New Phytologist

Research

Differential responses and mechanistic controls of soil phosphorus transformation in *Eucalyptus* plantations with N fertilization and introduced N_2-fixing tree species

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Received: *26 October 2022*
Accepted: *2 December 2022*

New Phytologist (2023) **237:** 2039–2053
doi: 10.1111/nph.18673

Key words: arbuscular mycorrhiza fungi, *Eucalyptus* plantation, N_2-fixing trees, phosphatase, soil phosphorus fractions, sustainable forestry.

Summary

- Introducing N_2-fixing tree species into *Eucalyptus* plantations could replace nitrogen (N) fertilization to maintain high levels of N consumption and productivity. However, N enrichment may exacerbate phosphorus (P) limitation as *Eucalyptus* robusta Smith is extensively planted in P-poor tropical and subtropical soils.
- We conducted a field experiment in a pure plantation of *Eucalyptus urophylla* × *grandis* to investigate the impacts of N fertilization and introduced an N_2-fixing tree of *Dalbergia odorifera* T. Chen on soil P transformation.
- Nitrogen fertilization significantly enhanced soil occluded P pool and reduced the other P pools due to acidification-induced pH-sensitive geochemical processes, lowering *Eucalyptus* leaf P concentration with higher N : P ratio. By contrast, introduced N_2-fixing tree species did not change soil pH, labile inorganic P pool, and *Eucalyptus* leaf N : P ratio, even enhanced organic P pools and reduced occluded P pool probably due to altering microbial community composition particularly stimulating arbuscular mycorrhiza fungal abundance.
- Our results revealed differential responses and mechanistic controls of soil P transformation in *Eucalyptus* plantations with N fertilization and introduced N_2-fixing tree species. The dissolution of occluded P pool along with organic P accumulation observed in the mixed plantations may represent a promising future to better manage soil P availability.

Introduction

Eucalyptus robusta Smith is one of the most extensively planted commercial plantation timber genera in tropics and subtropics throughout the world (Lino *et al.*, 2016). Because of high nutrient consumption during eucalypt growth and substantial nutrients removal at the end of each rotation, nitrogen (N) fertilization in *Eucalyptus* plantations is common to ensure high and sustainable stand production (Epron *et al.*, 2013). However, a massive application of N fertilizers not only increases economic costs, but also leads to low nutrient use efficiency and negative impacts on the environment (Goncalves *et al.*, 1997). In recent years, introducing N_2-fixing tree species into *Eucalyptus* plantations has been widely considered as one of the optimal silvicultural practices to balance nitrogen losses due to timber harvesting and reduce the demand for N fertilizer (May & Attiwill, 2003; Forrester *et al.*, 2006). It is worth noting that, in the highly weathered tropical and subtropical soils, large amounts of phosphorus (P) are often occluded in the insoluble iron (Fe) and aluminum (Al)-bearing minerals and thus not available for immediate biological uptake (Schachtman *et al.*, 1998; Gross *et al.*, 2020). Unlike N fertilizers, P fertilizer is a non-renewable resource and its decline in global supply has been posing a challenge to traditional agroforestry (Hammond *et al.*, 2004). Moreover, different P-acquisition strategies may be relevant for species coexistence and plant performance in terrestrial communities on P-deficient soils (Yu *et al.*, 2020). Therefore, understanding how N_2-fixing tree species impact soil P transformation is essential for sustainable nutrient management in *Eucalyptus* plantations.

Traditional belief and experimental evidence both hold that adding N into the soils generally exacerbates ecosystem P limitation (Li *et al.*, 2016; Deng *et al.*, 2017). For example, nitrogen addition generally stimulates plant growth, inevitably enhancing P demand (Zhao & Zeng, 2019). Nitrogen addition also increases soil N storage, amplifying the nutrient imbalance between nitrogen and P (Marklein & Houlton, 2012; Luo *et al.*, 2019; Liu *et al.*, 2021a). Moreover, nitrogen addition may cause soil acidification, limiting P availability in acidic soils where the high concentrations of reactive Al and Fe phases at low pH favor the soluble P being bound to their surfaces (Carreira *et al.*, 2000; Wan *et al.*, 2021; J. Ma *et al.*, 2021). Intriguingly, a recent study showed that N_2-fixing tree species could trigger soil

acidification and weathering in secondary Neotropical forests, and also change soil microbial community to locally unlock the occluded mineral nutrients including P for uptake of themselves and adjacent non-N_2-fixing tree species (Epihov *et al.*, 2021). Several studies also revealed that introduced N_2-fixing tree species could promote soil organic P (Po) accumulation and transformation in *Eucalyptus* plantations (Cabreira *et al.*, 2020). However, little information is available so far about introduced N_2-fixing tree species impacts on soil P transformation and the underlying microbial mechanisms in *Eucalyptus* plantations.

