



Differential aboveground-belowground adaptive strategies to alleviate N addition-induced P deficiency in two alpine coniferous forests



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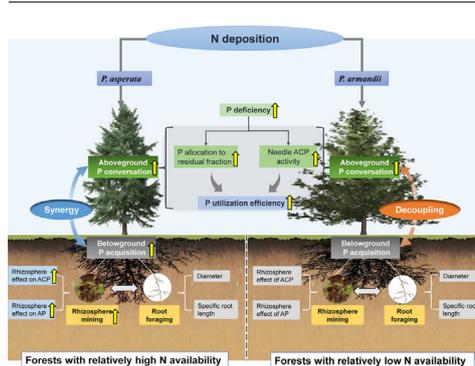
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HIGHLIGHTS

- Nitrogen (N) addition aggravated phosphorus (P) deficiency in two alpine coniferous plantations.
- Plant strategies to cope with N-induced P deficiency varied by initial soil N availability.
- Aboveground P conservation and belowground P acquisition were synergistic in N-rich stands but decoupled in N-poor stands.

GRAPHICAL ABSTRACT



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ABSTRACT

Increasing atmospheric nitrogen (N) deposition has resulted in phosphorus (P) limitation in multiple terrestrial ecosystems, yet how plants coordinate aboveground and belowground strategies to adapt to such P deficiency remains unclear. In this study, we conducted a field N fertilization experiment in two alpine coniferous plantations (*Picea asperata* Mast. and *Pinus armandii* Franch.) with different soil N availability on the eastern Tibetan Plateau of China, to examine N addition effects on plant nutrient limiting status and plant adaptive strategies corresponding to aboveground P conservation and belowground P acquisition. The results showed that N addition aggravated P deficiency in both plantations, as indicated by decreased needle P concentrations and increased N:P ratios, and that plant strategies for addressing such P deficiency differed in the two plantations with different initial soil N availabilities. In the *P. asperata* plantation with relatively high N availability, significantly enhanced needle phosphatase activity and shifts in P fraction allocation (downregulation of the structural P fraction and increased allocation to the residual P fraction) co-occurred with increased rhizosphere effects on phosphatase activity under N addition, indicating a synergistic strategy of aboveground P conservation and belowground P mining to alleviate P deficiency. In the *P. armandii* plantation with relatively low N availability, however, N addition only enhanced phosphatase activity and increased allocation to residual P fraction in the aboveground but had little effect on belowground P acquisition-associated traits, suggesting a decoupling relationship between aboveground P conservation and belowground P acquisition. This study highlights

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the vital significance of initial soil nutrient availability in regulating the coordination of aboveground and belowground strategic alternatives, emphasizing the need to integrate soil nutrient conditions for a holistic understanding of forest adaptation to anthropogenic N enrichment.

1. Introduction

Nitrogen (N) and phosphorus (P) are two dominant nutrients that limit the productivity of terrestrial plants (Elser et al., 2007; Tang et al., 2018). A balanced soil N and P supply is critical for maintaining ecosystem structure and function, as a relatively constant ratio of N and P is required for plant metabolism and growth (Finzi et al., 2011). Unlike the massive anthropogenic N deposition by fossil combustion and artificial fertilizer application over the past century, P deposition derived mostly from dust and mineral aerosols is typically small compared with N inputs (Galloway et al., 2008; Peñuelas et al., 2013). Increased N loading generally boosts plant photosynthesis and growth, thereby initially stimulating productivity in N-limited terrestrial ecosystems (Matson et al., 2002; Thomas et al., 2010). However, persistent disproportionate inputs of N and P would cause strong stoichiometric imbalances in ecosystems over time, potentially switching terrestrial ecosystems from N limitation to P limitation (Goswami et al., 2018; Heuck et al., 2018; Peñuelas et al., 2013). Such nutrient-limitation patterns in forests are usually depicted by soil available P, foliar N:P ratio, and the N resorption efficiency (NRE):P resorption efficiency (PRE) ratio (Du et al., 2020; Tessier and Raynal, 2003). Although growing evidence of N enrichment-induced P limitation has been documented in diverse terrestrial ecosystems (Gradowski and Thomas, 2006; Li et al., 2016), whether and how plants can adapt to such P deficiency remains unclear, hampering our prediction of vegetation dynamics following future N enrichment.

Improvements in aboveground P-use efficiency and expansion of belowground P acquisition are empirically considered the most important pathways to cope with P limitation in plants (Deng et al., 2016; Lambers, 2022; Lin et al., 2020). In the aboveground, enhanced P-use efficiency can be achieved by the extended residence time of P within the canopy (Kitayama, 2013; Veneklaas et al., 2012), increased P resorption in leaves before senescence (Hayes et al., 2014; Hofmann et al., 2016), or optimized allocation of foliar P fractions and associated phosphatase, allowing maximum plant fitness (Hidaka and Kitayama, 2011; Mo et al., 2019). These pathways are important nutrient conservation mechanisms that make plants less dependent on external nutrient availability. Among them, leaf P resorption alone supplies approximately 40 % of annual plant P demands (Cleveland et al., 2013; Yuan and Chen, 2009). Apart from aboveground P conservation, vascular plants have also evolved diverse belowground strategies to increase P acquisition, mainly shaped by traits of fine roots (Lambers, 2022; Lugli et al., 2020). Typical root pathways to improve P foraging include more carbon investment in absorptive fine roots, higher specific root length, thinner roots, and increased frequency and length of root hairs (Konoplenko et al., 2017). Additionally, plants can also employ root physiological or P-mining strategies such as increased acid phosphatase release into the rhizosphere or promoting phosphatase production by stimulating rhizosphere microbial activities to mobilize unavailable P forms (such as organic P) (Chen et al., 2020; Pang et al., 2018; Wen et al., 2019). All these P-saving or acquisition strategies have been recognized in some fertilization experiments or ecosystems with low soil P availability. However, neutral or even negative effects of N addition on leaf P resorption, nonstructural P allocation, and traits of fine roots are still widely observed (Li et al., 2015; Deng et al., 2016). These inconsistencies suggest that these nutrient adaptive strategies may vary by plant species and soil nutrient conditions.

