest and some broadleaf species together with *Pinus massoniana* co-dominated in the mixed forest. The three forests have similar elevation (50–250 m a.s.l.), slope aspect and slope degree¹⁵.

Experimental design of nutrient additions. The full factorial N×P manipulation experiment started in February 2007 in the three forests. In each forest, there were four treatments, i.e., control (without N or P additions), +N (150 kg N ha⁻¹ yr⁻¹ as NH₄NO₃), +P (150 kg P ha⁻¹ yr⁻¹ as NaH₂PO₄), and +NP (150 kg N ha⁻¹ yr⁻¹ N + 150 kg P ha⁻¹ yr⁻¹), with 5 replicates for each treatment. Plots of 5 × 5 m² were randomly selected to receive treatments. NH₄NO₃ and NaH₂PO₄ solutions were sprayed once every other month to the forest floor with a backpack sprayer starting from February 2007 and continued through August 2009. Fertilizer was weighed and mixed with 5L water for each plot. Each control plot received 5L water without fertilizer²⁵.

Field sampling and chemical analysis. In this study, the 0–10 cm mineral soils were collected in August 2009 from control, and the +N, +P and +NP treatment plots (treated for 2.5 years). In each plot, 3 soil cores (5 cm in diameter) were collected randomly and mixed to one composite sample. Soil samples were then passed through a 2 mm mesh sieve to remove other coarse fragments. One 10 g sub-sample from each composite sample was shaken for 1 hour in 50 ml of 1 mol/L KCl, and filtered; the filtrate was frozen immediately for later analysis. Extractable NH₄⁺-N was determined by the indophenol blue method followed by colorimetry³⁰. NO₃⁻-N was determined after cadmium reduction to NO₂⁻-N, followed by sulfanilamide-NAD reaction³⁰. Available P was extracted by acid-ammonium fluoride solution (0.025 mol L⁻¹ HCL + 0.03 mol L⁻¹ NH₄F)⁴⁴. Soil microbial biomass carbon (MBC) was estimated by chloroform fumigation extraction⁴⁵.

The remained samples were air-dried and used to measure pH (soil : water = 1 : 2.5) and the nutrient contents. Exchangeable elements (K⁺, Ca²⁺, Mg²⁺) were extracted with 1 mol/L NH₄OAC¹⁸, and exchangeable Al³⁺ was extracted with 1 mol/L KCl (soil : solution = 1 : 10)⁴⁶. All base cations were determined by inductively coupled plasma optical emission spectrometer (Perkin Elmer, USA).

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

Statistical analysis. Data were tested for normality using the Kolmogorov-Smirnov test and for equality of error variance using Levene's test. Two-way analysis of variance (ANOVA), with N and P as the main factors, was performed to test the effects of N and P treatments and potential N × P interactions on soil pH, available N and P, exchangeable base cations and Al³⁺, total litterfall production, and microbial biomass carbon in each forest. One-way ANOVA with Tukey's b test was used to test differences of all variables other than sum of exchangeable (K⁺ + Ca²⁺ + Mg²⁺) among treatments in each forest, as well as differences among forests (control plots). For sum of exchangeable (K⁺ + Ca²⁺ + Mg²⁺), One-way ANOVA with LSD test was adopted to test the difference between N/P treatment plots relative to control. We also used general linear model to analyze relationship between exchangeable Ca²⁺ and Al³⁺ in the mixed and pine forests. All analyses were conducted using SPSS 16.0 (SPSS Inc., Chicago, USA) for Windows. Level of significance was set with P < 0.05 unless otherwise stated.

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Author contributions

F.Z. carried out the field experiment and analyzed the data. J.M. designed the experiment. F.Z., X.L. and J.M. proposed the scientific hypothesis. L.L. performed the microbial analysis. All the authors contributed to the writing of the manuscript.

Additional information

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