Here, we conducted a field experiment including a pure plantation of *E. urophylla* × *E. grandis* without nitrogen fertilization (Pure) or with N-fertilization (Fert N), and a mixed plantation of *E. urophylla* × *E. grandis* by introducing an N_2-fixing tree of *Dalbergia odorifera* T. Chen without fertilization (N-fixer). The great N_2 fixation efficiency and interspecific N transfer of *D. odorifera* have been reported in many previous studies (Lu *et al.*, 2013; Yao *et al.*, 2019), which has been widely considered as one of the effective measures for improving *Eucalyptus* plantations in China (Yao *et al.*, 2021; Zhang *et al.*, 2021). The main aim of this study was to explore how N fertilization or introduced N_2-fixing tree species to impact soil P transformation in *Eucalyptus* plantations. Specifically, we hypothesized that (1) N fertilization would reduce soil P availability due to acidification-induced pH-sensitive geochemical processes and (2) introduced N_2-fixing tree species would improve soil P availability through stimulating microbially mediated P transformation. Moreover, to identify the general patterns of P fraction transformation, we further combined this field evidence with a mini meta-analysis of introducing N fixers mixed with the *Eucalyptus* plantations or N addition in tropical and subtropical soils.

Materials and Methods

Study sites and experimental design

The study site was located at the Experimental Center of Tropical Forestry, Chinese Academy of Forestry (22°04′N, 106°56′E), Pingxiang city, Guangxi Zhuang Autonomous Region, China. The mean annual precipitation is approximately 1400 mm and rainfall is concentrated from April to September. The mean annual temperature is 21°C. The soils were formed from granite, classified as red soil in the Chinese soil classification, equivalent to oxisols in the USDA Soil Taxonomy. Before the establishment of the current plantations, it was covered by a planted *Pinus massoniana* forest and clear harvested in June 2014. The soil before planting had an organic matter content of 27.11 g kg^{-1}, the concentration of total N with 1.33 g kg^{-1}, total P with 0.52 g kg^{-1}, nitrate N (NO_3^-–N) with 5.39 mg kg^{-1}, ammonium N (NH_4^+–N) with 12.21 mg kg^{-1}, available P with 5.76 mg kg^{-1}, and a pH of 5.54.

The experiment was established by planting tree-month-old seedlings in March 2015 and laid out as using a completely randomized block design with five replications, and each block contained three treatment plots (Supporting Information Fig. S1a). Three treatments were randomly assigned among the three plots within each block, including a pure plantation of *E. urophylla* × *E. grandis* without N fertilization (Pure) or with N fertilization (urea solution) of 100 kg N ha^{-1} (Fert N), and a mixed plantation of *E. urophylla* × *E. grandis* by introducing an N_2-fixing tree of *Dalbergia odorifera* without fertilization (N-fixer). To acquire enough *Eucalyptus* timber and maintain ecological sustainable management, we planted a ratio of 2 : 1 with *E. urophylla* × *E. grandis*: *D. odorifera* (67E : 33D), because *D. odorifera* can be harvested after planting decades. The descriptions of stand characteristics are shown in Table S1. The urea solution with 40 l water was evenly sprayed below the canopy in each Fert N plot using a backpack sprayer; each pure or N-fixer plot received 40 l water with no N addition, respectively. In the N-fixer plots, the two species were planted alternately at 2 m spacing in the row and 2.5 m between two rows (Fig. S1b), giving a total planting density of 2000 trees ha^{-1}, the same as the density of *E. urophylla* × *E. grandis* as in the monoculture.

Plant sample collection and analysis

Five *Eucalyptus* aboveground biomass in each plot were harvested in September 2020 and April 2021. All the materials were collected and weighed up fresh weight immediately, and then 500 g of every composition sample was taken in laboratory. The harvested material was dried at 65°C until constant dry weight analyses. Plant leaves were ground and passed through a 0.2-mm sieve after oven-dried, then weighed 0.1000 g leaf samples digestion with 5 ml 98% H_2SO_4, with no added water at 180°C, until white fumes appeared. After heating for a further 5 min, 2 ml of 30% H_2O_2 was added dropwise through a capillary funnel and the mixture was heated until transparent, which was recommended by Dayton *et al.* (2017) with an average total P percent recovery > 90%. The N and P concentrations of leaves were determined using a continuous-flow chemical analyzer (AA3; Seal Analytical, Norderstedt, Germany) and Mo-Sb-Vc colorimetry with the liquid supernatant, respectively (Murphy & Riley, 1962).

Soil sample collection

Soil samples in each plot were randomly collected with cores (2.5 cm diam) from five points at 0–20 cm between the planting lines (Fig. S1b). The composite soil samples were immediately put into insulated ice containers and were divided into three portions: one portion of the fresh soil samples was used for exchangeable ammonium N (NH_4^+–N), nitrate N (NO_3^-–N) concentrations, microbial P and acid phosphatase activity; the second portion was air-dried at room temperature, sieved to 2 mm, and stored at 4°C for general soil total N concentration and soil P fractions analyses; and the last portion was used for the analysis of phospholipid fatty acid (PLFA) with freeze-dried soil samples.

Soil properties analysis

Soil samples were prepared in a 1 : 5 soil/water suspension for pH measurement. Soil water content was measured using a sub-

sample dried in oven at 105°C until the sample reached a constant weight. Soil organic carbon (SOC) content was determined by dichromate oxidation and titration with ferrous ammonium sulfate (Bao, 2000). Soil total N was determined using the liquid supernatant with a continuous-flow analytical system (AA3; SEAL Analytical) (Reis *et al.*, 1980; Bao, 2000). Soil NH_4^+–N and NO_3^-–N concentrations were calorimetrically determined in a continuous flow analyzer after 10 g fresh mass of soil had been extracted in 50 ml of 2 M KCl (You *et al.*, 2014).

