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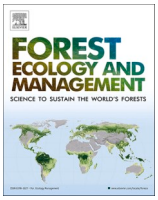

Seven years phosphorus addition has no effect on soil acidity in two tropical plantations

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ABSTRACT

Strong acidity accompanied by low phosphorus (P) availability occurs in tropical soils especially under high nitrogen (N) deposition. Commonly, improved P availability can mitigate soil acidity in tropical natural forests via direct reaction with acidic cations or impacting plant uptake and soil microbial community to indirectly regulate soil cations. However, whether P input can alleviate soil acidity in tropical plantations remains unknown. This study selected two tropical typical plantations (dominated by *Acacia auriculiformis* and *Eucalyptus urophylla*, respectively), to investigate the effects of seven years of P addition on soil acidity and the underlying mechanisms. The results showed that P addition did not change soil pH or cation concentrations, suggesting no variations in soil acidity under P addition. Moreover, P addition did not affect acidic cation concentrations within plant tissues, soil microbial community, litterfall input, and fine-root biomass, indicating that P addition did not impact the processes of plant and soil microbes to regulate the dynamics of soil cations to alleviate acidity. However, we found significant increases in soil available P, soil total P, and plant P concentrations, indicating that added P led to P enrichment in soil and plants. In addition, a consistent response of soil acidity in two tropical plantation ecosystems was observed under combined N with P addition. Our findings improve the understanding of the relationships between P availability and soil acidification in tropical forests and highlight the necessity to accurately evaluate the role of P in acidic tropical forest soils.

1. Introduction

Acidic soils have a pH of 5.5 or lower, they are widely distributed in tropical and subtropical regions, constituting approximately 30 % of the total area of the planet, and 50 % of the arable land in the world (Sade et al., 2016; Yadav et al., 2020). Strong soil acidity concomitant with phosphorus (P) deficiency, characterized by low pH, high levels of acidic cations (Huang et al., 2014), and very low inorganic P levels (Vitousek et al., 2010), are well documented in the tropics. High soil acidity heavily impacts plants growth, survival, and productivity, and threatens the health and functioning of forest ecosystems, hence soil acidification has become a serious global environmental issue (Guo et al., 2010; Smith, 1989).

Phosphorus addition has been reported to increase soil pH and non-acidic cations (Mao et al., 2017; Zhu et al., 2015), thus alleviating soil acidification, especially under conditions of high N deposition. This effect occurs due to the following three reasons: first, added P directly reacts with iron (Fe) and aluminum (Al) compounds, thereby increasing pH values and reducing Al toxicity in soils (Fujii, 2014; Huang et al., 2005; Sloan et al., 1995). Second, P addition promotes the uptake of toxic elements by plants and then indirectly reduces the soil acidity and potential toxicity of soil acidic cations (Cui, 2016; Huang et al., 2005). Third, P addition indirectly alleviates soil acidity via regulating the responses of soil microbes (Cleveland et al., 2002; Liu et al., 2012; Li et al., 2015). Soil microbial communities greatly contribute to the decomposition of organic matter and litterfall via secreting extracellular enzymes (e.g., phenol oxidase, β-glucosidase, and β-*N*-acetyl-glucosaminidase), changing macromolecular plant substrates into small pieces (Liang and Zhu, 2021), resulting in the release of nutrient and the production of organic acid in the soil (Burns et al., 2013; Lin et al., 2019; Mori et al., 2018; Ostertag, 2001; Weand et al., 2010). Organic acid could effectively chelate soil acidic cations (Huang et al., 2005) and soil organic

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matter is the major source of negative charge in most soils and therefore determines the retention and bioavailability of non-acidic cations, such as Ca^{2+}, K^{+}, Mg^{2+} (Londron et al., 2010). Therefore, the response of soil microbes to P addition indirectly regulates soil acidity via impacting the processes of fine root turnover, litterfall decomposition, and soil C dynamics.

It is well documented that soil acidity aggravated under short-term (≤4 yr) but not long-term (>4 yr) P-addition treatments in tropical natural forests (Mao et al., 2017; Niu et al., 2020; Turner et al., 2013; Zarif et al., 2020; Zhang et al., 2011). The different effects on soil acidity due to treatment duration were further validated by a consecutive seven year P-addition experiment in a tropical natural forest, which showed that two- or four-year P addition significantly increased soil pH but after the fifth-year P addition did not affect soil pH (Mao et al., 2017). Such similar effect was also observed on soil microbes. In a tropical natural forest, for example, 2-year P addition increased soil microbial biomass and altered the composition of soil microbial community (Liu et al., 2012), but this effect disappeared after 4 years of fertilization (Liu et al., 2013). In a tropical pine (*Pinus massoniana*) plantation and a mixed coniferous and broad-leaved forest, 2-year P addition had no effects on soil pH and microbial community (Liu et al., 2012; Zhu et al., 2015). By far, it remains inconclusive whether P inputs can alleviate soil acidity of plantation ecosystems, especially under long-term P treatments.

In addition, plantation ecosystems composed of a single, dominant tree species have a greater P demand compared to tropical natural forests (Zhong and Reddell, 1994; Webb et al., 1997), especially in the tropics under conditions of high N deposition. Hence, added P to the soils is more efficiently absorbed and largely accumulates in trees of plantation ecosystems (Chen et al., 1996; Gong and Liao, 2009), because trees have a strong capacity for P absorption and translocation (Fife et al., 2008; Saur et al., 2000). Consequently, long-term P addition results in greater effects on plant P uptake than participating in soil acidification process in the plantation ecosystems. However, it is still limiting for the information on the effects of long-term P addition on the soil acidity of plantation ecosystems. Further, the mechanisms underlying P addition effects on soil acidity remain unknown.

China has the world's largest total area of plantation resources (Luo et al., 2013), with an area of up to 80 million hm^2 in the 2010s (NFGA, 2019). In South China, Eucalyptus and Acacia plantations represent two typical broadleaf plantations. Our previous study showed that the two types of plantations could maintain a stable soil acidity (< 4.0) and a seriously acidified status under long-term N addition (Huang et al., 2021) and suffering heavy atmospheric N deposition (43 kg N ha^{-1} yr^{-1}, Huang et al., 2015). Here, the two typical tropical plantations were chosen to explore the effects of seven consecutive years of P addition on soil acidity and its possible mechanisms. The results from this study will help evaluate the application of P fertilization and guide the scientific management of plantation ecosystems under the scenarios of acid rain and elevated N deposition.

2. Materials and methods

2.1. Study site

This study was conducted in two subtropical plantations at the Heshan National Field Research Station of Forest Ecosystems, Heshan County, Guangdong Province, Southern China (22°34′N; 112°50′E). The elevation of the studied sites ranges from 60 to 80 m. The station has a typical monsoon climate with a mean annual temperature and precipitation of 21.7 °C and 1295 mm, respectively. Background N deposition during precipitation was approximately 43.1 kg N ha^{-1} yr^{-1} from July 2010 to June 2012 (Huang et al., 2015).

One plantation is dominated by the legume N_2-fixing tree species *A. auriculiformis* (AA) and the other by the non-legume tree species *E. urophylla* (EU). Both plantations were established in 1985 with the areas of 4.6 and 1.9 ha, respectively. The soils at both study sites are lateritic red earth with strongly acidic pH values (< 4.0), concomitant with strong P-limitation (Vitousek et al., 2010), as evidenced by the low soil available-P concentration (< 5 mg kg^{-1}). Soil bulk density was 1.3 and 1.2 g cm^{-3} in the AA and the EU plantations, respectively, and soil organic-carbon concentrations were 19.0 and 24.0 g kg^{-1}, respectively. General soil properties (soil organic C, total N, available P, ammonium N, and nitrate N) did not differ among treatment plots in each plantation before N treatment (Table S1, Zhang et al., 2012). Moreover, our previous study found no N-addition effects on soil pH in both AA and EU plantations (Huang et al., 2021).

2.2. Experimental treatments

An experiment with P and N + P addition was established in the two plantations (Fig. S1) in July 2010, including the following treatments: control (C, no fertilization), P addition (P, 100 kg P ha^{-1} yr^{-1}), and N + P addition (NP, 100 kg N ha^{-1} yr^{-1} + 100 kg P ha^{-1} yr^{-1}). The experiment was laid in a randomized complete block design: each of the two treatments and a control were randomly located in three blocks per plantation, with a total of nine plots, each with an area of 10 × 10 m. Each plot was surrounded by a 10-m wide buffer strip. The two plantations were located 500 m apart. Ammonium nitrate (NH_4NO_3) and sodium biphosphate (NaH_2PO_4) were used as N and P sources, respectively. Materials added to the soil were weighed and dissolved in 10 L of water for each plot. The solutions were sprayed bi-monthly on the ground surface in each plot using a backpack sprayer, beginning in August 2010. Simultaneously, each control plot received 10 L of water.

