Nitrogen -addition accelerates phosphorus cycling and changes phosphorus use strategy in a subtropical Moso bamboo forest

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Abstract

Ecosystem-level effects of increasing atmospheric nitrogen (N) deposition on the phosphorus (P) cycle and P use strategy are poorly understood. Here, we conducted a seven year N-addition experiment to comprehensively evaluate the effects of N deposition on P limitation, cycling, and use strategy in a subtropical Moso bamboo forest. N addition significantly increased foliar litterfall by 4.7%–21.7% and subsequent P return to the soil by 49.0%–70.1%. It also increased soil acidity, acid phosphatase activity, and soil microbial biomass P, which substantially contributed to a significantly increased soil P availability and largely alleviated the P limitation. This resulted in a significant decrease in the foliar P-resorption efficiency and the abundance and colonization of arbuscular mycorrhizal fungi. Our results indicate that N deposition can reduce plant internal cycling while enhancing ecosystem-scale cycling of P in Moso bamboo forests. This suggests a shift in P use from a ‘conservative consumption’ strategy to a ‘resource spending’ strategy. Our findings shed new light on N deposition effects on P cycle processes and P use strategy at the ecosystem scale under increasing atmospheric N deposition.

1. Introduction

Phosphorus (P) alone or in combination with nitrogen (N) regulates plant growth and primary productivity in most terrestrial ecosystems (Treseder and Vitousek 2001, Marklein and Houlton 2012, Li et al 2016). P limitation is widespread, occurring in 43% of natural terrestrial ecosystems, especially in subtropical and tropical regions due to the highly weathered soils (Du et al 2020). Soil available P (AP) and plant P (leaf P) concentrations; the foliar N:P ratio; and the N resorption efficiency (NRE):P resorption efficiency (PRE) ratio are usually regarded as representative indicators for P limitation (Li et al 2016, Deng et al 2017, Du et al 2020). Plants can take up P directly from the soil via the roots and indirectly via mycorrhizae (which are a symbiosis between roots and arbuscular mycorrhizal (AM) fungi), with acid phosphatase (APase) hydrolyzing the ester-phosphate bonds in soil organic P (Treseder and Vitousek 2001, Taylor et al 2017). P can also be resorbed from foliage prior to abscission, particularly in P-limited environments (Yuan and Chen 2015, Hofmann et al 2016, He et al 2020). Nutrient resorption efficiency (RE) is defined as the percentage of leaf nutrients resorbed before senescence and reflects the relative degree to which plants conserve nutrients invested in physiological and metabolic processes (Yuan and
Chen 2015, He et al 2020, Wu et al 2020). There is a trade-off among these P use strategies depending on soil P availability, plant nutrient demand induced by growth rate, and environmental conditions (Li et al 2016, Wu et al 2020). In general, as a result of long-term adaptation, plants in nutrient-limited environments or with a high relative growth rate (RGR) show a high RE and adopt a ‘conservative consumption’ strategy, whereas those in nutrient-enriched conditions or with a low RGR display a low RE and adopt a ‘resource spending’ strategy (Aerts and Chapin 2000, Wright and Westoby 2003, Wu et al 2020). Whether and how P use strategies change in response to external nutrient inputs, such as N deposition, are not well understood.

Human-induced N inputs are predicted to induce greater P limitation in terrestrial ecosystems (Vitousek et al 2010, Peñuelas et al 2013, Li et al 2016) or induce a shift in nutrient limitation patterns from N to P in forests (Crowley et al 2012). This could further regulate the magnitude of terrestrial carbon (C) uptake in response to climate change (Du et al 2020). A meta-analysis concluded that N input could induce P limitation in terrestrial ecosystems, decreasing plant P concentrations while accelerating P cycling due to the stimulation of phosphatase activity, particularly in tropical forests (Deng et al 2017). Another meta-analysis found that N deposition may aggravate P limitation under N-limited conditions, but alleviate P limitation under P-limited conditions; this was primarily caused by the different responses to N addition by certain plants regarding their nutrient uptakes and nutrient use efficiencies (You et al 2018).