Analysis of soil P fractions

Soil P distribution was assessed by sequential extraction (Hedley *et al.*, 1982) and modified by Tiessen & Moir (2007) and was used to measure P fractions in the soil. Concisely, 0.5 g of soil was extracted with 30 ml deionized water, 0.5 M $NaHCO_3$ (pH 8.5), 0.1 M NaOH, 1 M HCl, and hot concentrated HCl (for 16 h each to extract $NaHCO_3$–P, NaOH–P, HCl–P, and HCl_{con}–P extractable P fractions, respectively). Final extraction was done using H_2SO_4 and H_2O_2 at 360°C for residual P determination and shown in Fig. S2. The extraction process was repeated for 16 h in a rotary shaker for each extraction, and the soil and water were then separated by centrifugation for 15 min at 8000 ***g***. For the determination of inorganic P and total P, the filtrates of $NaHCO_3$–P, NaOH–P, and HCl_{con}–P were divided into two sets of subsamples. Inorganic P was estimated by molybdate colorimetry with a UV–vis spectrophotometer at 880 nm (Murphy & Riley, 1962). Total P in aliquots was determined by the same procedure following acid-persulfate digestion (80°C, 16 h) (Rowland & Haygarth, 1997). The organic P (Po) was calculated as the difference between total P and Pi in each of these extracts. The sum of P extracted with the resin and $NaHCO_3$–Pi was considered to be labile Pi, $NaHCO_3$–Po was considered to be labile Po; NaOH–Pi and NaOH–Po were considered to be moderately labile Pi and Po, respectively; HCl–Pi was considered to be primary mineral Pi; and the sum of P extracted with the concentrated HCl–Pi/Po and residual P was considered to be occluded P. Soil total P was the sum of the nine P fractions (Tiessen *et al.*, 1983; Turrión *et al.*, 2007). To verify the accuracy of the method of acid-persulfate digestion, the total P percent recovery rate was measured by the same method on three repeated soil samples and the average value was 92.93 ± 7.31%.

Analysis of soil microbial properties

Soil microbial P was measured using the chloroform fumigation extraction method: a 15 g subsample of fresh soil was fumigated for 24 h at 25°C and then extracted with 0.5 M K_2SO_4 for measurement of soil microbial P (soil: extractant ratio (w/v) = 1 : 4) (Brookes *et al.*, 1985; Wu *et al.*, 2000). To evaluate the relative magnitude of soil P stored in microbial biomass, soil microbial P was expressed as a percent of total soil P. As soil microbial P is considered a potentially plant-available P pool in weathered soils (Ayaga *et al.*, 2006), the ratio of soil microbial P to labile P was calculated to evaluate the relative proportions of these two measures of potentially available P.

Phospholipid fatty acid analysis was commonly used for analyzing major microbial groups in soil such as biomass of bacteria, fungal, and arbuscular mycorrhizal fungi (AMF; Bossio & Scow, 1998). Phospholipid fatty acid assays were adopted to analyze the soil microbial community composition using freeze-dried soil samples. Lipids were extracted from the soils as well as from the bacterial pellets with a one-phase mixture (1 : 2 : 0.8 v/v/v) of chloroform, methanol, and citrate buffer (0.15 M, pH 4.0), and fractionated into neutral, glyco-, and phospholipids on columns containing silicic acid, then, the phospholipids were subjected to a mild alkaline methanolysis. After that, the fatty acid methyl esters were separated on a capillary gas chromatograph and identified using gas chromatography/mass spectrometry. The sum of 16:1ω7, cy17:0, cy19:0, i14:0, i15:0, a15:0, i16:0, 14:0, 15:0, 17:0 and i17:0, a17:0 as relative marker for total bacteria abundance (Zelles, 1999; Francisco *et al.*, 2016), the sum of 18:2ω6c and 18:1ω9c were used to indicate fungal abundance (Cusack *et al.*, 2011), and 16:1ω5c was used as a marker for AMF abundance (Lekberg *et al.*, 2013). The microbial PLFA was expressed as nmol per gram of dry soil (nmol g^{-1}).

Analysis of acid phosphatase activity analysis

Soil microbial function was represented by soil extracellular enzyme activities related to P cycling. The acid phosphatase activity was determined by conventional p-nitrophenol (pNPP) assays. Briefly, fresh soil (typically sieved to < 2 mm) with universal buffer (MUB, pH 5.0) and substrate of pNPP as solutions. After incubation at 37°C for 1 h, the absorbance of the soil filtrates was measured with a spectrophotometer at 410 nm (Tabatabai & Bremner, 1969).