2.3. Samples collection and determination

Soil samples were collected from the 0–10 cm topsoil layer in July 2010 (0 years), July 2011 (1 year), December 2014 (4 years), and December 2017 (7 years), using a 5-cm (inner diameter) soil sampling borer. Three randomly marked locations were sampled in each plot. Soil samples were mixed thoroughly by hand to make composite samples, passed through a 2-mm sieve after removing roots and stones, and divided into two portions. One portion was air-dried for analyzing pH, exchangeable non-acidic and acidic cations, available P, and total P, while the other was used for analyzing soil available N. In turn, soil pH was measured with a glass electrode using a 1:2.5 soil-deionized CO_2-free water suspension. Exchangeable non-acidic (K^{+}, Na^{+}, Ca^{2+}, and Mg^{2+}) and acidic cations (Al^{3+}, Fe^{3+}, and Mn^{2+}) were extracted with 0.1 mol/L $BaCl_2$ (50:1, solution: soil) and the contents were determined using an inductively coupled plasma optical emission spectrometer (ICP-ES, Optima 2000, Perkin Elmer, USA). Cation exchange capacity (CEC) was calculated as the sum of the charge equivalents of all exchangeable cations. Soil available P was extracted with an acid-ammonium fluoride solution and analyzed spectrophotometrically. The air-dried soil was further ground through a 1-mm sieve for the analysis of total P content. Soil total P was determined using inductively coupled plasma (ICP-OES, Optima 2000, Perkin Elmer, USA) after digestion with nitric acid and perchloric acid. Soil ammonium N (NH_4^+-N) and nitrate N (NO_3^--N) were measured using the indophenol blue method followed by colorimetry and a spectrophotometer (Metash Instruments, Shanghai, China), respectively (Liu et al., 1996). Available soil N content was estimated as the sum of the concentrations of ammonium N and nitrate N.

Leaf samples were collected from three fully-illuminated branches of each tree. At least three individuals from each plot of the two plantations were selected in December 2019 for sampling. Branches and roots (diameter > 5 cm) from the previous year were also sampled. All samples were washed with deionized water, oven-dried at 65 ℃ for 48 h, and ground to a fine powder for mineral element analysis. The concentrations of leaf nutrient metals (i.e., K, Na, Ca, and Mg) and toxic metals (i.e., Al, Fe, and Mn) were measured using an inductively coupled plasma optical emission spectrometer (Perkin Elmer, Waltham, MA, USA) after digestion with a mixture of nitric acid and perchloric acid (1

mL nitric acid: 4 mL perchloric acid). N concentrations in the leaves, branches, and roots were measured using a mineral element analyzer. In turn, P contents were analyzed spectrophotometrically after digestion with a mixture of sulfuric acid with perchloric acid (Liu et al., 1996).

Soil phospholipid fatty acids (PLFAs) were analyzed using the method described by Bossio and Scow (1998). Fresh soil samples were extracted with a mixture of chloroform, methanol, and phosphate buffer (1:2:0.8, v/v/v), and then successively eluted with chloroform, acetone, and methanol on silica columns. Finally, the samples were subjected to mild alkaline methanolysis to turn the fatty acids into free methyl esters and then determined by gas chromatography (GC7890, Agilent, USA). The abundance of individual fatty acids was regarded as nmol per gram of dry soil (Tunlid et al., 1989). The concentration of each PLFA was quantitated based on the 19:0 internal standard concentrations. Fungi, actinomycetes, gram-positive (GP) bacteria, gram-negative (GN) bacteria, and arbuscular mycorrhizal fungi (AMF) were identified based on their PLFA biomarkers, and these PLFAs were considered to be representative of the total PLFAs of soil microbial community. The ratios of fungal to bacterial biomass (F:B) were also calculated (Liu et al., 2012).

Fine-root biomass was determined in both plantations. First, soil cores were obtained from the 0–10 cm topsoil layer using a 30 cm ID soil sampling borer at a distance of 1 m from the trunk. Fine roots (diameter $\leq$ 2 mm) were sorted from the soil cores by hand and washed with distilled water. Finally, fine root samples were dried at 65 ℃ for 48 h and weighed.

Litterfall production was calculated for both plantations. A litter trap (0.5 × 0.5 m) with a mesh size of 1 mm was randomly placed in each plot at 0.5 m above the ground surface. All traps were emptied once a month in 2017. Litterfall samples were dried at 65 ℃ for 48 h and then weighed.

2.4. Statistics

One-way analysis of variance (ANOVA) was performed to examine the effects of treatment (P and NP addition) on soil pH, exchangeable non-acidic and acidic cations, CEC, available N and P, microbial community, fine root biomass, litterfall production, nutrients, and toxic metal elements, and tissue N and P. An independent-sample *t*-test was applied to the differences in each of these parameters in both the AA and the EU plantations. Repeated-measures ANOVA was used to examine the effects of P and NP addition on soil parameters across the seven-year treatment period. All data analyses were conducted using SPSS (version 26.0) for Windows (SPSS, Chicago, IL, USA), with statistical significance set at $P < 0.05$, unless otherwise stated. Data shown are mean ± standard error of the mean.

3. Results

3.1. Soil pH

From 2010 to 2017, soil pH values at 10-cm depth in all plots ranged from 3.5 to 4.0. Furthermore, no significant differences between AA and EU plantations ($P > 0.05$) were detected despite P or N + P addition for seven years. Neither treatment significantly affected soil pH at 0–10 cm depth in either plantation ($P > 0.05$), except for the 4-yr P addition treatment in the AA plantation (Fig. 1, $P < 0.05$). Furthermore, there were no differences in pH between 7-yr treatments of P or N + P ($P > 0.05$). Repeated-measures analysis indicated that there were no significant effects of P or N + P addition on soil pH in either plantation ($P > 0.05$).

3.2. Soil non-acidic and acidic cations

Very low concentrations (mostly < 4 mmol kg^{-1} soil) of soil non-acidic cations (i.e., K^+, Na^+, Ca^{2+}, and Mg^{2+}) were detected in the AA and EU plantations; however, Ca^{2+} was consistently the most abundant non-acidic cation species, followed by K^+, Na^+, and Mg^{2+} (Fig. 2). Additionally, there were no significant P effects on the concentrations of non-acidic cations in either plantation ($P > 0.05$), except for Na^+ concentrations in the EU plantation, which significantly increased ($P < 0.05$) when treated with 1-yr P addition. Similarly, there were no significant effects of N + P on the concentrations of non-acidic cations in either plantation ($P > 0.05$), except for Ca^{2+} and K^+ in the EU plantation under the 4-yr and 7-yr N + P addition, respectively, when these two ions showed significant reductions (Fig. 2, $P < 0.05$).

The concentrations of Al^{3+} in both the AA and EU plantations were consistently much greater than those of other soil exchangeable cations, accounting for > 80 % of total soil exchangeable cations in both plantations at 0–10 cm depth, whereas Mn^{2+} was the least abundant species, followed by Fe^{3+} (Fig. 3). Compared with the control plots, neither P nor N + P addition had a significant impact on the concentrations of the three acidic cations (Al^{3+}, Fe^{3+}, and Mn^{2+}) in the two plantations ($P >$