More importantly, the aforementioned strategies for alleviating P limitation involve variable energy investments by plants, thus synergistic or tradeoff relationships may occur between aboveground P conservation and belowground P acquisition (Kou et al., 2017; Lin et al., 2020;

Zhao et al., 2020). Several recent studies have shown that plants increase P conservation or acquisition independently to overcome N enrichment-caused P deficiency (Deng et al., 2016; Kou et al., 2017), while synergistic relationships have also been found in other studies (Lin et al., 2020). One potential explanation for such distinct findings may be the variabilities in local soil nutrient conditions and plant stoichiometric homeostasis. The type and magnitude of initial nutrient limitation, as well as plant stoichiometric homeostasis, which essentially dictates the degree of plant P limitation under N addition (You et al., 2018), could therefore substantially drive plant adaptive strategies related to P use and uptake. Furthermore, since the main pathways to alleviate plant P limitation are enzyme-regulated degradation or carbon consumption behaviors that have different N and energy requirements (Hogan et al., 2010; Marklein and Houlton, 2012), resource investment in specific strategies may rely on background N supply levels. The increased soil phosphatase activity, for example, was more pronounced in ecosystems with greater soil N availability, where N limitation of plant growth could be easily lifted with increased N supply (Chen et al., 2020; Li et al., 2016). Such impacts of local soil fertility or the strength and type of nutrient limitation on the responses of leaf P resorption, allocation of P fractions, root-associated acid phosphatase, and root traits to N deposition have been found in divergent studies (Kou et al., 2017; Li et al., 2021; Zheng et al., 2020). However, most previous studies focused on individual changes in aboveground or belowground strategies and paid less attention to the associations of plant strategies with local soil fertility, resulting in a very limited understanding of plant adaptive mechanisms under changing soil nutrient conditions characterized by N deposition.

Here, we carried out a 4-year N-addition field experiment in two alpine coniferous plantations (*Picea asperata* Mast. and *Pinus armandii* Franch.) on the eastern Tibetan Plateau of China to examine nutrient limiting characteristics and plant adaptive mechanisms under N deposition. These two plantations are typical N-limited forests, and are characterized by distinct local soil N availability as a consequence of the accumulation of biological differences in tree species (i.e., litter quantity and quality) over 40 years (Yin et al., 2014; Guo et al., 2021). By investigating the responses of aboveground and belowground traits related to P use and acquisition processes to N addition in these two plantations, we were able to detect the interactions between aboveground P conservation and belowground P acquisition strategies to alleviate N enrichment-induced P limitation and assess the rules of local soil nutrients in such strategy-selection. Specifically, we aimed to test the following hypotheses: (i) N addition would induce or aggravate plant P deficiency, given that increased N availability always increases plant P demand due to stimulated growth in N-limited forests (Li et al., 2016); (ii) aboveground P conservation and belowground P acquisition strategies would work concurrently to alleviate P deficiency induced by N addition, and this coordination may be dependent on soil nutrient conditions.

2. Materials and methods

2.1. Study site

Our study was conducted in two ca. 40-year-old alpine coniferous (*P. asperata* Mast. and *P. armandii* Franch.) plantations located in the Maoxian Ecosystem Research Station of the Chinese Academy of Sciences on the eastern Tibetan Plateau in Sichuan, China (31°42'N, 103°54'E, 2200 m a.s.l.). The area has a typical alpine monsoon climate with warm, humid summers and cold, dry winters. The average annual temperature is 8.9 °C, ranging from -1.1 °C in January to 18.8 °C in July, and the mean annual

precipitation is 920 mm with most rainfall occurring in the periods of June to August. The two plantations are about 1 km apart with similar land-use histories and close phylogenetic relationships (Ran et al., 2018). They were established in two previous similar and separate areas that experienced complete shrub deforestation in the 1980s. Due to the accumulation of biological differences in tree species over 40 years, these two plantations had different soil nutrient availabilities, with *P. asperata* plantation having a relatively higher soil N availability than the *P. armandii* plantation (Table S1). The understory of the plantations is dominated by herbs and shrubs, including *Impatiens potaninii* Maxim., *Lamium barbatum* Sieb. et Zucc., *Polygonum sieboldii* Meissn., and *Rubus setchuenensis* Bureau et Franch. The soil is classified as a Typic Calcic Luvisol in the IUSS classification.

2.2. Experimental design

A randomized block design with three replicated blocks of two treatments (N addition and ambient) was applied in the two plantations (Fig. 1; Yin et al., 2021). Each block contained a pair of 10 m × 10 m plots that were randomly assigned to the N addition treatment or ambient control, and separated by a 10-m buffer zone. Since May 2017, for the N-addition plots, artificial N fertilization was applied by ammonium nitrate (NH₄NO₃) solution evenly sprayed on the forest floor in the first week of every month during the growing season (from May to October). Given that the measured ambient nitrogen deposition rate in this region is 26.45 kg N ha⁻¹ yr⁻¹ (Zhu et al., 2015) and the ‘critical load threshold’ above which significant adverse effects on specific environmentally sensitive elements would occur is 50 kg N ha⁻¹ yr⁻¹ (Yu et al., 2019; Zhu et al., 2020), the N application level was set to 25 kg N ha⁻¹ yr⁻¹ to simulate the possible impacts of N deposition on forest ecosystems when N deposition exceeds the “critical load threshold” in the future. Meanwhile, ambient plots received 20 L of water to match the same amount of water input caused by N fertilization. The understory vegetation in each

plot was removed once a month to minimize the interference of non-target species with the experimental results.

2.3. Needle sampling and measurements

In the middle growing season (August) of 2019 and 2020, three healthy individuals of similar diameters at breast height were randomly selected for sampling in each plot. For each individual, 1- and 2-year-old twigs with needles of the sun-exposed mid-upper canopy were accessed through climbing and sampled using a pole pruner. Freshly fallen senesced needles during September–October of 2019 and 2020 were collected by three litter traps (1 m × 1 m × 0.5 m) installed in each plot. All needle samples were immediately placed in a 0–4 °C cooler with ice once collected and returned to the laboratory within 3 h. After being taken back to the laboratory, green (fresh) needles from the same plot were pooled together and divided into two samples. The first green needle samples were stored at 4 °C for the determination of enzyme activities; the second green needle samples and the senesced needle samples were freeze-dried to constant weight, and ground into powders with a ball mill for analysis of P fractions and total N and P concentrations.

2.3.1. Needle acid phosphatase (ACP) activity

Following the method of Ushio et al. (2015), green needle samples homogenized with acetate buffer were mixed with a reaction medium consisting of 5 mM *p*-nitrophenyl phosphate (pNPP) and 50 mM acetate buffer. After incubation in the dark at 20 °C for 1 h, the reaction was terminated by the addition of 0.5 M NaOH and 0.5 M CaCl₂. The absorbance of the filtrates was measured with a spectrophotometer at 410 nm, and converted to the amounts of *p*-nitrophenol (pNP) generated by incubation on the basis of a standard curve of *p*-nitrophenol. The activity of acid phosphatase was expressed as μmol pNP per gram fresh weight per hour (μmol pNP g⁻¹ FW h⁻¹).