Data collection for mini meta-analysis

The peer-reviewed articles published before September 2022 were searched from the Web of Science (http://apps.webofknowledge.com/) and China Knowledge Resource Integrated Database (http://www.cnki.net/). We divided into two groups to search using the following the keywords (1): 'Nitrogen fertilization, Nitrogen addition, Nitrogen input or Nitrogen application' and 'phosphorus fractions or soil phosphorus'; (2) '*Eucalyptus*' and 'phosphorus fractions or soil phosphorus', and 'nitrogen-fixing trees or N_2-fixing trees or legume species', resulting in a list of 21 and 8 published papers, respectively (Dataset S1; Notes S1, S2). To avoid bias in the selection and obtain more relevant data, we extracted papers based on the following criteria: (1) there were few studies of N fertilization on P transformation in *Eucalyptus* plantations; we only collected the data in tropical and subtropical soils because *Eucalyptus* generally are planted in these areas; (2) the treatment and control groups started with the same soil type, and were conducted under equal spatial and temporal scales (Canarini *et al.*, 2017); (3) for several studies, measurements were taken at multiple time points within a year, we only chose the last set of measurements (Ren *et al.*, 2017); (4) if more than one experiment was reported in an

article with different soil or environmental conditions, each experiment was regarded as an independent study (Deng *et al.*, 2021); and (5) at least one response variable related to soil P fractions should be reported in both treatment and control groups.

Statistical analyses

Before the analysis, all data and residuals were tested for normality. One-way analysis of variance (ANOVA) and Duncan's multiple comparisons were used to determine the plant properties (biomass, leaf P concentrations and N : P ratio), soil properties (soil pH, soil water content, NH_4^+–N, NO_3^-–N, SOC, soil total N, soil total phosphorus, and their stoichiometry), soil P fractions, acid phosphatase, and microbial community composition. And then used the two-way ANOVA to test the effects of treatment, season, and their interaction on above the indicators. The correlations between soil properties, microbial properties, and soil P fractions were tested using Pearson's correlation analysis. All analyses were performed using SPSS software (SPSS Inc., Chicago, IL, USA). Structural equation modeling (SEM) approach was used to test the conceptual model for soil P transformation in Fert N vs Pure and N-fixer vs Pure, respectively. The SEM analysis was performed with the IBM SPSS Amos 20.0 using the maximum likelihood estimation method. Several tests were used to assess model fit: the chi-square (χ^2)-test, comparative fit index and root square mean error of approximation. The best fitted and most parsimonious model was obtained after excluding all non-significant parameters.

For the mini meta-analysis, the effects of N fertilization or introducing N_2-fixing tree species into the *Eucalyptus* plantations were estimated based on the natural log-transformed response ratio (RR): For each study, the RR was calculated as follows:

$$RR = \log_e\left(\overline{Xt}/\overline{Xc}\right) \quad (1)$$

where $\overline{Xt}$ and $\overline{Xc}$ are the mean values of a given variable in treatment and control, respectively. A weighted RR was computed from individual RR by giving greater weight to studies whose estimates have greater precision, that is, lower variance (Hedges & Olkin, 1985; Luo *et al.*, 2006). It was considered to be significant if the 95% confidence interval of RR did not overlap with zero.

Results

Changes in soil physical–chemical properties

All soil physical–chemical properties significantly differed among the three treatments except C : P ratio, whereas the interactive effect between treatment and season was found to be significant only on NO_3^-–N concentration (Table 1; $P < 0.05$). When compared to the Pure treatment, soil pH values in both the seasons were significantly decreased under the Fert N treatment but did not change under the N-fixer treatments, whereas soil water content was significantly increased under the N-fixer treatment but did not change under Fert N treatment (Table 1). The SOC contents in both the seasons were also significantly increased under N-fixer treatments but did not change under Fert N treatment (Table 1). There was no significant difference of soil total N content between the N-fixer and Fert N treatments, while their soil total N contents in both the seasons were significantly higher than at the Pure treatment (Table 1). The soil total P contents were significantly lower under the Pure and Fert N treatments in the wet-hot season only, but did not differ between the N-fixer and Pure treatments (Table 1). The NO_3^-–N contents in both the seasons were significantly higher under the N-fixer and Fert N treatments when compared to the pure treatment (Table 1). When compared to the Pure treatment, the NH_4^+–N content in the dry-cold season was also significantly higher at both N-fixer and Fert N treatments, whereas in the wet-hot season it was significantly higher only under the N-fixer treatment (Table 1). The soil C : P ratio under the Pure treatment was significantly lower than those under the N-fixer and Fert N treatments in the wet-hot season, while it was significantly lower than those under the N-fixer treatment in the dry-cold season only (Table 1). When compared to the Pure treatment, the soil C : N ratio under the Fert N treatment was significantly decreased in both the seasons, while under the N-fixer treatment it was significantly lower in the dry-cold season only (Table 1). The soil N : P ratio under the Pure treatment was significantly lower than those under N-fixer and Fert N treatments in both the seasons (Table 1).

Changes in plant dry weight and leaf nutrient concentrations

The aboveground biomass, leaf N, P concentration, and N : P ratio of *Eucalyptus* differed among the three treatments, whereas no significant interactive effect was found between treatment and season (Table 2). In both the seasons, the *Eucalyptus* aboveground biomass and leaf N concentration under N-fixer and Fert N treatments were significantly higher than those of the Pure treatment (Table 2). The *Eucalyptus* leaf P concentrations in both the seasons were significantly decreased under the Fert N treatment in relation to Pure and N-fixer treatments, whereas the leaf N : P ratio was significantly increased (Table 2). However, under N-fixer treatment, the *Eucalyptus* leaf P concentrations and the N : P ratio did not change compared to the Pure treatment (Table 2).