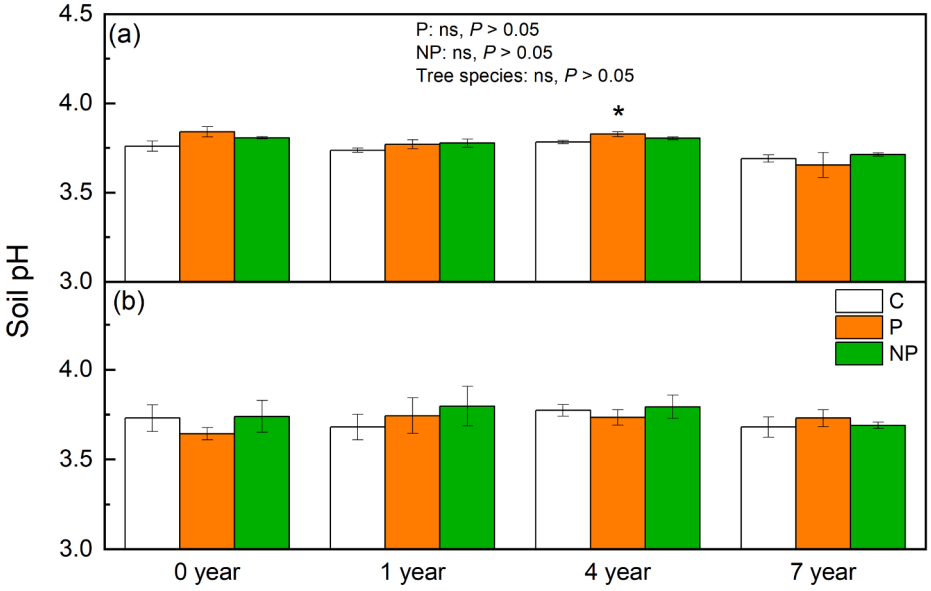

Fig. 1. Changes in soil pH at 10-cm depth of AA (a) and EU (b) plantations across 7-yr P and combined N with P addition. Error bars indicate ±1S.E. (N = 3). 'ns' and '*' indicate non-significant and significant differences among different treatments at $P < 0.05$, respectively.

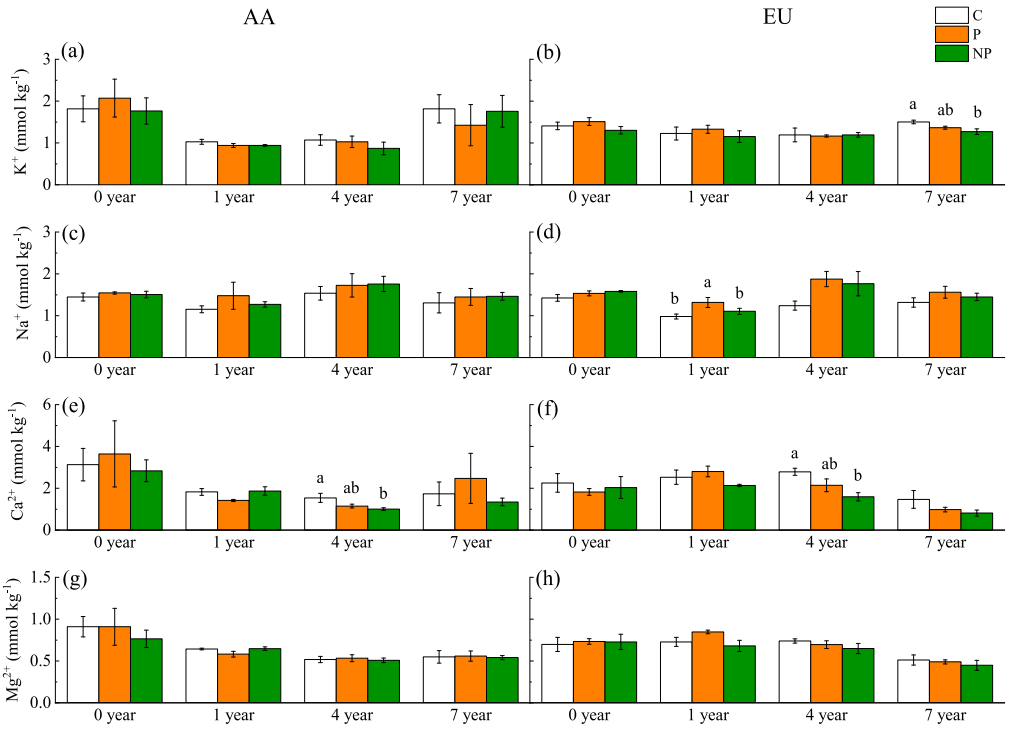

Fig. 2. Changes in soil non-acidic cations concentrations at 0–10 cm depth in AA and EU plantations across 7-yr P addition and combined N with P addition. K^+ (a, b), Na^+ (c, d), Ca^{2+} (e, f), and Mg^{2+} (g, h) in AA and EU plantations, respectively. Error bars indicate ±1S.E. ($N = 3$). Different letters indicate significant differences in non-acidic cations concentrations among different treatments at $P < 0.05$.

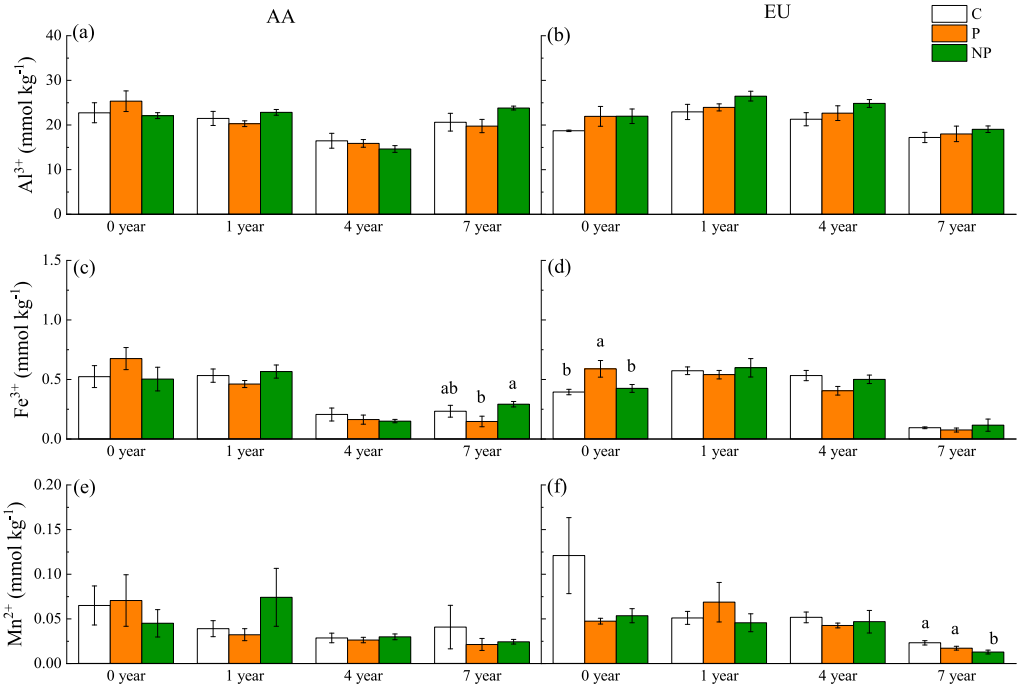

Fig. 3. Changes in soil acidic cations concentrations at 0–10 cm depth in AA and EU plantations across 7-yr P addition and combined N with P addition. Al^{3+} (a, b), Fe^{3+} (c, d), and Mn^{2+} (e, f) in AA and EU plantations, respectively. Error bars indicate ±1S.E. ($N = 3$). Different letters indicate significant differences in non-acidic cations concentrations among different treatments at $P < 0.05$.

0.05), except for Mn^{2+} in the EU plantation under 7-yr N + P addition (Fig. 3).

Moreover, 7-yr P or N + P addition had no effect on CEC in either plantation ($P > 0.05$). Furthermore, there were no significant differences in CEC between the two plantations (Fig. S2, $P > 0.05$).

3.3. Plant nutrients and toxic metal elements

Leaf mineral-nutrient (i.e., K, Na, Ca, and Mg) contents in trees of the AA plantation showed a greater response to 7-yr P and N + P addition than those in the EU plantation. In the AA plantation, 7-yr P treatment

had a significant negative effect on leaf K, Ca, and Mg ($P < 0.05$) contents but not on Na content and had no impact on leaf mineral contents in trees in the EU plantation ($P > 0.05$). Similarly, the 7-yr treatment with N + P negatively affected leaf K and Mg ($P < 0.05$) contents in trees in the AA plantation but had no impact on leaf K, Ca, or Mg contents in trees in the EU plantation but did affect leaf Na content (Fig. 4a-d). Further, lower leaf concentrations of toxic elements (i.e., Al, Fe, and Mn) were observed in AA than in EU trees under the 7-yr P and N + P treatment ($P < 0.05$). However, there were no significant effects of P addition or combined N and P addition on these toxic metal contents (Fig. 4e-g).