N addition can have positive (Olander and Vitousek 2000, Wang et al 2014), negative (Yang et al 2015, Lü et al 2016), or neutral (Lü et al 2013, Li et al 2016, Deng et al 2017) effects on soil AP and leaf PRE as a result of variations in phosphatase activity (Deng et al 2017, Chen et al 2020), which suggests that N addition can influence leaf PRE (plant P use strategies) by changing soil AP. These inconsistencies indicate that the effects of N input on P vary with plant species and soil P conditions. Furthermore, previous studies have observed only part of the P-cycle, such as soil AP, plant P concentration, and phosphatase activity (Li et al 2016, Deng et al 2017); few studies have simultaneously considered the total P-cycle, including soil total P, soil AP, microbial biomass P, plant biomass, plant P concentration, PRE, and phosphatase activity. This substantially limits our understanding of the effects of increasing N deposition on P cycling and absorption strategies at the ecosystem scale and also constrains efforts to improve the accuracy of global ecological models for quantifying N deposition effects.

As a major bamboo species, Moso bamboo (Phyllostachys edulis) is widely distributed in the tropical and subtropical regions of east and southeast Asia (Song et al 2011). There are about 4.68 Mha of Moso bamboo forest located in its geographic origin in subtropical China, accounting for 72.7% of the Chinese total bamboo forest area (Li and Feng 2019). Due to its rapid growth and strong regeneration ability, Moso bamboo forests have a greater potential for C sequestration than other forests in subtropical China and can play an important role in mitigating climate change (Song et al 2017, Li et al 2019). Moso bamboo forests suffer from P limitation (Song et al 2016a) and are also affected by an N deposition rate of 30 kg N ha$^{-1}$ yr$^{-1}$, a greater deposition rate than that in Western Europe (8–11 kg N ha$^{-1}$ yr$^{-1}$) or the United States (4–5 kg N ha$^{-1}$ yr$^{-1}$) (Holland et al 2003, Jia et al 2014). This region is projected to receive more than 50 kg ha$^{-1}$ yr$^{-1}$ by 2050, with an increase in rate of 1.1 kg N ha$^{-1}$ yr$^{-1}$ (Galloway et al 2004, Ackerman et al 2019). How dramatic increases in N deposition will influence P cycling and uptake strategy in Moso bamboo forests consisting of uneven-aged bamboo plants is poorly understood.

We conducted a 7 year N-addition field experiment in a Moso bamboo forest to test four key aspects of the P cycling process. First, we considered whether N addition can significantly decrease soil P availability and aggravate P limitation by decreasing phosphatase activity. Second, we considered the influence of N addition on plant dependence on arbuscular mycorrhizal fungi (AMF) for P absorption. Third, we examined whether N addition can increase foliar P resorption. Finally, we aimed to characterize whether N increase would result in a change of P use strategy from a ‘conservative consumption’ strategy to a ‘resource spending’ one. In contrast to previous studies focusing only on a single aspect of the P cycle, this study takes a broad view of the full P cycle to comprehensively describe N deposition effects at the ecosystem scale, including leaf P resorption, litterfall P return, soil microbial activity, enzyme activity, soil P availability and P limitation, P absorption strategy, and underlying mechanisms.

2. Materials and methods

2.1. Study site

The field site is located in Qingshan Town, Hangzhou City (30°14′ N, 119°42′ E), Zhejiang Province, China, which is in the northern edge of the subtropical monsoon climate zone. It has four distinct seasons, a mild climate, abundant rainfall with a mean annual precipitation of 1420 mm, an average annual temperature of 15.6 °C, and an annual frost-free period of about 230 d. The topography is characterized by low hills, with an elevation of 100–300 m. The Moso bamboo plantation surveyed was established on a previous native evergreen broadleaf forest in the late 1970s, and the soil here was classified as a ferrosol derived from granite (Li et al 2019). N deposition over most parts of southern China, the main distribution area of Moso bamboo forests, has reached 30 kg N ha$^{-1}$ yr$^{-1}$.
The understory vegetation is sparse with a cover of approximately 5% and total herbaceous biomass of 14.6 kg ha\(^{-1}\). A detailed description of the field site is provided in Li et al (2019).