Changes in soil P fractions

All soil P fraction concentrations differed among the three treatments (Fig. 1), while only the labile Po concentration significantly affected by season (Fig. 1d; $P < 0.05$) and no significant interactive effect was found between treatment and season for all measurements ($P > 0.05$). The labile Pi concentration under the Fert N treatment was significantly lower than those under the N-fixer and Pure treatments in the wet-hot season, but did not change among the three treatments in the dry-cold season (Fig. 1a). On the contrary, the concentrations of moderately labile Pi and primary mineral Pi under the Fert N treatment were

Table 1 Soil physical–chemical properties as affected by mixed N_2-fixing trees and N fertilization in *Eucalyptus* plantations.

Source of variation	pH	SWC (%)	SOC (g kg^{-1})	TN (g kg^{-1})	TP (mg kg^{-1})	NO_3^-–N (mg kg^{-1})	NH_4^+–N (mg kg^{-1})	C : N ratio	C : P ratio	N : P ratio
Wet-hot										
Pure	4.21 ± 0.30 A	21.77 ± 1.28 AB	16.38 ± 1.13 B	1.18 ± 0.11 B	462.37 ± 16.78 A	2.64 ± 0.26 B	10.07 ± 1.37 B	14.04 ± 1.95 A	35.55 ± 5.26 B	2.55 ± 0.31 B
Fert N	3.97 ± 0.17 B	19.81 ± 1.53 B	18.37 ± 3.11 AB	1.69 ± 0.31 A	441.71 ± 28.72 B	3.56 ± 0.44 A	11.79 ± 1.52 B	11.11 ± 1.78 B	41.90 ± 6.09 A	3.86 ± 0.91 A
N-fixer	4.14 ± 0.19 A	25.66 ± 1.42 A	19.96 ± 0.91 A	1.61 ± 0.11 A	467.87 ± 13.62 A	3.59 ± 0.30 A	13.34 ± 2.27 A	12.72 ± 0.82 B	42.71 ± 2.86 A	3.45 ± 0.43 A
Dry-cold										
Pure	4.24 ± 0.20 a	18.55 ± 1.27 b	14.83 ± 1.07 b	0.91 ± 0.13 b	482.77 ± 16.56 ab	2.26 ± 0.27 b	8.39 ± 1.23 b	16.23 ± 1.81 a	30.40 ± 4.64 b	1.88 ± 0.21 b
Fert N	3.91 ± 0.21 b	17.38 ± 2.01 b	15.58 ± 2.03 b	1.30 ± 0.19 a	472.63 ± 27.54 b	4.08 ± 0.28 a	10.81 ± 1.64 a	12.02 ± 1.98 b	32.73 ± 2.60 ab	2.79 ± 0.42 a
N-fixer	4.20 ± 0.20 a	22.18 ± 2.51 a	17.07 ± 1.78 a	1.33 ± 0.18 a	495.71 ± 13.52 a	3.39 ± 0.31 a	11.20 ± 1.53 a	13.52 ± 2.23 b	35.62 ± 3.91 a	2.68 ± 0.36 a
Season	ns	21.72***	9.28**	14.86**	5.05*	ns	ns	ns	13.85**	17.04***
Treatment	5.39*	24.73***	4.13*	13.26***	4.70*	51.39***	8.35**	7.82**	ns	11.25***
Season × Treatment	ns	ns	ns	ns	ns	5.73**	ns	ns	ns	ns

Data are means ± SD ($n = 5$). Different capital letters (A, B, and C) and lowercase letters (a, b, and c) indicate a significant difference among different N treatments as defined by ANOVA ($P < 0.05$) in the wet-hot and the dry-cold seasons. *F*-value significant at: ns, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$, as defined by two-way ANOVA. C : N ratio, soil organic carbon: total nitrogen ratio; C : P ratio, soil organic carbon: total phosphorus ratio; Fert N, N fertilization in the *Eucalyptus* plantations; N : P ratio, soil total nitrogen: total phosphorus ratio; N-fixer, mixed with N_2-fixing species and *Eucalyptus* plantations; NH_4^+–N, ammonium nitrogen; NO_3^-–N, nitrate nitrogen; Pure, pure *Eucalyptus* plantations; SOC, soil organic carbon; SWC, soil water content; TN, soil total nitrogen; TP, soil total phosphorus.

lower than those under the N-fixer and Pure treatments in the dry-cold season, but did not change among the three treatments in the wet-hot season (Fig. 1b,c). When compared to the Pure treatment, the labile Po concentration in the both the seasons significantly increased under the N-fixer treatment but significantly decreased under the Fert N treatment (Fig. 1d). The moderately labile Po under the Fert N treatment was significantly lower than those under the N-fixer and Pure treatments in the wet-hot season (Fig. 1e). However, in the dry-cold season, the moderately labile Po under the N-fixer treatment was significant higher than those under the Fert N and Pure treatments (Fig. 1e). The occluded P in the both the seasons significantly decreased under the N-fixer treatment but significantly increased under the Fert N treatment compared to the Pure treatment (Fig. 1f).