3.4. Soil microbial community

In both AA and EU, bacteria were the most abundant species, followed by actinomycetes and fungi, and AMF were the least abundant species. However, 7-yr P and N + P addition did not significantly affect bacteria, actinomycetes, fungi, AMF, or total microbial biomass (indicated by bacteria PLFAs, actinomycetes PLFAs, fungi PLFAs, AMF PLFAs, and total PLFAs, respectively) in AA or EU (Fig. 5a-e, $P > 0.05$). In addition, there were no significant differences in GP and GN biomass and the ratios of fungi to bacteria (F:B) in AA and EU under 7-yr P and N + P addition (Fig. 5f-g, $P > 0.05$). Moreover, there were no differences in soil microbial biomass between AA and EU, indicated by no differences in their PLFAs between AA and EU ($P > 0.05$).

The relative abundances of bacteria PLFAs, actinomycetes PLFAs, and fungi PLFAs, in AA and EU did not respond to 7-yr P or N + P addition (Fig. S3, $P > 0.05$). In addition, there were no significant differences in the relative abundances of bacteria PLFAs, actinomycetes PLFAs, and fungi PLFAs between AA and EU ($P > 0.05$).

3.5. Fine root biomass and litterfall production

Fine root biomass was higher in trees of the AA plantation than in those of the EU plantation ($P < 0.01$), regardless of treatment with P or N + P. Furthermore, there were no significant changes in fine root biomass in trees of either of the plantations in response to the 7-yr P or N + P addition treatments (Fig. 6a, $P > 0.10$).

The 7-yr P or N + P treatments did not significantly affect monthly litterfall production in either plantation (Fig. S4, $P > 0.05$). There were no differences in total, annual litterfall production between the plantations regardless of treatment (Fig. 6b, $P > 0.05$).

3.6. Soil available N and P concentration

Soil available N did not significantly change in response to P or N + P addition over seven years in the soils of the AA plantation ($P > 0.05$). Conversely, a significant reduction in soil available N was observed after the 7-yr P addition treatment in the soils of the EU plantation (Fig. 7a, $P < 0.05$). However, soil available P consistently and significantly increased in response to P or N + P addition over seven years in both plantations (Fig. 7b, $P < 0.05$). In turn, regardless of treatment, total soil-P concentration remained within the range of 0.24–0.25 g kg^{-1} and did not differ between the AA and EU plantations; however, total P was significantly enhanced by 7-yr P and N + P addition treatments in both plantations (Fig. S5, $P < 0.05$).

3.7. Plant N and P contents

Organ-level N and P contents were significantly higher in trees of the AA plantation than in those of the EU plantation, regardless of treatment ($P < 0.01$), except for leaf P content ($P > 0.05$). However, plant N and P contents in the two tree species showed different responses to 7-yr P and N + P addition treatments (Fig. 8).

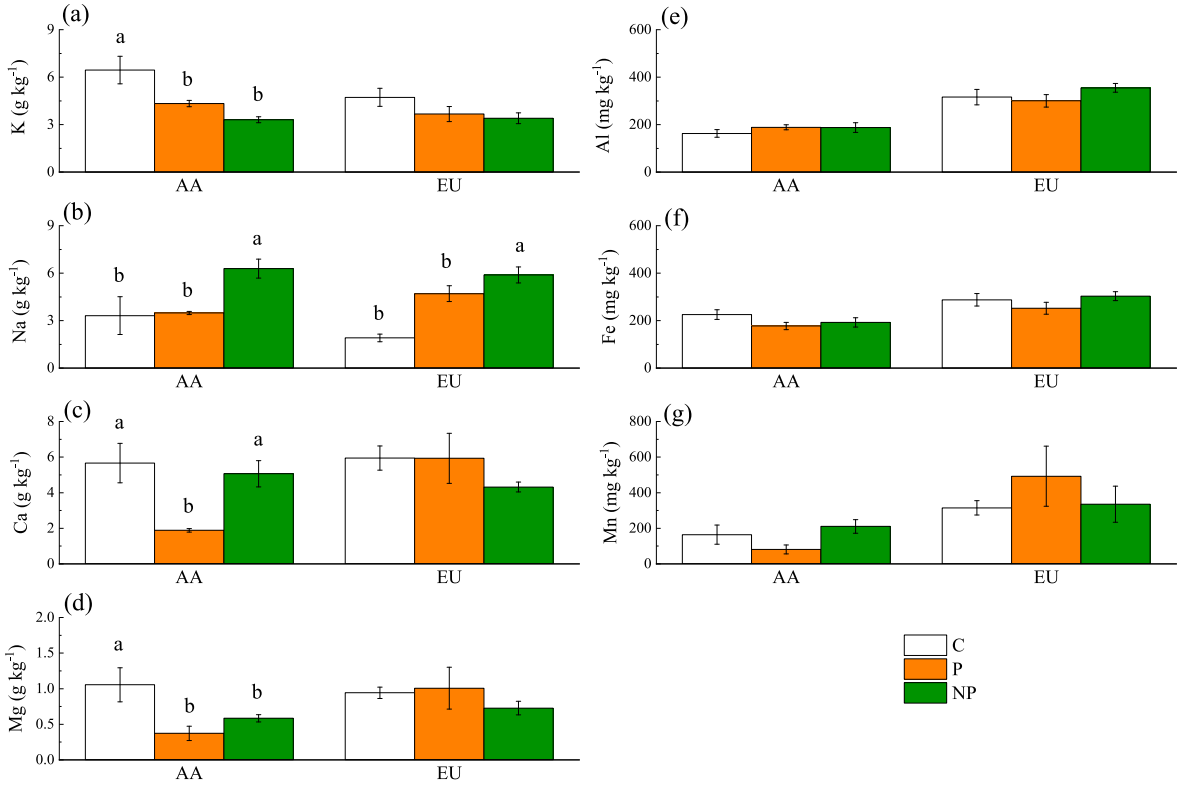

Fig. 4. Contents of leaf nutrient and toxic mental elements in AA (*Acacia auriculiformis*) and EU (*Eucalyptus urophylla*) responding to 7-yr P addition and combined N with P addition. Nutrient metal element: K (a), Na (b), Ca (c), and Mg (d); toxic metal element: Al (e), Fe (f), and Mn (g). *Error bars* indicate ±1S.E. (EU: N = 7–9, AA: N = 3–5). Different letters indicate significant differences among different treatments at $P < 0.05$.