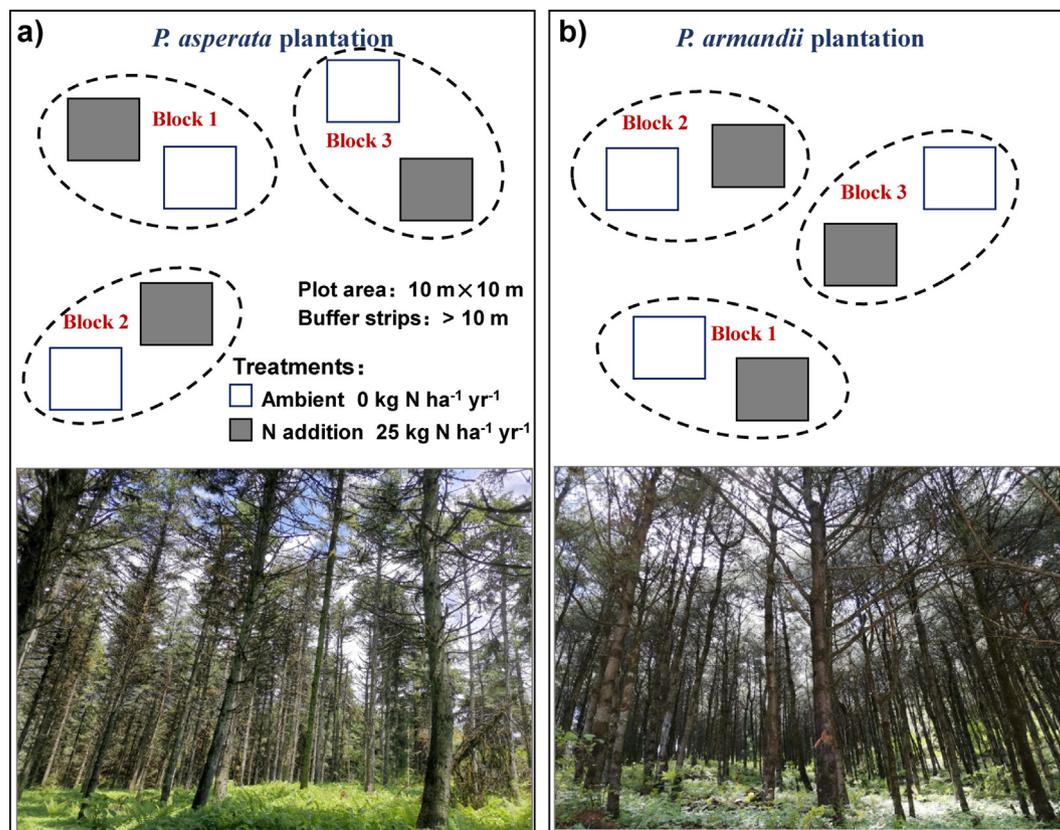


Fig. 1. Schematic diagram of *P. asperata* and *P. armandii* plots and the experimental design.

2.3.2. Needle total N and P concentrations and resorption efficiency

Total N concentrations of green and senesced needles were analyzed by an elemental analyzer (Vario Macro cube, Elementar). Total P concentrations of them were determined colorimetrically by the molybdenum blue method after digestion with sulfuric acid and hydrogen peroxide. Needle nutrient resorption efficiency (NuRE) refers to the proportion of nutrients in green needles that were resorbed during senescence, which was calculated using the equation below:

$$\text{NuRE} = \left(1 - \frac{X_{\text{green}}}{X_{\text{senesced}}} \times \text{MCLF} \right) \times 100\%$$

where X_{green} and X_{senesced} represent the N or P concentrations in green and senesced needles, respectively. MCLF is the mass loss correction factor with a value of 0.745 for conifer species (Vergutz et al., 2012).

2.3.3. Needle P fractions

Concentrations and proportions of four major P fractions, including structural P, metabolic P, nucleic acid P, and residual P in fresh needles were quantified using a modified sequential extraction method (Hidaka and Kitayama, 2011; Mo et al., 2019). Briefly, each freeze-dried ground sample was extracted sequentially with 12:6:1 CMF (chloroform, methanol, formic acid, v/v/v), 1:2:0.8 CMW (chloroform, methanol, water, v/v/v) and water-washed chloroform. The supernatants obtained by three groups of extraction were combined and the lower lipid-rich phase was used for the structural P determination. The upper lipid-insoluble phase and the remaining residue were mixed with methanol (85 % v/v) and extracted with pure TCA (trichloroacetic acid). Then, the supernatant obtained by centrifugation was used for metabolic P analysis. The TCA-insoluble residue was further extracted by hot water bath with TCA solution (2.5 %, w/v), and the liquid layer was used to measure nucleic acid P. The residue after the final extraction was used for determining residual P. Following these sequential extraction steps, the P concentrations of each fraction were measured colorimetrically by the method for total P measurements described above and expressed on a dry mass basis (mg/g). The proportion of each P fraction was calculated by dividing by the total P concentrations.

2.4. Root, soil sampling and measurements

On the same day of needle sampling, root, rhizosphere soil, and bulk soil for each target tree were collected through the soil block method described by Guo et al. (2008). For each target tree, three 30 cm (length) × 20 cm (width) × 20 cm (depth) soil blocks with intact terminal branch orders were carefully collected near the trunk by tracing the intact root systems to the lateral root clusters. Each harvested soil block was immediately placed into a rigid plastic box to keep the intactness of the root branches as well as the attached rhizosphere soil, and transported to the laboratory by a cooler with ice. Based on our previous studies, the fine roots of 1–3 orders were identified as absorptive roots predominantly responsible for resource acquisition (Wang et al., 2021), and thus were used for our analysis. In the laboratory, the intact roots of 1–3 orders were separated carefully following Pregitzer et al. (2002). Soil that adhered to the terminal 1–3 orders of roots was defined as rhizosphere soil, while soil non-adhering to roots was defined as the bulk soil (Phillips and Fahey, 2006; Zhu et al., 2020). The roots or soil samples from the same plot were pooled together, and stored at 4 °C for further morphological and chemical trait analysis.

2.4.1. Root morphological traits

Intact root branches were scanned using a scanner at a resolution of 400 dpi (Epson Expression 11000XL, Seiko Epson Corp., Nagano, Japan), and were then analyzed with WinRhizo software (Regent Instruments, Inc., 2012) for the average diameter, total length, and volume. The scanned root samples were then oven-dried at 65 °C to constant mass and weighed. Specific root length (SRL) was calculated as root length divided by dry mass; root tissue density (RTD) was calculated as dry mass divided by total volume. Afterward, the oven-dried absorptive roots were milled to

fine powders to determine the total N and total P concentrations by the method described above.

2.4.2. Soil acid phosphatase, available P, and rhizosphere effects

To assess the rhizosphere effect on soil P transformation, the activity of a key extracellular enzyme involved (i.e., acid phosphatase (ACP)), and the available P (AP) concentration of bulk soil and rhizosphere soil were measured. The acid phosphatase activity of soils was measured following the same method for needle acid phosphatase described above, and expressed as $\mu\text{mol pNP per gram dry soil per hour}$ ($\mu\text{mol pNP g}^{-1} \text{h}^{-1}$). Soil available P concentrations were determined colorimetrically using the molybdenum blue method after extraction with 0.5 M NaHCO_3 . The rhizosphere effects on ACP and AP were calculated as percentage differences between paired bulk and rhizosphere soils, respectively (Shan et al., 2018).