2.2. Experimental design

Twelve plots (20 × 20 m) were randomly established in the study site in November 2012. Each plot was surrounded by a 20 m wide buffer zone to avoid disturbing nearby plots. Based on a method used in previous studies simulating N deposition (Fang et al. 2007), N addition treatments were designed as follows in reference to local N deposition rates (30 kg N ha\(^{-1}\) yr\(^{-1}\), jia et al. 2014): low-N addition of 30 kg ha\(^{-1}\) yr\(^{-1}\) (N30), medium-N addition of 60 kg ha\(^{-1}\) yr\(^{-1}\) (N60), and high-N addition of 90 kg ha\(^{-1}\) yr\(^{-1}\) (N90). These treatments were each randomly applied to three replicate plots, with three control plots left untreated. The details of the initial plot setup are shown in table S1 (available online at stacks.iop.org/ERL/16/024023/mmedia). Based on a report stating that NH\(_4^+\) and NO\(_3^-\) account for 56.1% and 43.9% of the wet N deposition in China, respectively, and an average NH\(_4^+\):NO\(_3^-\) of 1.28 (Song et al. 2020), we selected NH\(_4\)NO\(_3\) as the N source. The required quantity of NH\(_4\)NO\(_3\) was dissolved in 10 l water, which was then sprayed evenly on the forest floor of each plot beginning in January 2013 and each month thereafter. Each control plot received the forest floor of each plot beginning in January 2013.

2.3. Leaf and foliar litterfall sampling and analysis

Moso bamboo leaves have a unique biological rhythm. New shoots usually emerge in April and leaves appear in June of the same year. These initial leaves fall the following spring, and new leaves quickly emerge. The new leaves then have a life span of 2 years and are replaced biennially in spring (Zhang et al. 2017a). Moso bamboo forests are characterized by years of alternating high and low recruitment of new bamboo, resulting in unevenly aged forests with leaves covering a 2 year interval (Song et al. 2016a). In the Moso bamboo stand at our study site, new bamboo emerges in the beginning of April in every even-numbered year (2014, 2016, and so on), reaches a maximum height in late May, and new leaf growth is complete within a month. All leaves from young and mature bamboo fall together in the spring of every odd-numbered year. We collected fresh leaf samples in July 2016 and senescing leaf samples in March 2017 in order to respectively capture the maturation and senescence stages of the bamboo leaf lifecycle. Based on the previous inventory (Song et al. 2020), three representative young Moso bamboo plants with diameter at breast height (DBH) close to average emerging in April 2016 (1 year old) and three representative mature Moso bamboo plants with DBH close to average emerging in April 2014 (3 year old) in each plot were selected for sampling. Twenty healthy leaves on the south-facing side in the mid-upper canopy were collected from each bamboo plant, placed in a cooler, and then transported to the laboratory. Samples were dried at 105 °C for 30 min, then at 65 °C to a constant weight. They were then milled for measurements of N and P concentrations.

Previous studies have demonstrated that the cumulative amount of foliar litterfall from March to June accounts for about 80% of the annual foliar litterfall in Moso bamboo forests (Zhang et al. 2017a). Six sample collection frames of 100 × 50 cm and a depth of 30 cm were randomly established in each plot to measure leaf litterfall amount and P return. Leaf litter was collected monthly from March to June 2017, samples were dried at 65 °C to a constant weight, and total P and N concentrations were measured (Song et al. 2015).