Different from the concentrations of P fractions, the proportion of labile Pi in both the seasons did not change among the three treatments (Fig. S3a). In both the seasons, the proportion of moderately labile Pi under the Fert N treatment was significantly lower than those under the N-fixer and Pure treatments (Fig. S3b). The proportion of moderately labile Pi did not change among the three treatments in the wet-hot season, while in the dry-cold season it was significantly lower under the Fert N treatment than the other two treatments (Fig. S3c). When compared to the Pure treatment, the proportion of labile Po in both the seasons significantly increased under the N-fixer treatment, but decreased under the Fert N treatment (Fig. S3d). In the wet-hot season, the proportion of moderately labile Po under the Fert N treatment was significantly lower than those under the N-fixer and Pure treatments (Fig. S3e). The change in the proportion of occluded P among the three treatments also showed opposite trends of that of the labile Po (Fig. S3f).

Changes in soil microbial properties

Soil microbial PFLAs, microbial phosphorus, and acid phosphatase activity were significantly affected by introduction N_2-fixing tree species or N fertilization ($P < 0.05$). Except for soil fungi : bacterial ratio, the other microbial indexes were also significantly affected by season. No significant interactive effect was found between treatment and seasons (Fig. 2). In both the seasons, the bacterial, fungi : bacterial ratio, AMF abundance, and microbial phosphorus under the N fixer treatment were significantly greater than those under the Pure and Fert N treatments ($P < 0.05$, Fig. 2a,c,d,e). However, when compared to the Pure treatment, the fungi under the N-fixer and Fert N treatments were significantly increased in both the seasons. Moreover, the fungi under the N-fixer treatment were significantly higher than those under the Fert N treatment (Fig. 2b). Particularly, the acid phosphatase activity did not differ between the N-fixer and Fert N treatments, but both of them were significantly higher than those under the Pure treatment in both the seasons (Fig. 2f).

Pathway and correlation analysis

The SEM models showed the different regulatory pathways of soil P fractions transformation under Fert N vs Pure and N-fixer

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Table 2 The *Eucalyptus* aboveground biomass, and the nitrogen (N), phosphorus (P) concentration, N : P ratio of leaf in *Eucalyptus* and *Dalbergia odorifera* under different treatments.

Source of variation	Aboveground biomass (kg plant^{-1})	Leaf N concentration (g kg^{-1})		Leaf P concentration (g kg^{-1})		Leaf N : P ratio	
	Eucalyptus	*Eucalyptus*	*D. odorifera*	*Eucalyptus*	*D. odorifera*	*Eucalyptus*	*D. odorifera*
Wet-hot							
Pure	62.74 ± 2.68 B	12.03 ± 1.09 B	/	0.92 ± 0.17 A	/	13.01 ± 0.46 B	/
Fert N	77.02 ± 4.82 A	14.36 ± 2.17 A	/	0.80 ± 0.10 B	/	18.10 ± 2.28 A	/
N-fixer	73.05 ± 4.34 A	15.27 ± 1.90 A	26.02 ± 1.60	1.03 ± 0.10 A	1.54 ± 0.14	14.80 ± 0.48 B	17.10 ± 2.53
Dry-cold							
Pure	65.61 ± 3.31 b	14.05 ± 1.70 b	/	1.05 ± 0.05 a	/	13.35 ± 1.41 b	/
Fert N	80.47 ± 7.93 a	18.17 ± 2.27 a	/	0.89 ± 0.13 b	/	20.10 ± 2.07 a	/
N-fixer	78.99 ± 2.41a	19.79 ± 1.80 a	27.57 ± 2.01	1.23 ± 0.07 a	1.52 ± 0.12	16.05 ± 1.24 b	18.14 ± 2.50
Season	7.78*	15.82**	/	12.70**	/	4.72*	/
Treatment	33.97***	9.51**	/	16.97***	/	39.94***	/
Season × Treatment	ns	ns	/	ns	/	ns	/

Data are means ± SD ($n = 5$). Different capital letters (A, B, and C) and lowercase letters (a, b, and c) indicate a significant difference among different N treatments as defined by ANOVA ($P < 0.05$) in the wet-hot and the dry-cold seasons. *F*-value significant at: ns, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$, as defined by two-way ANOVA. Fert N, N fertilization in the pure *Eucalyptus* plantations; N-fixer, mixed with *D. odorifera* and *Eucalyptus* plantations; Pure, pure *Eucalyptus* plantations.