2.5. Statistical analyses

Fixed effects of species, treatments, and their interactions on needle nutrient concentrations, nutrient resorption efficiency, their ratios, needle ACP, root traits, and rhizosphere effects on AP and ACP were tested by two-way analysis of variance (ANOVA). Data was transformed to obtain the normality of residuals. Differences in these indicators between the ambient and N addition treatments were evaluated by Student's *t*-test with plots as replicates. N addition effects on aboveground and belowground traits across the two sampling years were defined as the change in traits from the N added plots relative to the ambient plots, and their differences from zero were tested by *t*-test. Additionally, a principal component analysis (PCA) was conducted with the R package “FactoMineR” to assess the relationships among N-addition effect on traits of aboveground and belowground and needle nutrients for each species. To diminish the interference of interannual effects on traits, each value for PCA was standardized and divided by the averaged value from the ambient plots for each sampling year. The traits were classified into three types: needle conservation (PRE, proportions of needle P fractions, and needle ACP), root foraging/conservation (root diameters, SRL and RTD) and rhizosphere mining (rhizosphere effects on soil AP and ACP), and then the variance partitioning analysis was used with the “varpart” function of the R package “vegan” to determine the explanation degree for these traits in regulating needle P nutrients. All data analyses were conducted with the software R v. 4.0.4 (R Development Core Team, 2021).

3. Results

3.1. Needle nutrient concentrations and P resorption efficiency

Averaged across the two sampling years, needle N did not change substantially, while needle P concentrations decreased by $15.26 \pm 2.37\%$ and $15.85 \pm 3.07\%$ in the *P. asperata* and *P. armandii* plantations, respectively, under N addition ($P < 0.05$; Fig. 2). This resulted in an increase of $9.75 \pm 7.74\%$ in the needle N:P ratios of *P. asperata* from 14.05 ± 0.56 to 15.42 ± 1.02 , and an increase of $6.34 \pm 5.53\%$ in that of *P. armandii* from 11.71 ± 0.39 to 12.45 ± 0.55 , although the effect sizes were not statistically significant ($P > 0.05$; Fig. 2). In the case of needle nutrient resorption, N addition significantly decreased needle N resorption efficiency (NRE) in the *P. asperata* plantation but had no significant effect on P resorption efficiency (PRE) in either plantation (Fig. 2). As a result, only in the *P. asperata* plantation did the NRE: PRE ratio decrease significantly ($P < 0.05$; Fig. 2a). The changing trends of concentrations and resorption efficiency of needle N and P under N addition were consistent in the two sampling years (Fig. S1).

3.2. Needle P fractions and acid phosphatase

N addition significantly affected the allocation of needle P fractions ($P < 0.05$; Fig. 3). In the *P. asperata* plantation, N addition significantly decreased the structural P proportions by $9.32 \pm 2.27\%$ but increased

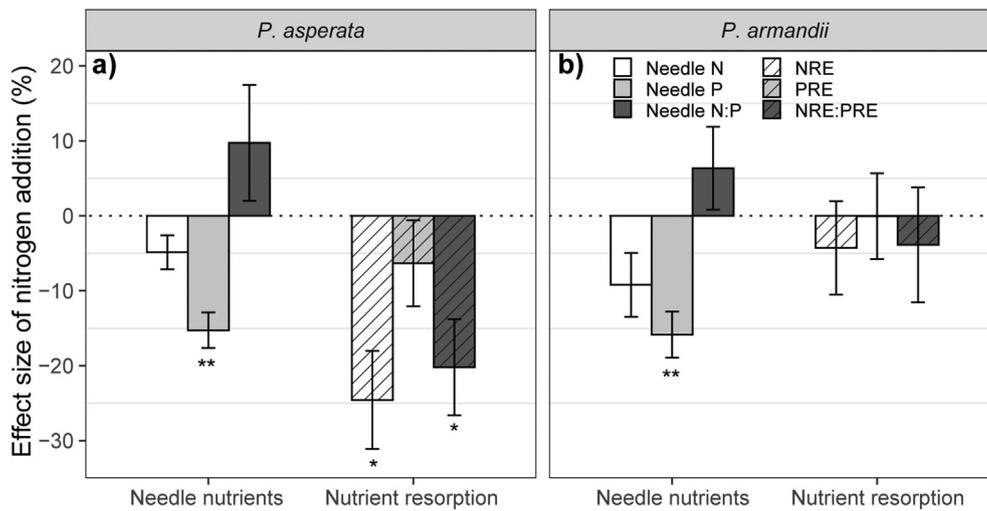


Fig. 2. Effect of N addition on needle nutrient concentrations and their ratios, and on nutrient resorption efficiency and their ratios (with slashes) in the *P. asperata* and *P. armandii* plantations. Values are means \pm standard errors across two growing seasons in 2019 and 2020. The horizontal dotted line was drawn at the effect = 0. Asterisks represent the significant effect of N addition treatments (*, $P < 0.05$; **, $P < 0.01$).

residual P proportions by $24.63 \pm 3.89\%$ ($P < 0.05$; Fig. 3a). This result was mainly due to a decrease of $24.00 \pm 3.19\%$ in structural P concentrations but an increase of $6.99 \pm 4.78\%$ in residual P concentrations in this plantation. In the *P. armandii* plantation, however, only the proportion of residual P showed a significant increase of $5.83 \pm 2.46\%$ with N addition ($P < 0.05$; Fig. 3b), while the proportions of the other P fractions were unaffected.

N addition also induced significant decreases in needle acid phosphatase activities during the two sampling years (Fig. S3). Averaged across the two years, needle acid phosphatase activities decreased by $40.93 \pm 7.70\%$ and $48.21 \pm 5.00\%$ in *P. asperata* and *P. armandii* plantations, respectively (Fig. 3).

3.3. Rhizosphere effects on soil P mining and root morphological traits

The key extracellular enzyme involved in soil P mining, acid phosphatase activities (ACP) in rhizosphere soil, and the rhizosphere effect on it under N addition were much higher than those under ambient treatments

in *P. asperata* plantation, with average increases of $39.28 \pm 5.36\%$ and $76.80 \pm 25.10\%$, respectively, across the sampling periods, although the differences were not statistically significant (Fig. 4). Similar trends were observed in the concomitant available P (AP) concentrations in rhizosphere soil and the rhizosphere effect on AP in the N-added *P. asperata* plantation (Fig. 4b). However, N addition did not affect rhizosphere ACP activities, AP concentrations, or rhizosphere effects in the *P. armandii* plantation. Such distinct effects of N addition among the two plantations were consistently observed over the two years (Fig. S4).

Regarding root traits related to nutrient acquisition, N addition significantly decreased root diameters in the *P. armandii* plantation, and decreased specific root length (SRL) in the *P. asperata* plantation in the year 2020 but not in the year 2019 (Fig. S4). The effect of N addition on root diameters and SRL was insignificant when averaged across the two sampling years (Fig. 4; $P > 0.05$). Additionally, N addition did not affect root tissue density (RTD) in the *P. asperata* plantation but increased RTD significantly in the *P. armandii* plantation over two years (Figs. 4, S4e).