2.4. Soil sampling and physicochemical analysis

We collected samples of the topsoil (0–20 cm depth) and subsoil (20–40 cm) in the May growing season every year from 2013 to 2019. Soil cores that were 5 cm in diameter to a depth of 20 cm were collected from five random locations in each plot. The samples were transported to the laboratory in a constant temperature box containing ice, sieved using <2 mm mesh, and divided into two portions. One portion was stored in a refrigerator for measuring microbial biomass phosphorus (MBP) within 3 d using the chloroform fumigation-extraction method using soil extractant (0.5 M NaHCO\(_3\)) at a ratio of 1:20 with a conversion factor (K\(_{P}\)) of 0.4 (Brookes et al. 1982). The other portion was air-dried for measuring soil AP and available N (AN) concentrations. Soil AP was extracted with 0.5 mol l\(^{-1}\) NaHCO\(_3\) and determined using the molybdenum blue method (Lu 2000). AN was determined using the alkali N-proliferation method (Lu 2000). The soil N:P ratio was calculated based on AN and AP. Some air-dried soil samples collected from 2015 onward were also used to determine APase (Enzyme Commission, EC 3.1–3.2) activity based on the concentration of p-nitrophenol released at 410 nm when soils were incubated with toluene and buffered sodium p-nitrophenyl phosphate solution in a buffer with pH 6.5 at 37 °C for 24 h (Dick 2011).

2.5. Assessment of AMF colonization and soil AMF abundance

Soil adhering to bamboo roots was collected by digging in five random locations in each plot in July and October of 2018, and January and April of 2019. The samples were stored at −80 °C for molecular analysis. After collection of rhizospheric soils, the roots were cleaned gently with distilled water and stored at 4 °C. Root samples were cut into approximately 1 cm segments, cleared with 10% (v/v) KOH at 90 °C for 30 min, and stained with trypsin blue. The AM fungal
colonization rate was determined using the grid cross method after staining with an improved acid fuchsin method (Kormanik et al. 1980). Soil DNA was extracted from 0.3 g soil using a Mobio Powersoil™ DNA Isolation Kit (Mobio Laboratories Inc., Carlsbad, CA, USA). The quality and concentration of the extraction was evaluated using a spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA), and extracted DNA was preserved at −80 ºC.

AM fungi gene copy numbers were determined using qPCR with an ABI-7300 (Applied Biosystems Inc. Carlsbad, CA, USA). Each reaction in 20 µl contained a specific primer set for AM fungi: AMV4.5NF/AMDGR (5′-AAGCTC GTAGTTGAATTTCG-3′/5′-CCCCAATCTCCCATATT AATCAT-3′) (Sato et al 2005). Each reaction mixture contained 10 µl SYBR® Premix Ex Taq™ (Vazyme Biotechnology, Nanjing, China), 0.8 µl of each primer, 2 µl diluted DNA (10–20 ng), and 6.4 µl nuclease-free deionized (DI) water. Amplification was initiated by denaturation at 95 ºC for 5 min, followed by 40 cycles of denaturation at 95 ºC for 5 s, annealing at 55 ºC for 30 s, and a final extension at 72 ºC for 40 s. Standard curves were created using a ten-fold dilution series of plasmid DNA containing the AM fungi gene. Serial dilution of the DNA template was used to determine whether the qPCR was inhibited during amplification. Amplification resulted in single peaks with efficiencies of 92.65% and R² values of 0.999.

2.6. Statistical analyses
Leaf mass-based nutrient concentration was used to estimate nutrient RE. We defined NRE and PRE as the percentage of leaf N or P resorbed prior to senescence, calculated as:

\[
\% \text{ RE} = \left(1 - \frac{\text{MLCF} \times [\text{N or P}]_{\text{senescent}}}{[\text{N or P}]_{\text{mature}}} \right) \times 100
\]

where \([\text{N or P}]_{\text{senescent}}\) and \([\text{N or P}]_{\text{mature}}\) are the N or P concentrations (mg g⁻¹) of senescent (March 2017) and mature (July 2016) leaves, respectively, MLCF (mass loss correction factor) is a correction factor that accounts for the mass loss during leaf senescence (Vergutz et al 2012, Achat et al 2018), calculated according to the ratio of the dry mass of 50 senesced leaves and the dry mass of 50 green leaves with three replications in each treatment (table S2).

All statistical analyses in this paper were conducted using the SPSS 22.0 software package for Windows (SPSS Inc., Chicago, IL, USA). All data were checked for normality and homogeneity of variance before testing for treatment differences. One-way analysis of variance and least significant difference calculations at a 5% confidence level were performed to investigate the significance of the effects of N addition rate on foliar P concentration; the N:P ratio; P resorption; the NRE:PRE ratio; litterfall and P return; and soil AP, total phosphorus (TP), MBP, APase, and pH. A Pearson correlation analysis was used to explore the relationships between soil AP and foliar P concentrations, P resorption, P return, soil MBP, APase, and pH.