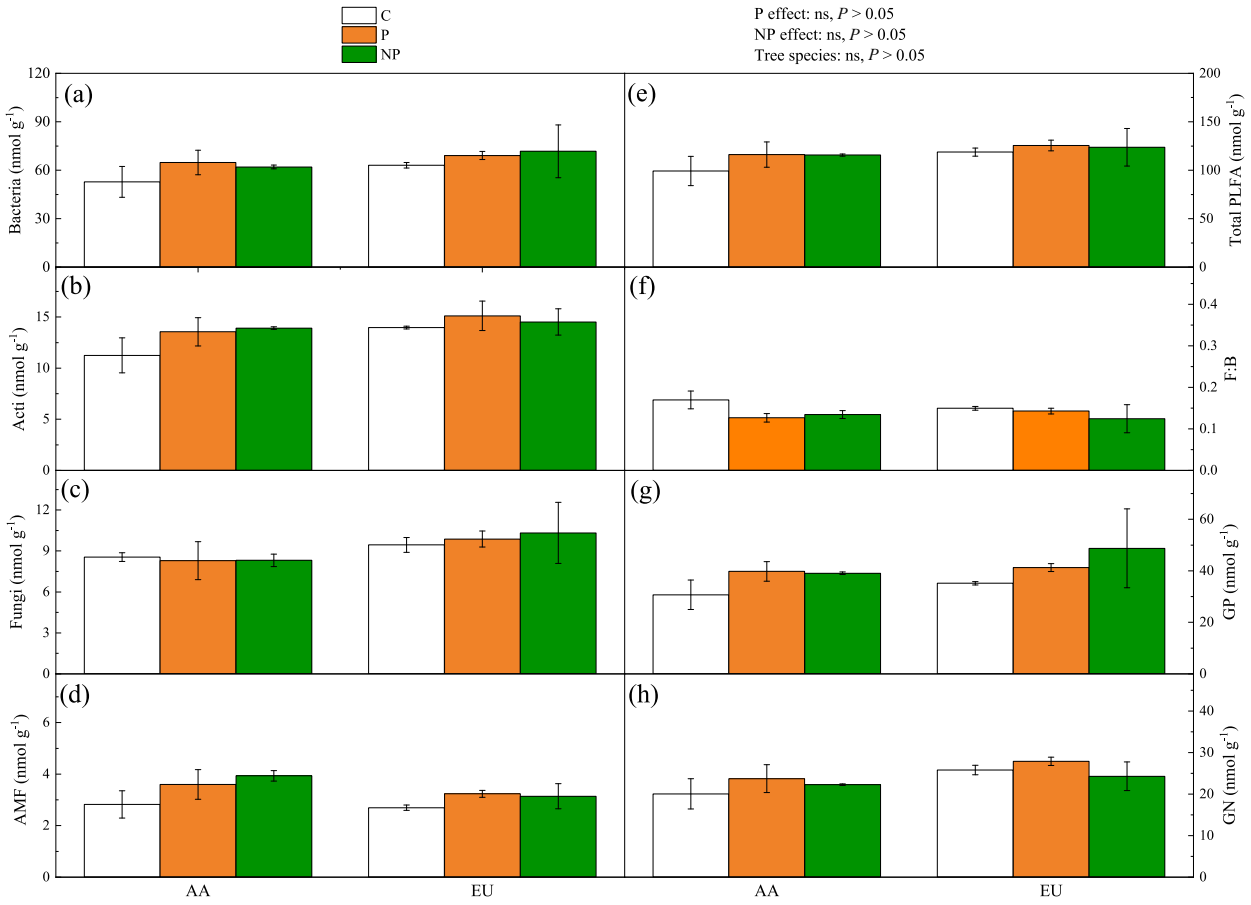

Fig. 5. Responses of soil microbial PLFAs in both AA and EU to long-term P addition and combined N with P addition. (a) Bacteria: bacterial PLFAs; (b) Acti: actinomycetes PLFAs; (c) Fungi: fungal PLFAs; (d) AMF: arbuscular mycorrhizal PLFAs; (e) Total: the total PLFAs; (f) F:B: ratios of fungal to bacteria PLFAs; (g) GN: the gram-negative bacterial PLFAs; and (h) GP: the gram-positive bacterial PLFAs. *Error bars* indicate ±1S.E. ($N = 3$). 'ns' indicates no significant differences at p < 0.05.

Further, the 7-yr P nor N + P treatments did not affect branch or root N contents ($P > 0.05$) but negatively affect leaf N content ($P < 0.05$) in the trees of the AA plantation. Similarly, neither 7-yr P nor N + P treatments affected the leaf or branch N content ($P > 0.05$) in trees of the EU plantation but N + P addition treatment significantly increased root N content (Fig. 8a, $P < 0.05$). Lastly, compared with N content, the organ-level P content showed stronger responses to both 7-yr P and N + P addition treatments in the two tree species, indicating significantly positive effects, especially for branches and roots (Fig. 8b, $P < 0.01$).

4. Discussion

4.1. Effects of P addition on soil acidity

Our study indicated that P addition to the soil for seven consecutive years did not affect soil acidity in either of the two plantations, as supported by the following results. First, soil pH values in the AA and EU plantations did not change after P treatment (Fig. 1). Second, there were no responses of soil non-acidic or acidic cations (Figs. 2, 3) or CEC (Fig. S2) to P addition in either of the plantations (Fig. 3c). Our results are supported by research in a tropical natural forest with long-term P addition (five or more years) showing no changes in soil pH or cation concentrations (Mao et al., 2017), but enhanced soil pH and non-acidic cations with short-term P addition (Mao et al., 2017; Zhu et al., 2015).

Phosphorus addition directly mitigates soil acidity via providing a large amount of inorganic P to react with soil acidic cations (Fujii, 2014; Huang et al., 2005; Sloan et al., 1995). In our study, soil acidic cations had no changes in response to P addition (Fig. 3, $P > 0.05$), suggesting added P rarely reacted with these acidic cations so that their concentrations maintained stable. Hence, there was no direct contribution to the alleviation of soil acidity from P addition in the two plantations.

Phosphorus addition favors plants absorbing elements to reduce toxicity damage caused by soil acidic cations (Huang et al., 2005; Štrbac et al., 2016), which is an important mechanism to mitigate soil acidity (Cui, 2016). However, our study showed that the concentrations of toxic metals (Al, Fe, and Mn) in leaf did not respond to long-term P treatments (Fig. 4e-g, $P > 0.05$), further indicating that P addition did not facilitate plant absorbing toxic elements in our sites. Leaf K, Ca, and Mg concentrations decreased in the AA plantation and remained unaltered in the EU plantation, which further indicated that plant does not help remove non-acidic cation in soil and the plant capacity of regulating soil acidity could be limiting (Duan et al., 2004).

Further, we explored whether P addition regulates soil microbial community, the growth of fine roots and the decomposition of litterfall, all of which may affect the release of non-acidic cations into soil and the absorption of acidic cations from the soil. Our results showed that, under P addition, the soil bacteria biomass, actinomycetes biomass, fungi biomass, AMF biomass, total microbial biomass, and F:B did not change (Fig. 5, $P > 0.05$) and the relative abundances of bacteria PLFAs, actinomycetes PLFAs, and fungi PLFAs remained stable in either of the plantations (Fig. S3, $P > 0.05$), suggesting that there were no significant P effects on soil microbial community and composition. Similar negligible P effects were observed in a subtropical Chinese fir (*Cunninghamia lanceolata*) plantation treated by 9-yr P addition (Zhang et al., 2021), a tropical pine plantation treated by 4-yr P addition (Liu et al., 2012), and a temperate coniferous plantation (*Pinus sylvestris* var. *mongolica*)

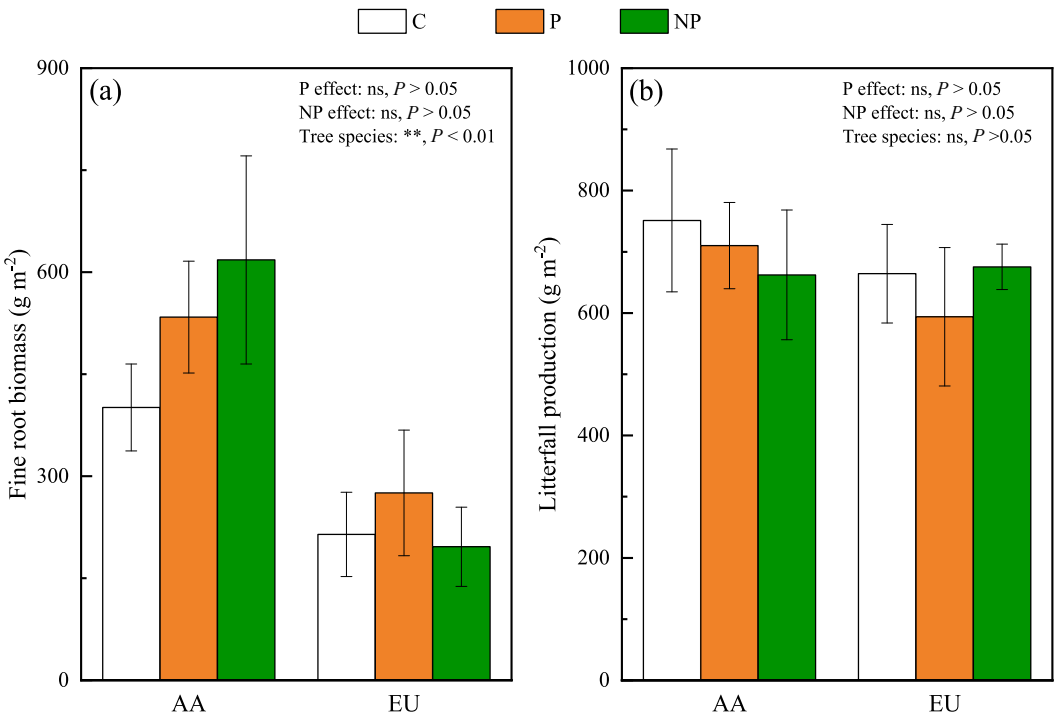

Fig. 6. Fine root biomass (a) and litterfall production (b) in AA and EU plantations under 7-yr P addition and combined N with P addition. Error bars show standard errors (N = 3). Fine root samples were collected in May 2021. Litter samples were collected from January to December 2017. 'ns' indicates no significant differences among different treatments at $P < 0.05$, and '**' indicates the significant differences in fine root biomass between AA and EU plantations at $P < 0.01$.