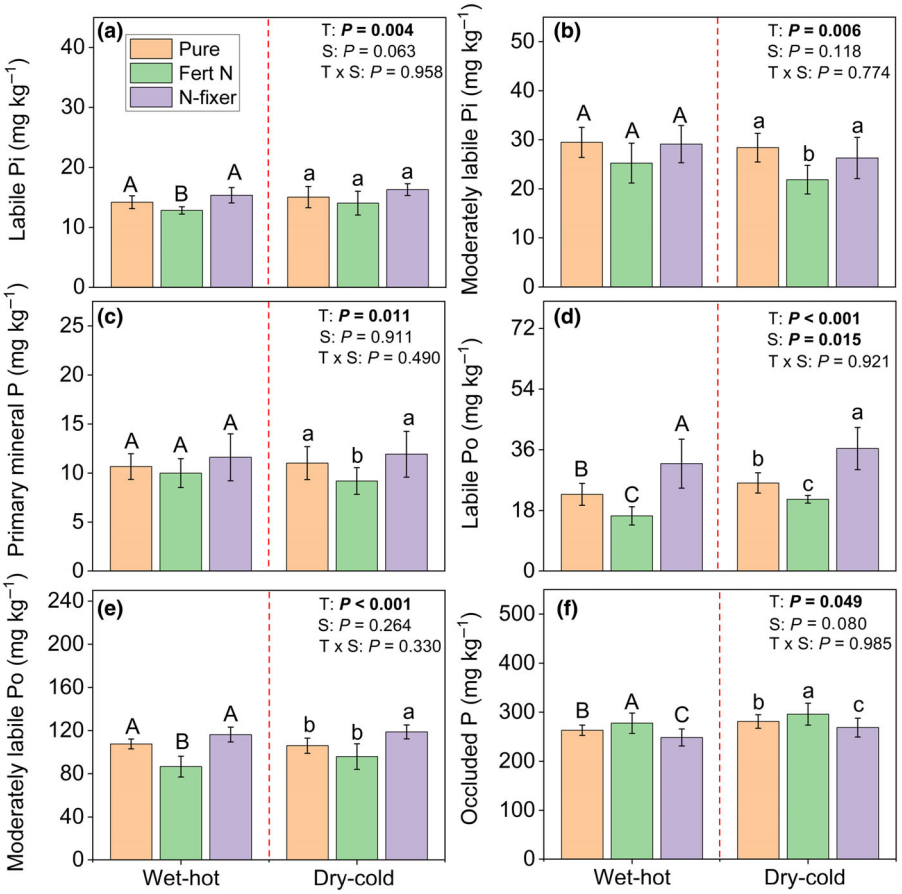

Fig. 1 Concentrations of soil phosphorus fractions as affected by introduction N_2-fixing tree species in *Eucalyptus* plantations or N fertilization treatment. (a) The change in soil labile Pi concentration, (b) the change in soil moderately labile Pi concentration, (c) the change in soil primary mineral Pi concentration, (d) the change in soil labile Po concentration, (e) the change in soil moderately labile Po concentration, and (f) the change in soil occluded phosphorus concentration under Fert N and N-fixer treatments. Data are means ± SD ($n = 5$). Different capital letters (A, B, and C) and lowercase letters (a, b, and c) on the bars indicate a significant difference ($P < 0.05$) under different N treatments in the wet-hot and the dry-cold seasons, respectively. S, season; T, treatments, 'ns' indicates there was no significant difference among treatments as defined by ANOVA ($P > 0.05$). Fert N, N fertilization in the pure *Eucalyptus* plantations; N-fixer, mixed with N_2-fixing species and *Eucalyptus* plantations; P, phosphorus; Pi, inorganic phosphorus; Po, organic phosphorus; Pure, pure *Eucalyptus* plantations.

vs Pure treatments (Fig. 3a,b). For Fert N vs Pure treatments, there were two regulatory pathways. One was that pH and soil water content significantly affected the Pi and primary mineral Pi, further significantly affected the occluded P and the labile Pi; the other was that soil nutrition significantly affected the microbial PFLA and acid phosphatase activity, and then significantly promoted the moderately labile and labile Po transformation to the labile Pi (Fig. 3a). For N-fixer vs Pure treatments, only the soil nutrition (including SOC, total N, and total P) significantly affected microbial PFLA and acid phosphatase activity.