3. Results

3.1. Soil AP, TP, MBP, and APase
AP concentrations of topsoil significantly increased under the N addition treatments compared with those of the control, especially after 2 years of N addition (\(P < 0.001\); figure 1(a)). This rapid increase tended to stabilize after 4 years of N addition. AP concentrations in subsoil showed a similar trend, although the increase was much smaller than that in topsoil (figure 1(b)). The trend became more obvious with N addition duration. After 4 years, AP concentrations in topsoil were greatest under the N30 treatment but tended to stabilize under the N60 and N90 treatments. By contrast, TP concentrations of both topsoil and subsoil did not significantly change with N addition (figure S1).

Soil MBP was significantly higher under the N treatments than in the control (\(P < 0.01\)), and was highest under the N60 treatment during most of the experiment, with this increase tending to stabilize after 3 years (figure S2(a)). In the topsoil, pH significantly declined with N addition but tended to stabilize after 4 years (figure S3(a)). The pH of the subsoil also declined with N addition, but these changes were not significant (figure S3(b)). Soil APase activities significantly increased under N addition compared with those in the control (figure 2(a)). APase activities under the N30 treatment tended to increase more than those under the N60 and N90 treatments, in which the increases in APase activities tended to level out over time. Soil AP showed a significantly positive correlation with soil APase (figure 2(b)) and MBP (figure S2(b)). Soil AP was significantly negatively correlated with topsoil pH after 2 years of N addition (figure S3(c)), but it was not significantly correlated with subsoil pH (figure S3(d)).

3.2. AMF colonization rate and gene copy number
AMF colonization rate of fine roots significantly declined under N addition compared with that of the control (\(P < 0.001\)), and the degree of decline decreased with N addition rate (figure 3(a)). Similarly, the number of AMF gene copies also significantly decreased under N addition treatments but were not significantly different among N treatments (figure 3(b)). Both AMF colonization rate and gene copy number showed significantly negative correlations with soil AP (figure S4).

3.3. Foliar P concentration, the N:P ratio, P resorption, and the NRE:PRE ratio
Fresh foliar P concentration in young bamboo (1 year old) was significantly higher (20.6%) than that in
mature bamboo (3 year old) under the N-free treatment. Compared with the control, N addition significantly increased fresh foliar P concentration of young bamboo (11.3%–44.8%, $P < 0.05$; figure 4(a)). Foliar P concentration of mature bamboo did not show a significant change with N addition, and foliar
P concentration even declined under N90 addition. There was a significant positive linear correlation between the foliar P concentration of young bamboo and AP concentration of topsoil ($P < 0.05$; figure S5(a)). The fresh foliar N:P mass ratio of young bamboo (16.9) was not significantly different from that of mature bamboo (17.5), and it significantly decreased with N addition but significantly increased in mature bamboo ($P < 0.05$; figure 4(b)). Foliar PRE of young bamboo was significantly greater by 100.1% than that of mature bamboo under the N-free treatment. Foliar PRE of both young and mature bamboo significantly declined with N addition ($P < 0.05$), by 23.3%–42.4% and 20.2%–25.7%, respectively (figure 5(a)). They both showed negative and significant linear correlations with both topsoil and subsoil AP (figure S6). N addition significantly decreased foliar NRE of both young and mature bamboo (figure S7). The foliar NRE:PRE ratio of mature bamboo (1.33) was significantly higher than that of young bamboo (0.96), and it significantly decreased with N addition but significantly increased in young bamboo under the N60 treatment ($P < 0.05$; figure 5(b)).