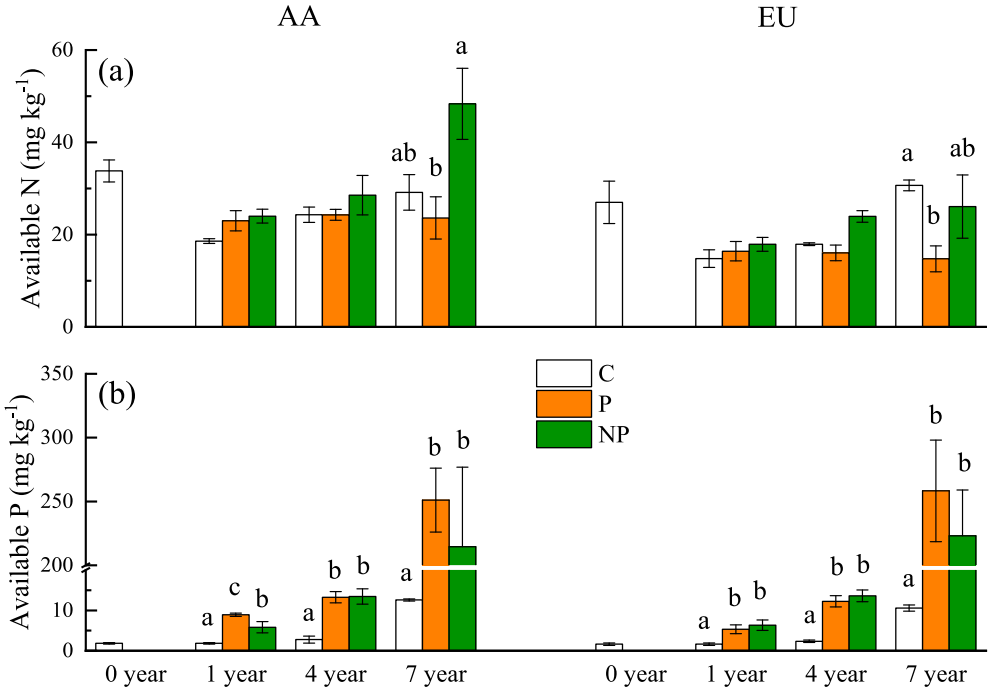

Fig. 7. Concentrations of soil available N at 0–10 cm (a) and P at 0–5 cm (b) in AA and EU plantations responding to 7-yr P addition and combined N with P addition. *Error bars* indicate ±1S.E. ($N = 3$). Different letters indicate significant differences at $P < 0.05$.

treated by 3-yr P addition (Zeng and Wang, 2015). The soil microbial community produces extracellular enzymes to the soil solution to either mineralize or immobilize essential nutrients and increase or decrease, respectively, their availability (Qi et al., 2016). And soil microbial biomass was associated with soil extracellular enzyme activities (Ai et al., 2012). No changes in the microbial biomass for total and single microbial species to P addition in our study suggested that the microbial impacts on soil acidification may be limiting. In addition, bacteria as the abundance species in soil microbial communities, is very sensitive to soil pH because of its relative narrow pH preference (Rousk et al., 2010). No responses in the biomass of bacteria, GP and GN and their relative abundance to P addition in turn suggested that soil pH is stable in the

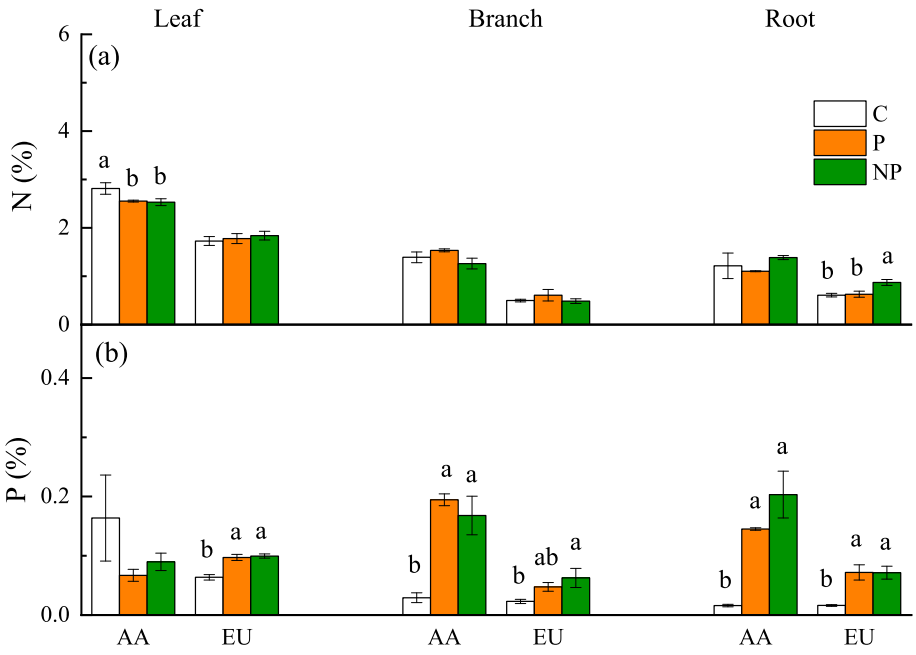

Fig. 8. Contents of N and P at the organ level in AA (*Acacia auriculiformis*) and EU (*Eucalyptus urophylla*) responding to 7-yr P addition and combined N with P addition. *Error bars* indicate ±1S.E. (EU: $N = 7–9$, AA: $N = 3–5$). Different letters indicate significant differences among different treatments at $P < 0.05$.

two plantations.

Fine root and litterfall are the primary source of soil organic matters, which are decomposed by soil microbes (Hopkins and Dungait, 2010). Extracellular enzymes, released by microbes, participate in the decomposition process of organic matters and further affect the release of soil cations (Burns et al., 2013; Lin et al., 2019; Ostertag, 2001; Weand et al., 2010). Hence, the changes in fine root biomass and litterfall production could indirectly impact the concentrations of soil cations and pH values. However, we found no changes in fine root biomass and litterfall production after 7-yr P addition in either plantation (Fig. 6, $P > 0.05$). Given that soil acidic cations may be partially derived from fine roots and litterfall, our results suggest that these sources from plant tissues changed little under P addition. Another study in our studied sites indicated that P addition did not affect the activities of soil extracellular enzymes, e.g., β-1,4-glucosidase, β-1,4-xylosidase, β-D-cellobiohydrolase, β-1,4-*N*-acetyl-glucosidase (Wang, 2021). In combination of the stable microbial community composition mentioned above, it is possible that P addition did not change soil microbial community and the activities of extracellular enzymes that may participate in the decomposition of fine root and litterfall as well as the release of acidic and non-acidic cation. The change in fine root biomass is considered an indicator of plant growth under soil acidification (Huang et al., 2021). No changes in fine root biomass were observed in both plantations under long-term P addition, which further indicated that no positive P effect on soil acidity and plant growth.

In addition, the responses of soil pH, CEC, soil microbes, fine root biomass, and litterfall production to 7-yr P addition had no differences between the AA and EU plantations ($P > 0.05$), suggesting that there was a consistent P effect on soil acidity in the plantations despite of tree species. Therein a conclusion was drawn that soil acidity in the tropical plantations maintained stable status under long-term P addition.

4.2. P enrichment in soil and plants

Although 7-yr P addition did not produce significant effects on soil acidity in either plantation, we found that P enrichment occurred in the soil and plants. The data indicated that P addition significantly increased the concentrations of soil available P (Fig. 6b, $P < 0.05$) and total P (Fig. S5) in the AA and EU plantations. Because P is limiting in our plantations, P addition resulted in the predominance of inorganic P (Ye et al., 2018), which facilitated plant uptake and the use of added P. Moreover, trees have a strong capacity for P absorption and translocation (Fife et al., 2008; Saur et al., 2000). The trees in both plantations can effectively absorb added P and relocate it to storage organs, as suggested by the significantly higher organ-P concentrations in both plantations, particularly in the branches and roots (Fig. 8b), which maintain buffering against large increases in leaf P concentrations (Shane et al., 2004; Zavišić et al., 2018). Therefore, long-term P addition induced a luxury P status in AA and EU, rather than favoring the plantation ecosystems.