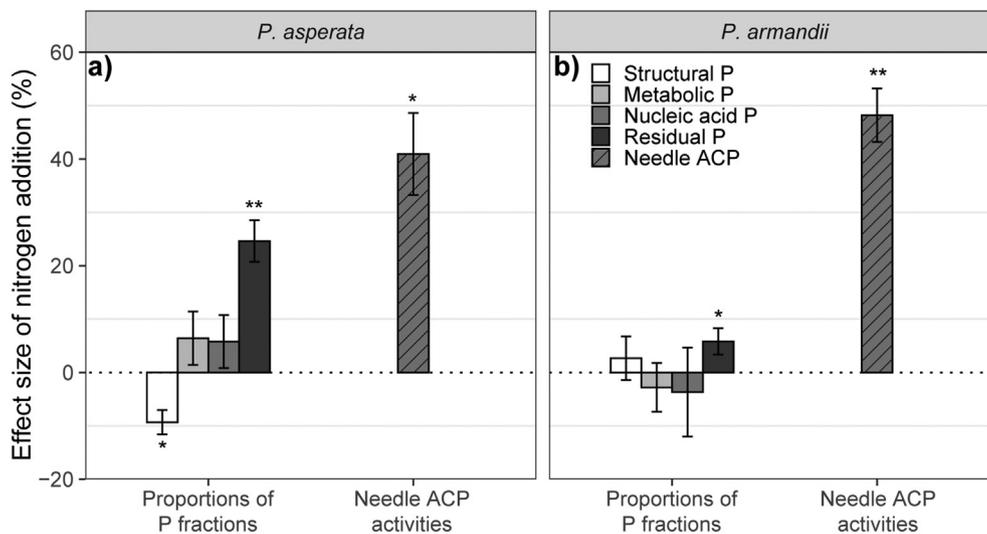


Fig. 3. Effect of N addition on proportions of P fractions and needle acid phosphatase activities (with slashes) in the *P. asperata* and *P. armandii* plantations. Values are means \pm standard errors across two growing seasons in 2019 and 2020. The horizontal dotted line was drawn at the effect = 0. Asterisks represent the significant effect of N addition treatments (*, $P < 0.05$; **, $P < 0.01$).

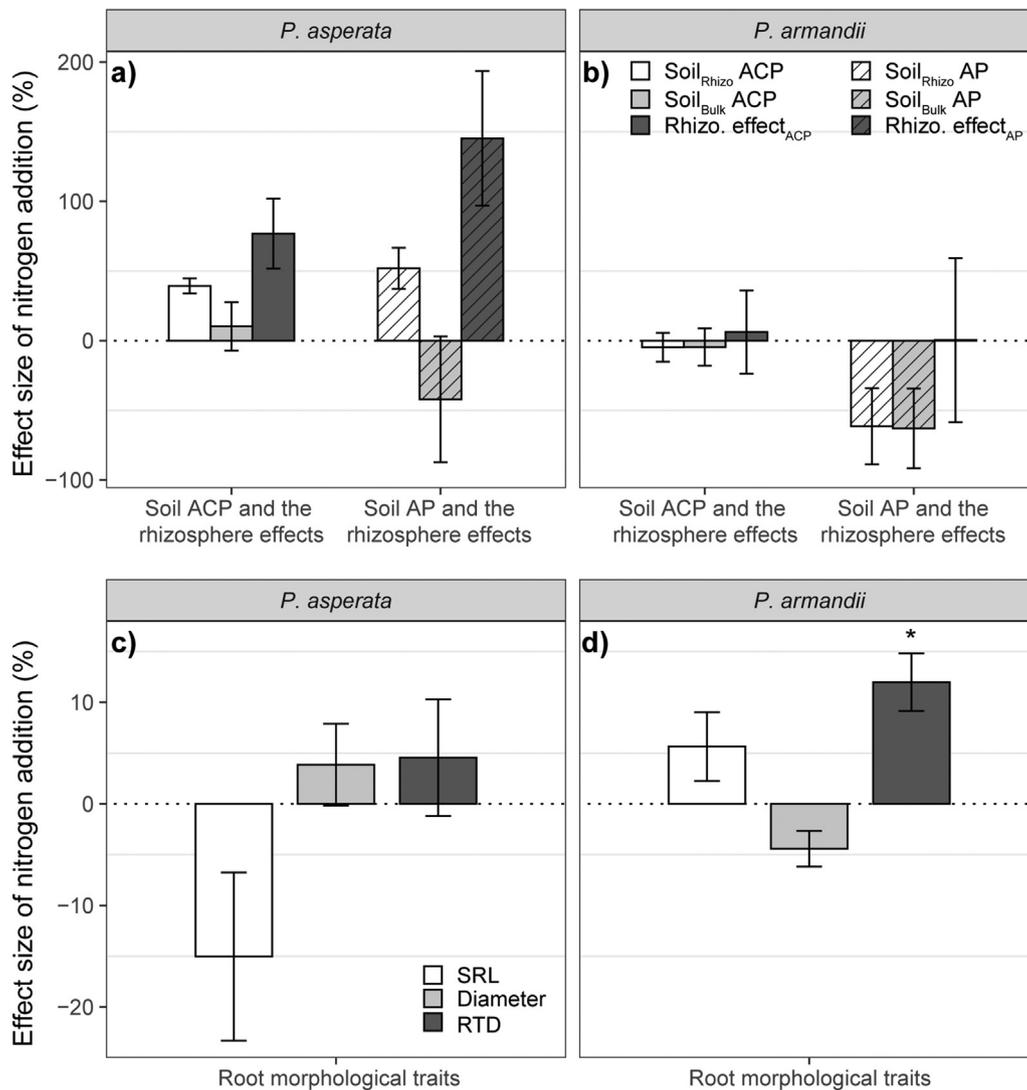


Fig. 4. Effect of N addition on soil P mining including soil acid phosphatase activities (ACP), available P contents (AP) in rhizosphere soil and bulk soil and the rhizosphere effect (a, b), and on root morphological traits including root diameter, specific root length (SRL) and root tissue density (RTD) in the *P. asperata* and *P. armandii* plantations. Values are means \pm standard errors across two growing seasons in 2019 and 2020. The horizontal dotted line was drawn at the effect = 0. Asterisks represent the significant effect of N addition treatments (*, $P < 0.05$; **, $P < 0.01$).

3.4. Relationships among N addition effects on aboveground P conservation traits, belowground P acquisition traits, and needle nutrients

Principal component analysis (PCA) clearly showed the relationships among N addition effects on traits of aboveground, belowground and needle nutrients. For *P. asperata* plantation, the first two axes accounted for 59.1 % of the total variation. The N-addition effect on needle N:P was negatively correlated with that of needle P contents, which was associated with the change in needle ACP, the rhizosphere effect of ACP, and the proportion of residual P under N addition (Fig. 5a). Variance partitioning showed that the change in traits related to needle conservation and the rhizosphere effects of soil P mining dominated the change in needle N and P nutrients, with individual explanatory contributions of 67.9 % and 64.0 %, respectively (Fig. 5c).