3.4. Foliar litterfall and P return
The amount of foliar litterfall significantly increased by 17.5% and 21.7% under the N30 and N60 treatments, respectively, compared with that of the control, but it increased only slightly (by 4.7%) under the N90 treatment (figure S8(a)). P return to soil via foliar litterfall significantly increased by 49.0%, 70.1%, and 56.9% under the N30, N60, and N90 treatments, respectively, compared with that of the control (figure S8(b)). Foliar P return showed a significantly positive correlation with soil AP (figure S9).

4. Discussion
4.1. Effects of N addition on P availability in soil
N addition significantly decreased topsoil pH (figure S3(a)). Soil pH has a considerable effect on the bioavailability of soil inorganic P, although the
extent and even direction of the effect are rather difficult to predict (Hinsinger 2001). Low pH may drive phosphate to bind to aluminum and iron oxides, leading to a decline in soil P availability (Yan et al 2017, Wu et al 2019). However, contrasting outcomes have also been observed (Hinsinger 2001, Barrow 2017). The decrease in soil pH may induce solubilization of phosphate by altering organic acids and thus increasing soil AP (Grinsted et al 1982, Marra et al 2012, Andersson et al 2015), which supports our finding of a significantly negative correlation between soil AP and pH (figure S3(c)). The mechanism by which soil pH affects soil AP content is complex and is attributed to multiple soil biological processes and abiotic factors; thus, this matter requires further study.

Soil APase can cleave ester bonds in a variety of organic P compounds to release phosphate, making them the dominant group of enzymes involved in mineralizing P in acidic soils (Duff et al 1994). However, this P acquisition process has a large N requirement, as phosphatases have relatively high N concentrations (about 15%) (Olander and Vitousek 2000, Treseder and Vitousek 2001, Marklein and Houlton 2012, Deng et al 2016). N addition could therefore substantially increase APase production and activity (Chen et al 2020), resulting in greater soil P availability, subsequently increasing foliar P concentrations. In the present study, N addition significantly increased soil APase activity (figure 2(a)). Furthermore, the significantly positive correlation between soil AP and APase (figure 2(b)) indicates that soil APase significantly contributed to increasing soil AP.

Soil microorganisms, after death, can release P to the soil, serving as an important source of soil AP. However, a few studies have shown that high MBP can decrease AP content due to AP being immobilized in microbial biomass when microbes do not suffer a C limitation (Chen et al 2016, Deng et al 2017). We found that N addition significantly increased soil MBP, which subsequently increased soil AP (figure S2). Similar results have been observed in tropical forests (Casack et al 2011). In addition, abundant NH$_4^+$ derived from NH$_4$NO$_3$ addition in this study could have also helped phosphate-solubilizing microorganisms to solubilize inorganic phosphate and contribute to the increase in soil AP; this was attributed to the decrease in soil pH induced by N addition, which further promoted the activities of phosphate-solubilizing microorganisms (Asea et al 1988). These positive effects of N addition together yielded a significant increase in soil AP, in contrast to our first objective. It is worth noting that the increase in soil AP, APase, and MBP tended to stabilize after 4 years of N addition, indicating that the priming effects of N addition on AP taper-off over time.

AMF can form mycorrhizae with about 80% of terrestrial plants and provide P to 70%–90% of plant species (Taylor et al 2017). AMF are sensitive to soil pH and P availability; low soil pH can inhibit AMF. Plants invest less C in AMF when the availability of P or N in soil is sufficient (Xiao et al 2019). In our study, N addition significantly decreased soil pH (figures S3(a) and (b)) but increased P availability (figure 1), resulting in a significant decrease in AMF gene copy number and colonization rate (figure 3). This indicates that Moso bamboo might decrease their C investment in AMF when N input is increased, causing a considerable decline in the dependence on AMF to obtain P. This is in opposition to our second objective.