4.3. N + P effects on soil acidity

Although our plantations have experienced long-term N deposition during the past decades, there were no N + P addition effects on soil acidity in both plantations, which was consistent with the effects of P addition, showing no changes in pH values (Fig. 1) or non-acidic or acidic cation concentrations, except for K^+ and Mn^{2+} (Figs. 2, 3). Moreover, there were no responses in soil microbial community (Fig. 5), fine root biomass or litterfall production (Fig. 6), or leaf toxic element (Al, Fe, and Mn) concentrations, showing no positive effects of 7-yr N + P addition on acidity mitigation in either plantation. Similar findings were reported by some previous studies (Liu et al., 2013; Mo et al., 2021). Second, N + P addition significantly stimulated soil P concentration that was consistent with that of P addition in both plantations, as evidenced by the enhanced concentrations of soil available P and total P in both plantations (Fig. 6b, S4, $P < 0.05$). Similar results were observed in other tropical forests (Mao et al., 2017; Mo et al., 2019) where N + P addition enhanced both soil available P and total P concentrations. Finally, there were no interactive effects of N + P on organ-level P concentrations (Fig. 7b) in either plantation, consistent with a report by Mo et al. (2020) who found that N + P addition did not change P enrichment in plants of tropical forests. Therein P addition still had no effects on the soil acidity in the two plantations even under N addition.

5. Conclusions

Seven-year P addition had no effects on soil acidity in the

experimental AA or EU plantations, as evidenced by the unaltered values for soil pH, non-acidic and acidic cations, and CEC. These observations may be due to the fact that P addition did not change plant uptake of non-acidic cations from soil and did not impact fine root biomass and litterfall production (i.e., the possible sources of soil acidic cations) and microbial community (i.e., decomposers of fine-roots and litterfall), either. However, P addition mainly resulted in P enrichment in the soil and plants, as supported by significantly enhanced P concentrations in soils and plants especially in branches and roots. Under conditions of N deposition, P addition still had no effects on soil acidity in either plantation. Therefore, our study demonstrated that soil treatment with P addition led to soil and tree P-enrichment rather than affecting soil acidity in the tropical plantation ecosystems. Our findings indicated that the P-limited tropical plantations have strong adaptation capacity to environmental changes, and warn about the cautious use of P fertilization in the process of scientific management of such ecosystems. However, there is a limitation. Our study is conducted at each single *Acacia* and *Eucalyptus* plantation, respectively. Thus, the study is lacking replication at the level required to assess whether the observed effects of fertilizer applications are generalizable beyond the specific location studied. We suggest more studies to validate our findings in other forest sites in the future.

Author contributions

Juan Huang: Conceptualization, Funding acquisition, Investigation, Validation, Writing-original draft, review & editing. **Lei Liu:** Data curation, Investigation, Resource. **Juxiu Liu:** Supervision, Funding acquisition, Writing-Review & editing. **Wei Zhang:** Investigation, Resource. **Senhao Wang:** Investigation, Resource. **Qing Ye:** Supervision, Writing-Review & editing. **Jiangming Mo:** Conceptualization, Funding acquisition, Supervision. **Mianhai Zheng:** Conceptualization, Funding acquisition, Supervision, Writing-Review & editing.

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foreco.2023.120822.

References

Ai, C., Liang, G., Sun, J., Wang, X., Zhou, W., 2012. Responses of extracellular enzyme activities and microbial community in both the rhizosphere and bulk soil to long-term fertilization practices in a fluvo-aquic soil. Geoderma 173–174, 330–338.

Bossio, D.A., Scow, K.M., 1998. Impacts of carbon and flooding on soil microbial communities: phospholipid fatty acid profiles and substrate utilization patterns. Microb. Ecol. 35, 265–278.

Burns, R.G., Deforest, J.L., Marxsen, J., Sinsabaugh, R.L., Stromberger, M.E., Wallenstein, M.D., Weintraub, M.N., Zoppini, A., 2013. Soil enzymes in a changing environment: current knowledge and future directions. Soil Biol. Biochem. 58, 216–234.

Chen, S., Wang, G., Xiang, D., 1996. Fertilizer response of Eucalyptus urophylla. For. Res. 06, 573–578 (in Chinese with English abstract).

Cleveland, C.C., Townsend, A.R., Schmidt, S.K., 2002. Phosphorus limitation of microbial processes in moist tropical forests: evidence from short-term laboratory incubations and field studies. Ecosystems 5, 680–691.

Cui, P., 2016. Effects of acid, aluminum, calcium and phosphorus on chemical forms of aluminum in soil, growth and nutrient uptake of Chinese fir seedlings. Master Dissertation, Fuzhou, Fujian Agriculture and Forestry University (in Chinese with English Abstract).

Duan, L., Huang, Y., Hao, J., Xie, S., Hou, M., 2004. Vegetation uptake of nitrogen and base cations in China and its role in soil acidification. Sci. Total Environ. 330, 187–198.

Fife, D.N., Nambiar, E.K.S., Saur, E., 2008. Retranslocation of foliar nutrients in evergreen tree species planted in a mediterranean environment. Tree Physiol. 28, 187–196.

Fujii, K., 2014. Soil acidification and adaptations of plants and microorganisms in Bornean tropical forests. Ecol. Res. 29, 371–381.

Gong, S., Liao, S., 2009. Soil nutrient characteristics in eucalyptus plantation and natural forest. J. Jiangsu Fore. Sci. Technol. 36 (3), 1–4 (in Chinese with English abstract).

Guo, J.H., Liu, X.J., Zhang, Y., Shen, J.L., Han, W.X., Zhang, W.F., Christie, P., Goulding, K.W., Vitousek, P.M., Zhang, F.S., 2010. Significant acidification in major Chinese croplands. Science 327, 1008–1010.

Hopkins, D.W., Dungait, J.A.J., 2010. Soil microbiology and nutrient cycling. In: Dixon, G.R., Tilston, E.L., eds. Soil Microbiology and Sustainable Crop Production. Springer, Netherlands, pp. 59-80.

Huang, J., Kong, G., Xia, H., 2005. Aluminum toxicity and its relationship with organic acids and phosphorus. Chin. J. Appl. Environ. Biol. 11 (4), 498–503 (in Chinese with English abstract).

Huang, J., Mo, J., Zhang, W., Lu, X., 2014. Research on acidification in forest soil driven by atmospheric nitrogen deposition. Acta Ecol. Sin. 34 (6), 302–310.

Huang, J., Zhang, W., Zhu, X., Gilliam, F.S., Chen, H., Lu, X., Mo, J., 2015. Urbanization in China changes the composition and main sources of wet inorganic nitrogen deposition. Environ. Sci. Pollut. R. 22, 6526–6534.

Huang, J., Zhang, W., Li, Y., Wang, S., Mao, J., Mo, J., Zheng, M., 2021. Long-term nitrogen deposition does not exacerbate soil acidification in tropical broadleaf plantations. Environ. Res. Lett. 16, 11402.

Li, J., Li, Z., Wang, F., Zou, B., Chen, Y., Zhao, J., Mo, Q., Li, Y., Li, X., Xia, H., 2015. Effects of nitrogen and phosphorus addition on soil microbial community in a secondary tropical forest of China. Biol. Fert. Soils 51, 207–215.

Liang, C., Zhu, X., 2021. The soil microbial carbon pump as a new concept for terrestrial carbon sequestration. Sci. China Earth Sci. 64 (4), 545–558.

Lin, C., Lin, W., Chen, S., Peng, J., Guo, J., Yang, Y., 2019. Phosphorus addition accelerates fine root decomposition by stimulating extracellular enzyme activity in a subtropical natural evergreen broad-leaved forest. Eur. J. Forest Res. 138, 917–928.

Liu, L., Gundersen, P., Zhang, T., Mo, J., 2012. Effects of phosphorus addition on soil microbial biomass and community composition in three forest types in tropical China. Soil Biol. Biochem. 44, 31–38.

Liu, G.S., Jiang, N.H., Zhang, L.D., Liu, Z.L., 1996. Soil Physical and Chemical Analysis and Description of Soil Profiles. China Standards Press, Beijing (in Chinese).

Liu, L., Zhang, T., Gilliam, F.S., Gundersen, P., Zhang, W., Hao, C., Mo, J., 2013. Interactive effects of nitrogen and phosphorus on soil microbial communities in a tropical forest. PLOS ONE 8 (4), e61188.

Londron, L., Stark, C., O'Callaghan, M., Clinton, P., Huang, Z., 2010. The role of microbial communities in the formation and decomposition of soil organic matter. In: Dixon, G.R., Tilston, E.L. (Eds.) Soil Microbiology and Sustainable Crop Production, Springer Dordrecht, pp. 81-118.