For the *P. armandii* plantation, the first axis explained 33.3 % of the total variance, and mainly represented the variation associated with the N addition effect on needle N. The second axis explained 20.3 % of the total variance, and mainly represented the variation related to the N-addition effect on needle P concentrations and N:P ratios. This axis was highly correlated with the N addition effect on needle ACP and RTD, with greater

needle ACP activity and RTD in more P-deficient plots. Overall, the change in traits associated with needle conservation and root foraging/conservation were the dominant contributors to needle nutrients with individual explanatory contributions of 39.7 % and 72.6 %, respectively (Fig. 5d).

4. Discussion

4.1. Effects of N addition on plant P nutrients

Leaf N and P nutrient concentrations and their ratios have proven useful to diagnose the types and degrees of plant nutrient limitation (Güsewell, 2004; Li et al., 2016). The N:P ratio threshold of 14:1 versus 16:1 or 10:1 versus 20:1 is generally recommended to indicate N limitation and P limitation (Güsewell, 2004; Koerselman and Meuleman, 1996; Yan et al., 2017). In the present study, although changes in N:P ratios did not indicate a shift in nutrient limitation types according to the thresholds, a significant decrease in needle P concentrations and an increase in N:P ratio during the two consecutive years could clearly reflect an insufficient P supply and increasing P deficiency in the two plantations under N addition, which is consistent with our first hypothesis. Moreover, compared to the

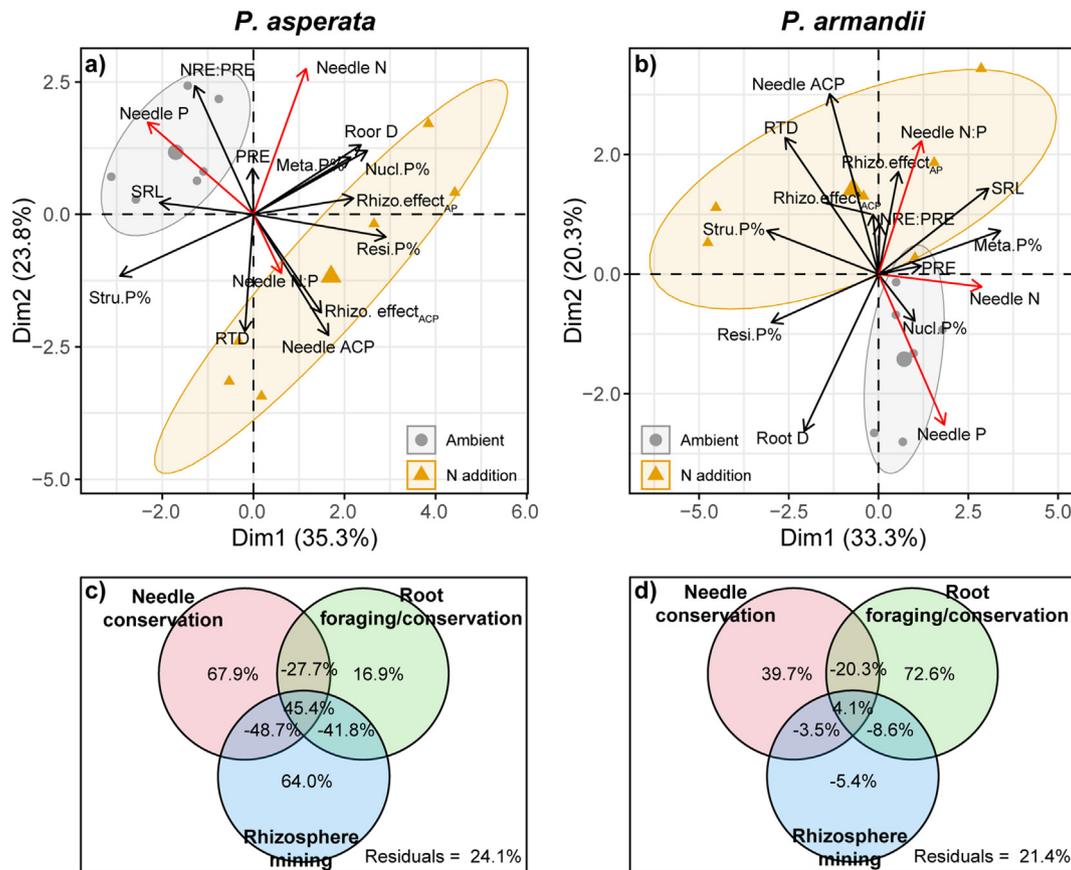


Fig. 5. Principal component analysis (PCA) for the relationships among N-addition effects on aboveground and belowground traits and needle P nutrients for each species in two plantations (a, b). The two panels below (c, d) show the variance partitioning analysis of the explanation for the relative contribution of needle conservation, root foraging/conservation, and rhizosphere mining in regulating needle P nutrients. PRE, phosphorus resorption efficiency; NRE: PRE, the ratio of nitrogen and phosphorus resorption efficiency; needle ACP, needle acid phosphatase activities; Stru. P%, proportions of structural P; Meta. P%, proportions of metabolic P; Nucl. P%, proportions of nucleic acid P; Resi. P%, proportions of residual P; Rhizo. effect_{ACP}, rhizosphere effects on soil acid phosphatase activities; Rhizo. effect_{AP}, rhizosphere effects on soil available phosphorus; Root D, root diameter; SRL, specific root length; and RTD, root tissue density.

P. armandii plantation, N addition was more effective in mitigating soil N limitation in the *P. asperata* plantation due to its relatively high soil N availability and needle N:P ratio (Fig. 2), resulting in a more pronounced P deficiency in this plantation. The significant decrease in NRE: PRE ratio occurring in the *P. asperata* plantation further provided additional evidence for such N enrichment-induced P deficiency (Fig. 2), as it was recently proposed that a lower NRE: PRE ratio indicated greater P deficiency (Du et al., 2020). In the *P. armandii* plantation, however, no significant changes in NRE: PRE ratio were observed. Such mismatches in results presented by the NRE: PRE ratio and N: P ratio call into question the universality and precision of the relative resorption efficiency of N vs. P in diagnosing the change in nutrient status, especially for nutrient fertilization experiments, in which the absence of a significant effect of N addition on nutrient resorption efficiencies has been widely reported (Chen et al., 2015; Deng et al., 2016; Yan et al., 2018).