### 4.2. P return through litterfall and resorption

Plants return P to the soil mainly through litterfall and subsequent decomposition. The litter contribution to soil nutrient return is usually greater in tropical forests than that in many temperate forests (Powers and Marin-Spiotta 2017). In previous research conducted in the same plots, we found that N addition significantly increased Moso bamboo productivity by 23.9%–36.8% (Song et al 2020), which contributed to a 4.7%–21.7% increase in litterfall, increasing P return by 49.0%–70.1% (figure S8) and ultimately replenishing soil P stocks. Organic P can be mineralized to soluble forms via soil microbes or rhizosphere microflora mediated by enzymes, especially phosphatases (Taraftar and Claassen 1988). The finding that AP concentration was significantly greater in topsoil than in subsoil supports this conclusion (figure 1). Meanwhile, the significantly positive correlation between soil AP and foliar P return (figure S9) indicates that high soil AP content increases plant productivity and eventually the turnover of leaf litter with high P concentrations in Moso bamboo forests. Our previous studies revealed that N addition and increased soil AP content significantly enhanced photosynthetic rates and productivity of Moso bamboo (with a higher increase in young plants than mature plants) (Zhang et al 2017b, Song et al 2020), which supports this theory. A similar result was observed in upland heathlands (dominated by Calluna vulgaris) in Wales (Kritzler and Johnson 2010).

Nutrient resorption from senescing foliage is a key strategy employed by perennial plants to overcome limitations and reduce dependence on soil nutrient availability, which plays a critical role in growth in nutrient-limited conditions where uptake via the roots usually costs more energy relative to resorption (Wright and Westoby 2003, He et al 2020). On average, about 50%–64.9% of leaf P is recycled via resorption (Vergutz et al 2012), with PRE from senesced leaves decreasing rapidly with increasing soil P availability (Hofmann et al 2016). We observed that N addition significantly increased soil AP, which may decrease the energy cost of direct P absorption from soil, leading to a significant decline in foliar PRE (figures 5 and S6). In addition, PRE decreases with increasing foliar P concentrations (Vergutz et al 2012). In the present study, N addition significantly
increased foliar P concentrations of young bamboo (figure 4(a)), which also led to a significant decline in foliar PRE (figure 5(a)). However, this effect was not found in mature bamboo, which may be attributed to the different growth stages of the plant. This is in contrast to our third objective. The foliar PRE (figure S6) and AMF colonization rate and gene number (figure S4) significantly declined with increasing soil AP, which showed that bamboo P uptake gradually shifted from foliar P resorption and AMF uptake to direct root absorption as a response to increasing atmospheric N deposition (figure 6). This indicates that the P use strategy of Moso bamboo changed from a ‘conservative consumption’ strategy to a ‘resource spending’ strategy, which supports our fourth objective. This change in P use strategy induced by N addition may drive bamboo to invest less photosynthates and energy into nutrient resorption and AMF for P uptake. The present results imply that N deposition reduced internal P cycling in plants via a decrease to the PRE. Conversely, N deposition enhanced ecosystem-scale P cycling by increasing P return to soil through litterfall and accelerating subsequent litter decomposition, which was due to higher foliar P concentrations induced by lower PRE compared with that of the control.

Plant nutrient demand and use strategy are closely correlated with RGR (Li et al. 2018, Wu et al. 2020). Young Moso bamboo completes its rapid height growth at the end of May and quickly begins to accumulate dry matter (Song et al. 2016b), which places a greater demand on P compared with mature bamboo, and thus yields a higher PRE (figure 5(a)). This drives young bamboo to adopt a ‘conservative consumption’ strategy (with high PRE) while mature bamboo tends to adopt a ‘resource spending’ strategy (with low PRE). A similar result was observed in a chronosequence of Chinese fir (Cunninghamia lanceolata (Lamb.) Hook., Taxodiaceae) plantations (Wu et al. 2020). Mature bamboo often provides nutrient elements, including P and N, to young bamboo via connected underground rhizomes to meet their rapid growth demands (Sun et al. 2019). Therefore, young bamboo have significantly greater foliar P concentrations than mature bamboo (figure 4(a)). Moreover, the fact that the only significant correlation between foliar P concentration and soil AP concentration was in young bamboo.
and the AP concentration of topsoil (P < 0.05; figure S5(a)) indicates that mature bamboo is insensitive to soil AP variation, while topsoil was the main nutrient source for young bamboo. More than 80% of Moso bamboo roots are localized in the top 30 cm of soil (Zhou and Fu 2004), which supports our above results that nutrition of bamboo mainly comes from the topsoil. Our previous study found that young bamboo showed significantly higher rates of photosynthesis and stronger responsivity to N addition than those in mature bamboo (Zhang et al. 2017b), supporting the above assumption that the growth rate of plants influences plant nutrient demand and use strategy. Although foliar N and P concentrations of young and mature bamboo varied, foliar N:P ratios did not show a significant difference between young and mature bamboo, which suggests that the growth stages of bamboo could alter foliar N and P concentrations but not N:P ratios.