Luo, Y., Sun, D., Lin, J., Guo, W., Lu, L., Wen, Y., 2013. Effect of close-nature management on the natural regeneration and species diversity in a masson pine plantation. Acta Ecol. Sin. 33 (19), 6154–6162 (in Chinese with English Abstract).

Mao, Q., Lu, X., Zhou, K., Chen, H., Zhu, X., Mori, T., Mo, J., 2017. Effects of long-term nitrogen and phosphorus additions on soil acidification in an N-rich tropical forest. Geoderma 285, 57–63.

Mo, Q., Li, Z., Sayer, E.J., Lambers, H., Li, Y., Zou, B., Tang, J., Heskel, M., Ding, Y., Wang, F., 2019. Foliar phosphorus fractions reveal how tropical plants maintain photosynthetic rates despite low soil phosphorus availability. Funct. Ecol. 33, 503–513.

Mo, Q., Wang, W., Chen, Y., Peng, Z., Zhou, Q., 2020. Response of foliar functional traits to experimental N and P addition among overstory and understory species in a tropical secondary forest. Glob. Ecol. Conserv. 23, e01109.

Mo, Q., Wang, W., Lambers, H., Chen, Y., Yu, S., Wu, C., Fan, Y., Zhou, Q., Li, Z., Wang, F., 2021. Response of foliar mineral nutrients to long-term nitrogen and phosphorus addition in a tropical forest. Funct. Ecol. 35, 2329–2341.

Mori, T., Lu, X., Aoyagi, R., Mo, J., 2018. Reconsidering the phosphorus limitation of soil microbial activity in tropical forests. Funct. Ecol. 32, 1145–1154.

National Forestry, N.-F.-G.-A., Administration, G., 2019. Forest resources inventory report of China (2014–2018). China Forestry Publishing House, Beijing (in Chinese).

Niu, Y., Zhang, M., Bai, S.H., Xu, Z., Liu, Y., Chen, F., Guo, X., Luo, H., Wang, S., Xie, J., Yuan, X., 2020. Successive mineral nitrogen or phosphorus fertilization alone significantly altered bacterial community rather than bacterial biomass in plantation soil. Appl. Microbiol. Biot. 104, 7213–7224.

Ostertag, R., 2001. Effects of nitrogen and phosphorus availability on fine-root dynamics in Hawaiian montane forests. Ecology 82, 485–499.

Qi, R., Li, J., Lin, Z., Li, Z., Li, Y., Yang, X., Zhang, J., Zhao, B., 2016. Temperature effects on soil organic carbon, soil labile organic carbon fractions, and soil enzyme activities under long-term fertilization regimes. Appl. Soil Ecol. 102, 36–45.

Rousk, J., Bååth, E., Brookes, P.C., Lauber, C.L., Lozupone, C., Caporaso, J.G., Knight, R., Fierer, N., 2010. Soil bacterial and fungal communities across a pH gradient in arable soil. ISME J. 10, 1340–1351.

Sade, H., Meriga, B., Surapu, V., Gadi, J., Sunita, M.S.L., Suravajhala, P., Kishor, P.B.K., 2016. Toxicity and tolerance of aluminum in plants: tailoring plants to suit to acid soils. Biometals 29, 187–210.

Saur, E., Nambiar, E.K.S., Fife, D.N., 2000. Foliar nutrient retranslocation in *Eucalyptus globulus*. Tree Physiol. 16, 1105–1112.

Shane, M.W., McCully, M.E., Lambers, H., 2004. Tissue and cellular phosphorus storage during development of phosphorus toxicity in *Hakea prostrate* (Proteaceae). J. Exp. Bot. 55, 1033–1044.

Sloan, J.J., Basta, N.T., Westerman, R.L., 1995. Aluminum transformations and solution equilibria induced by banded phosphorus-fertilizer in acid soil. Soil Sci. Soc. Am. J. 59, 357–364.

Smith, W.H., 1989. Effect of acidic precipitation on forest ecosystems in North America. In: Adriano, D.C., Johnson, A.H. (Eds.) Acidic Precipitation. Vol. 2. Biological and Ecological Effects. Springer-Verlag, New York, pp. 165-188.

Štrbac, S., Gavrilović, M., Budakov, L., 2016. Bioaccumulation of metals in the trees of Novi Sad Serbia. J. Toxicol. Environ. Heal. A 79, 804–807.

Tunlid, A., Hoitink, H.A.J., Low, C., White, D.C., 1989. Characterization of bacteria that suppress rhizoctonia damping-off in bark compost media by analysis of fatty-acid biomarkers. Appl. Environ. Microb. 55, 1368–1374.

Turner, B.L., Yavitt, J.B., Harms, K.E., Garcia, M.N., Romero, T.E., Wright, S.J., 2013. Seasonal changes and treatment effects on soil inorganic nutrients following a decade of fertilizer addition in a lowland tropical forest. Soil Sci. Soc. Am. J. 77, 1357–1369.

Vitousek, P.M., Porder, S., Houlton, B.Z., Chadwick, O.A., 2010. Terrestrial phosphorus limitation: mechanisms, implications, and nitrogen-phosphorus interactions. Ecol. Appl. 20, 5–15.

Wang, S., 2021. Effects of nitrogen and phosphorus additions on elemental dynamics and coupling in typical plantations of south subtropical China. Ph. D. Dissertation. Beijing, University of Chinese Academy of Sciences. (in Chinese with English abstract).

Weand, M.P., Arthur, M.A., Lovett, G.M., McCulley, R.L., Weathers, K.C., 2010. Effects of tree species and N additions on forest floor microbial communities and extracellular enzyme activities. Soil Biol. Biochem. 42, 2161–2173.

Webb, M.J., Reddell, P., Hambleton, A., Mazza, G.M., 1997. Nutritional constraints to growth of Australian red cedar (*Toona ciliata*) seedlings in five north Queensland soils. Aust. For. 60, 46–52.

Yadav, D.S., Jaiswal, B., Gautam, M., Agrawal, M., 2020. Soil acidification and its impact on plants. In: Singh, P., Singh, S.K., Prasad, S.M. (Eds.), Plant Responses to Soil Pollution. Springer, Singapore, pp. 1–26.

Ye, D., Li, T., Zheng, Z., Zhang, X., Yu, H., 2018. P uptake characteristics and root morphological responses in the mining ecotype of *Polygonum hydropiper* under high organic P media. Int. J. Phytoremediat. 20, 608–615.

Zarif, N., Khan, A., Wang, Q., 2020. Linking soil acidity to P fractions and exchangeable base cations under increased N and P fertilization of mono and mixed plantations in northeast China. Forests 11, 1274.

Zavišić, A., Yang, N., Marhan, S., Kandeler, E., Polle, A., 2018. Forest soil phosphorus resources and fertilization affect ectomycorrhizal community composition, beech P uptake efficiency, and photosynthesis. Front. Plant Sci. 9, 463.

Zeng, W., Wang, W., 2015. Combination of nitrogen and phosphorus fertilization enhance ecosystem carbon sequestration in a nitrogen-limited temperate plantation of Northern China. Forest Ecol. Manag. 341, 59–66.

Zhang, M., Niu, Y., Wang, W., Bai, S.H., Luo, H., Tang, L., Chen, F., Xu, Z., Guo, X., 2021. Responses of microbial function, biomass and heterotrophic respiration, and organic carbon fir plantation soil to successive nitrogen and phosphorus fertilization. Appl. Microbiol. Biot. 105, 8907–8920.

Zhang, T., Zhu, W., Mo, J., Liu, L., Dong, S., 2011. Increased phosphorus availability mitigates the inhibition of nitrogen deposition on CH_4 uptake in an old-growth tropical forest, southern China. Biogeosciences 8, 2805–2813.

Zhang, W., Zhu, X., Liu, L., Fu, S., Chen, H., Huang, J., Lu, X., Liu, Z., Mo, J., 2012. Large difference of inhibitive effect of nitrogen deposition on soil methane oxidation between plantations with N-fixing tree species and non-N-fixing tree species. J. Geophys. Res. 117, G00N16.

Zhong, C., Reddell, P., 1994. Determining soil nutrient limitations to tree growth: Nutrient omission experiments with *Eucalyptus grandis* on six forest soils. In: Brown, A.G. (Ed.) Australian Tree Species Research in China. Australian Centre Int Agricultural Research, Canberra, pp. 96-100.

Zhu, F., Lu, X., Liu, L., Mo, J., 2015. Phosphate addition enhanced soil inorganic nutrients to a large extent in three tropical forests. Sci. Rep. 5, 7923.