This result of P deficiency induced by N enrichment is in line with previous N addition experiments and meta-analyses on forests and has been attributed to altered nutrient demand and soil nutrient supply (Gonzales and Yanai, 2019; Goswami et al., 2018; Mao et al., 2020; Yuan and Chen, 2015). First, the increased N supply promotes plant growth so that the demand for P increases. Therefore, plant P deficiency can be induced or aggravated when soil available P cannot meet plant P demands (Deng et al., 2016; Li et al., 2016). Second, external N deposition can diminish soil P availability by driving P to bond to aluminum and iron oxides caused by soil acidification (Tian and Niu, 2015; Vitousek et al., 2010), or through promoting P retention by microorganisms and increasing the

accumulation of soil organic matter (Gradowski and Thomas, 2006; Lu et al., 2012). Alternatively, N deposition can increase soil available P through enhanced P solubilization or mineralization with organic acids and by stimulating soil phosphatase activities (Andersson et al., 2015; Chen et al., 2020). In this study, phosphatase activities in the bulk soils did not change, while soil P availability slightly decreased in the two plantations after N addition (Fig. 4), indicative of an inhibition of soil P availability by N addition in both forests. In addition, the marginal increase of soil DIN:AP in the two N-added plantations also indicated an enhancement of soil P shortages (Fig. S5). Therefore, the decline in soil P supply may be an essential reason for N addition-induced plant P deficiency in the two plantations, which may ultimately result in the shift in nutrient limitation if N deposition persists.

4.2. Strategies to alleviate N-induced P deficiency depend on soil fertility

The two N-added plantations examined in this study showed similar aboveground P conservation strategies. Under N addition, needle acid phosphatase activities increased dramatically, accompanied by a significant change in needle P fraction allocation by increasing concentrations and proportions of residual P fractions, while PRE remained unaffected by N addition (Figs. 2, 3), indicating the adoption of optimization of internal P allocation and regulation of phosphatase activities to acclimate to low P availability induced by N addition. The steady PRE was against previously observed higher P resorption under lower soil P availabilities, but consistent with several studies showing that resorption efficiency was generally

unaffected by nutrient addition (Chen et al., 2015; Huang et al., 2018; Zheng et al., 2020). A reasonable explanation for these findings is that nutrient resorption efficiency reflects the long-term evolutionary adaptability of plants to nutrient availability, and it may be influenced more by the energy cost of resorbing nutrients than by instant soil nutrient variations (Gerdol et al., 2019; Yuan and Chen, 2015). In contrast, changes in internal P allocations and regulation of phosphatase activities are functionally linked to photosynthesis, metabolism, growth and P use efficiency, which may play important roles in conserving P and improving plant adaptation (Lambers, 2022). Among the four P fractions, structural P is in membrane phospholipids (Hidaka and Kitayama, 2011). Under a low-P environment, replacing phospholipids with P-free lipids, such as galactolipids and sulfolipids, has been experimentally confirmed, particularly in P-efficient crop plants (Andersson et al., 2003; Lambers et al., 2012; Kuppasamy et al., 2014; Lambers, 2022). Therefore, our finding of the decreased allocation to the structural P fraction in N-added *P. asperata* plots may be an important mechanism for plants to increase P use efficiency. Combined with phosphatase mobilization of organic P, the downregulation of structural P could provide P for key metabolites (e.g., ADP, ATP, and sugar phosphates), phosphorylated proteins, and ribosomal RNA, thereby sustaining key physiological processes such as Calvin–Benson cycle, photosynthesis assimilation and protein synthesis (Hidaka and Kitayama, 2011; Mo et al., 2019). Additionally, residual P is an insoluble P compound comprised primarily of phosphorylated proteins, which has been proven to play essential roles not only in phosphate uptake, distribution, and remobilization, but also in many metabolic processes, such as carbohydrate metabolism, phytohormones, and signal transduction (Parra-Almuna et al., 2019; Ma et al., 2021). Therefore, in this study, the significant increase in P investment to this P fraction may be associated with improved plant adaptation to P deficiency, which is consistent with the results of one previous fertilization experiment that found that residual P was the only fraction that increased after N addition (Mo et al., 2019). These findings suggest a pivotal functional role of residual P fractions in alleviating N-induced P deficiency. In such cases, shifting internal P fraction allocations may be a more plastic and cost-effective strategy than increasing nutrient resorption efficiency to conserve P under instant soil nutrient status, and ultimately plays a critical role in improving plant fitness for the alpine coniferous forests.

Different from aboveground strategies, plant belowground strategies to cope with P deficiency differed in the two plantations. In the *P. asperata* plantation, N addition considerably boosted the rhizosphere effect on acid phosphatase and available P concentrations, but had little effect on root traits (Fig. 4), indicating a reliance on mining strategies to increase P acquisition. However, in the *P. armandii* plantation, N addition did not affect the rhizosphere effect on soil P transformation, but increased root tissue density, implying a conservative P use rather than enhanced P acquisition in roots. This result suggests a synergistic aboveground P conservation with belowground P acquisition strategy in *P. asperata* plantation but not in *P. armandii* plantation, partly supporting our second hypothesis that aboveground P conservation and belowground P acquisition would work in synergy to alleviate N addition-induced P deficiency. The PCA further confirmed the close cooperation of aboveground P conservation with belowground P mining in the *P. asperata* plantation, but they were decoupled in the *P. armandii* plantation (Fig. 5). Such synergistic or decoupled P conservation-acquisition strategies have been reported in various species through N fertilization experiments, where N addition affected P conservation or acquisition alone or both (Kou et al., 2017; Lin et al., 2020). Moreover, even for the same species, such as larch, the coordination between aboveground P conservation and belowground P acquisition to cope with N addition-induced P deficiency is diverse, as both synergistic P conservation-acquisition strategies and decoupled responses of aboveground PRE and belowground P acquisition were observed in different stands (Deng et al., 2016; Lin et al., 2020). These results imply that apart from the internal factors such as species-specific characteristics, external factors such as N addition levels and duration as well as the local soil nutrient conditions may also have great impacts on

strategic plant options to alleviate P-deficiency. However, our knowledge of the underlying mechanisms driving this diversity remains very restricted.

Here we propose a framework illustrating the differential strategies to alleviate N addition-induced P deficiency in the two coniferous plantations. In the *P. asperata* plantation with relatively high N availability, the aboveground acted in synergy with belowground via greatly increased P mining to enhance P conservation and acquisition. Trees in the *P. armandii* plantation with relatively low N availability relied only on the P conservation strategy to overcome N addition-induced P deficiency (Fig. 6). The disparate strategies in the two plantations support our hypothesis that plant solutions to alleviate N addition-induced P deficiency may be dependent on initial soil nutrient status. The *P. asperata* plantation had a more pronounced P deficiency than the *P. armandii* plantation due to its relatively high N availability. Increasing aboveground P conservation alone may not be adaptive, thus a synergistic conservation-acquisition strategy was adopted to improve plant fitness for *P. asperata* trees. In the *P. armandii* plantation, however, plant P deficiency caused by N addition was relatively modest, and conservative P use might suffice. On the other hand, the rise in rhizosphere effects on soil P cycling was assumed to be generated by enhanced P-associated enzyme synthesis in roots or by stimulating effect of root exudates (e.g., carboxylates) on microbial activities and their interactions. This increase in phosphatase, either by the root itself or by the rhizosphere microorganisms, imposes higher N costs, and thus occurs more easily in ecosystems that already have relatively high soil N contents such as the *P. asperata* plantation (Chen et al., 2020; Mineau et al., 2014; Ratliff and Fisk, 2016). In contrast, the lack of N effects on rhizosphere effects in the *P. armandii* plantation may be attributable to the fact that N addition levels have not yet reversed the type of ecosystem nutrient limitation due to its relatively low N availability, which means plants and microbes remain N-limited with increased N inputs; thus, N loading would be used preferentially for growth rather than phosphatase production or exudation (Kuz'yakov and Xu, 2013; Pii et al., 2015). The finding that root exudation was downregulated in the N-added *P. armandii* plantation further supported this explanation (Jiang et al., 2021). It should be stressed that plant strategies to cope with N-induced P deficiency may be affected by the plant species as well as initial soil nutrients, and thus caution should be exercised in attributing the differences in plant strategies to soil N status. However, given that the two tree species in this study have a close phylogenetic relationship (Ran et al., 2018), the potential effect of species on their adaptation strategy may be minor. Accordingly, although our study could not accurately exclude the impacts of species, our results leave open the idea that soil nutrient status profoundly controls plant strategic options for alleviating N addition-induced P deficiency.