### 4.3. The shift in nutrient limitation

Several approaches have been used to infer nutrient limitations, including fertilization experiments, thresholds of leaf N:P ratios, and leaf NRE:PRE ratios (Güsewell 2004, Du et al. 2020). Fertilization experiments can be used to directly test nutrient limitations (Du et al. 2020). In our previous study at the same site, N addition significantly increased the productivity of young and mature bamboo by 50.0%–62.6% and 16.7%–45.1%, respectively, indicating that the Moso bamboo at this site suffers from N limitation (Song et al. 2020). However, no study has investigated the effects of P addition alone on the productivity of Moso bamboo. The N:P ratio has been widely applied as a threshold for identifying nutrient limitations in terrestrial plants (Güsewell 2004). It has been suggested that plants are N-limited at a biomass N:P ratio <14 and P-limited at >16, with an ambiguous boundary between the two (Koerselman and Meuleman 1996). However, different plants and species will have varying nutrient demands, calling into question the universality of the N:P thresholds. Some studies suggest that leaf N:P thresholds of 10 and 20 could better indicate N or P limitation (Güsewell 2004, Yan et al. 2017). In the present study, the N:P ratio of young bamboo (16.9) was similar to that of mature bamboo (17.5) in the control, but under N addition, young bamboo had significantly greater foliar P concentrations and reduced N:P ratios (15.7–12.7). However, high N addition (90 kg ha\(^{-1}\) yr\(^{-1}\)) significantly decreased foliar P concentrations of mature bamboo and increased N:P ratios accordingly. The N:P ratios of both young and mature bamboo in all treatments were between 10 and 20 (figure 4(b)), which suggests that the Moso bamboo simultaneously suffered from N and P limitation. Furthermore, a recent study demonstrated that the leaf NRE:PRE ratio can be used to estimate N and P limitations at the ecosystem scale (Du et al. 2020). An NRE:PRE ratio >1 indicates a stronger N limitation than P limitation. Alternatively, P is more limiting when the NRE:PRE ratio is <1 (Du et al. 2020). In this study, the leaf NRE:PRE ratio of young bamboo was <1, which suggests that young bamboo suffers more from a P limitation. Conversely, the leaf NRE:PRE ratio of mature bamboo was >1, which suggests that mature bamboo suffers more from an N limitation. These results indicate that the estimation on nutrition limitations should consider plant species and growth stage. Additionally, medium-N addition increased the NRE:PRE ratio of young bamboo (>)1 (figure 5(b)), which suggests that medium-N addition alleviates P limitation. N addition significantly decreased the NRE:PRE ratio of mature bamboo (from 1.32 to 0.87), which indicates that mature bamboo shifted from an N limitation to a P limitation. Above all, our findings further confirm that the effects are heavily dependent on plant growth stage and environmental conditions.

### 5. Conclusion

N input significantly increased bamboo foliar litterfall, P return, soil acidity, soil APase activity, and MBP, contributing to a significant increase in soil P availability. This induced a significant decline in foliar P resorption, AMF colonization rate, and AMF abundance. The significant and positive effects of N addition on several key parts of the P cycle indicate that N deposition can alleviate P limitation, alter P use strategy from a ‘conservative consumption’ to a ‘resource spending’ strategy, and accelerate ecosystem-scale P cycling by weakening plant internal cycling in subtropical Moso bamboo forests. Our findings provide a broad perspective of N input effects on P cycling, P use strategy, and P limitation mitigation in subtropical forest ecosystems for a future with increased atmospheric N deposition.

### Data availability statement

All data that support the findings of this study are included within the article and/or the supplementary information files.

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