4.3. Implications for future modeling and forest management

Our study confirms that N deposition aggravates P deficiency in alpine coniferous plantations, and underlines the importance of initial soil nutrient conditions in regulating plant aboveground-belowground adaptive strategies to cope with such P deficiency. The coordination of aboveground and belowground strategic alternatives in response to N-induced P limitation will have great ecological consequences on the stability of forest structure and functioning (de Vries and Posch, 2011), but has been largely overlooked in current Earth system models, potentially leading to substantial uncertainties in model projections (Chen et al., 2020). Our results highlight the need to incorporate plant aboveground P conservation and belowground P acquisition associated with soil nutrient conditions into model simulations to better predict nutrient constraints on ecosystem productivity under the scenarios of future climate change.

More importantly, given that N-induced P limitation and the accompanying negative effects on forest productivity have been reported in many ecosystems, management interventions directed at specific stand nutrient conditions could be effective in maintaining forest functions and ecosystem services under N deposition (Du et al., 2019). Therefore, formulating efficient nutrient management practices guided by a more in-depth knowledge on plant aboveground-belowground P adaptive strategies associated

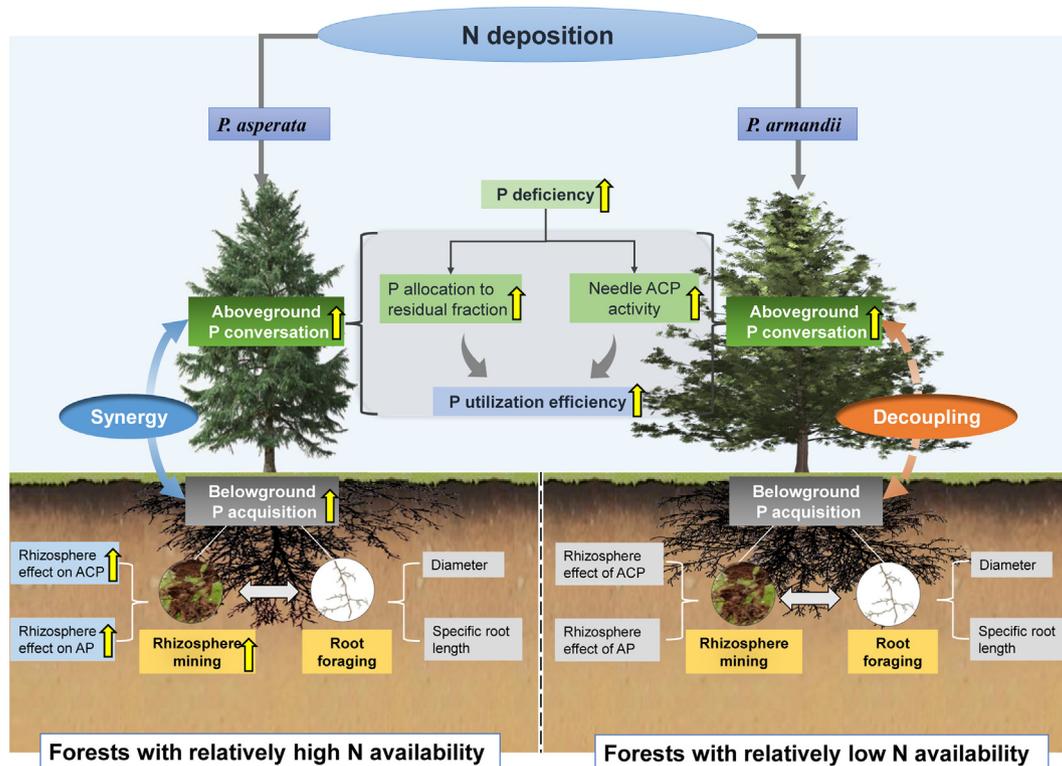


Fig. 6. A conceptual framework illustrating plant strategies of aboveground P conservation and belowground P acquisition to alleviate N addition-induced P deficiency in two coniferous plantations. The up arrows denote the enhanced effect of N addition. ACP, acid phosphatase activities.

with the local soil conditions would be critical for enhancing forest productivity and mitigating any negative climate change effects on forest ecosystems, especially in the hotspot regions of N deposition.

5. Conclusions

Through a simulated N deposition experiment in two alpine coniferous plantations with different soil N availability, we provide robust evidence for enhanced P deficiency under N enrichment and their differential strategies for alleviating such P deficiency. Trees in stands with relatively high N availability adopt a synergistic strategy of aboveground P conservation and belowground P acquisition, whereas those in stands with low N availability rely exclusively on a P conservation strategy. These findings suggest that plant strategies to alleviate N addition-induced P deficiency may depend on soil nutrient status. This result advances our understanding of how aboveground and belowground work concurrently to adapt to altered soil nutrient supply, and it has great implications for vegetation dynamics predictions and forest management in the face of rising atmospheric N deposition. Limited by the relatively short duration of the fertilization treatments and the single level of fertilization, our experiment did not capture the transition point where forests were from N-limited to P-limited. Given that our understanding may be limited by the small amount of forest and relatively short-term N fertilization treatments, it deserves further studies on various types of forests with different experimental schemes to better understand plant adaptation strategies and the underlying mechanisms.

CRedit authorship contribution statement

Peipei Zhang: Conceptualization, Formal analysis, Visualization, Writing – original draft, Writing – review & editing. **Mingzhen Yin:** Methodology, Investigation, Data curation, Writing – original draft. **Xinjun Zhang:** Formal analysis, Writing – review & editing. **Qitong Wang:** Formal

analysis, Writing – review & editing. **Ruihong Wang:** Conceptualization, Formal analysis, Writing – review & editing, Project administration, Funding acquisition. **Huajun Yin:** Conceptualization, Methodology, Formal analysis, Writing – review & editing, Supervision, Funding acquisition.

Data availability

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2022.157906>.

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