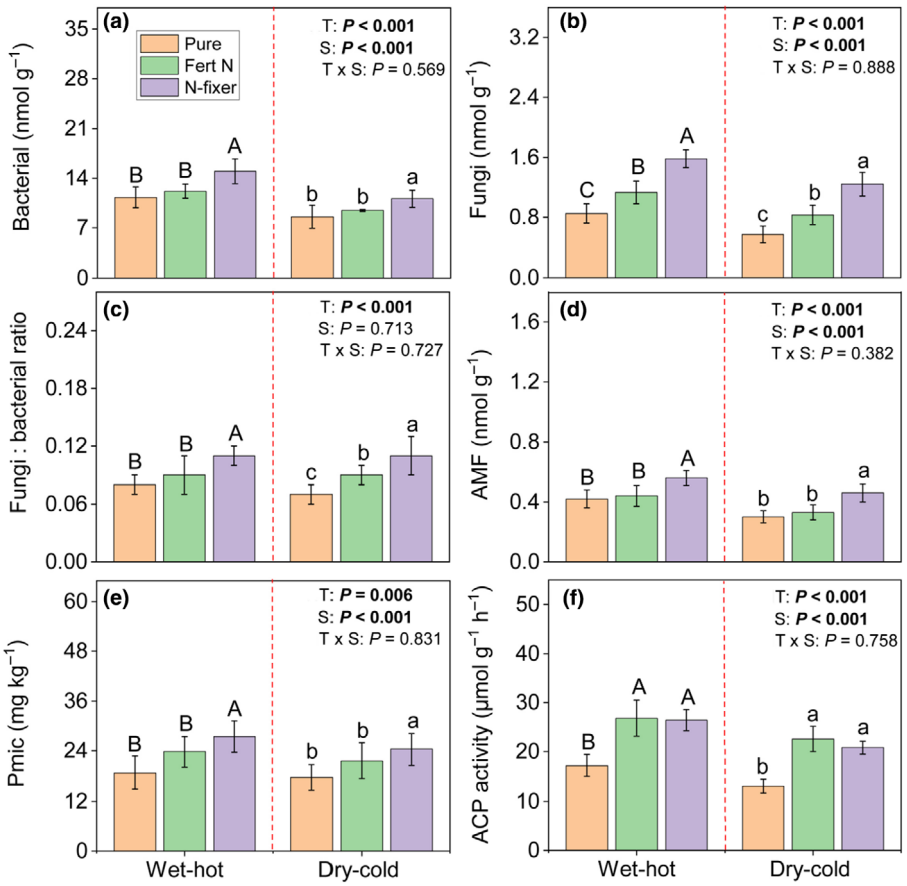

Fig. 2 Soil microbe groups and soil acid phosphatase activity as affected by introduction N_2-fixing tree species in *Eucalyptus* plantations or N fertilization treatment. (a) The change in soil bacterial PFLA, (b) the change in soil fungi PFLA, (c) the change in soil the fungi : bacterial PFLA ratio, (d) the change in soil arbuscular mycorrhizal fungi (AMF) PFLA, (e) the change in soil microbial biomass P (Pmic), and (f) the change in soil acid phosphatase activity under Fert N and N-fixer treatments. Data are means ± SD ($n = 5$). Different capital letters (A, B, and C) and lowercase letters (a, b, and c) on the bars indicate a significant difference ($P < 0.05$) under different N treatments in the wet-hot and the dry-cold seasons, respectively. S, season; T, treatments, 'ns' indicates there was no significant difference among treatments as defined by ANOVA ($P > 0.05$). ACP, Acid phosphatase; AMF, Arbuscular mycorrhizal fungal biomass; Fert N, N fertilization in the pure *Eucalyptus* plantations; N-fixer, mixed with N_2-fixing species and *Eucalyptus* plantations; Pmic, microbial phosphorus biomass; Pure, pure *Eucalyptus* plantations.

and then microbial PFLA also significantly affected occluded P and acid phosphatase activity. Intriguingly, we showed that the occluded P rather than acid phosphatase activity was related to the moderately labile and labile Po (Fig. 3b).

Pearson correlation analyses further showed that change in bacteria was primarily related to soil fungi under the Fert N vs Pure treatments, where under the N-fixer vs Pure treatments it was related to soil NH_4^+ (Fig. S4). Change in fungi was related to soil acid phosphatase under the Fert N vs Pure treatments, where under the N-fixer vs Pure treatments it was related to soil total N, NH_4^+, and N : P ratio (Fig. S4). Change in AMF did not correlate with any soil physical–chemical properties under the Fert N vs Pure treatments, where under the N-fixer vs Pure treatments it was related to soil total N, NO_3^- and N : P ratio (Fig. S4). Change in fungi : bacterial ratio was primarily related to soil pH under the Fert N vs Pure treatments and was related to soil total N under the N-fixer vs Pure treatments, respectively (Fig. S4). Changes in acid phosphatase activity under the N-fixer vs Pure and Fert N vs Pure treatments were primarily related to soil fungi and total N (Fig. S4). For P fractions, change in labile Pi was primarily related to soil pH, fungi, microbial P, and the occluded P under the Fert N vs Pure treatments but not under the N-fixer vs Pure treatments (Figs S5–S7). Change in moderately labile Pi was correlated with soil pH, SOC, NO_3^-, and the occluded P under Fert N vs Pure treatments, whereas under the N-fixer vs Pure treatments, it was correlated with soil pH only (Figs S5, S7). Change in labile Po was also primarily related to soil C : P ratio, AMF, and acid phosphatase under the Fert N vs Pure treatments, and it was related to only AMF under the N-fixer vs Pure treatments (Figs S5–S7). Change in moderately labile Po was correlated with fungi, acid phosphatase, and microbial P under Fert N vs Pure treatments, whereas under N-fixer vs Pure treatments it was correlated with fungi, AMF, labile Po, and the occluded P (Figs S5–S7). Change in occluded P was related to soil pH, moderately labile Pi and labile Pi under Fert N vs Pure treatments, but it was primarily related to soil total N, C : N and N : P ratio, AMF, moderately labile Po, and labile Po under the N-fixer vs Pure treatments (Figs S5–S7).

A mini meta-analysis

The meta-analysis showed that the effect of N addition on the labile Pi, labile Po, moderately labile Po, and primary mineral Pi was significantly decreased of 7.96%, 9.57%, 1.52% and 5.99%, respectively. However, the effect of N addition on the occluded P was positive, with an average increase of 1.00%. Although the moderately labile Pi pool was increased by 1.11% under N addition, there was no significant effect in the global dataset (Fig. 4a). Another mini meta-analysis showed that there was no change in the Pi pools when introducing N_2-fixing tree species into the *Eucalyptus* plantations (Fig. 4b). However, soil labile Po and moderately labile Po were increased by an average of 31.47% and

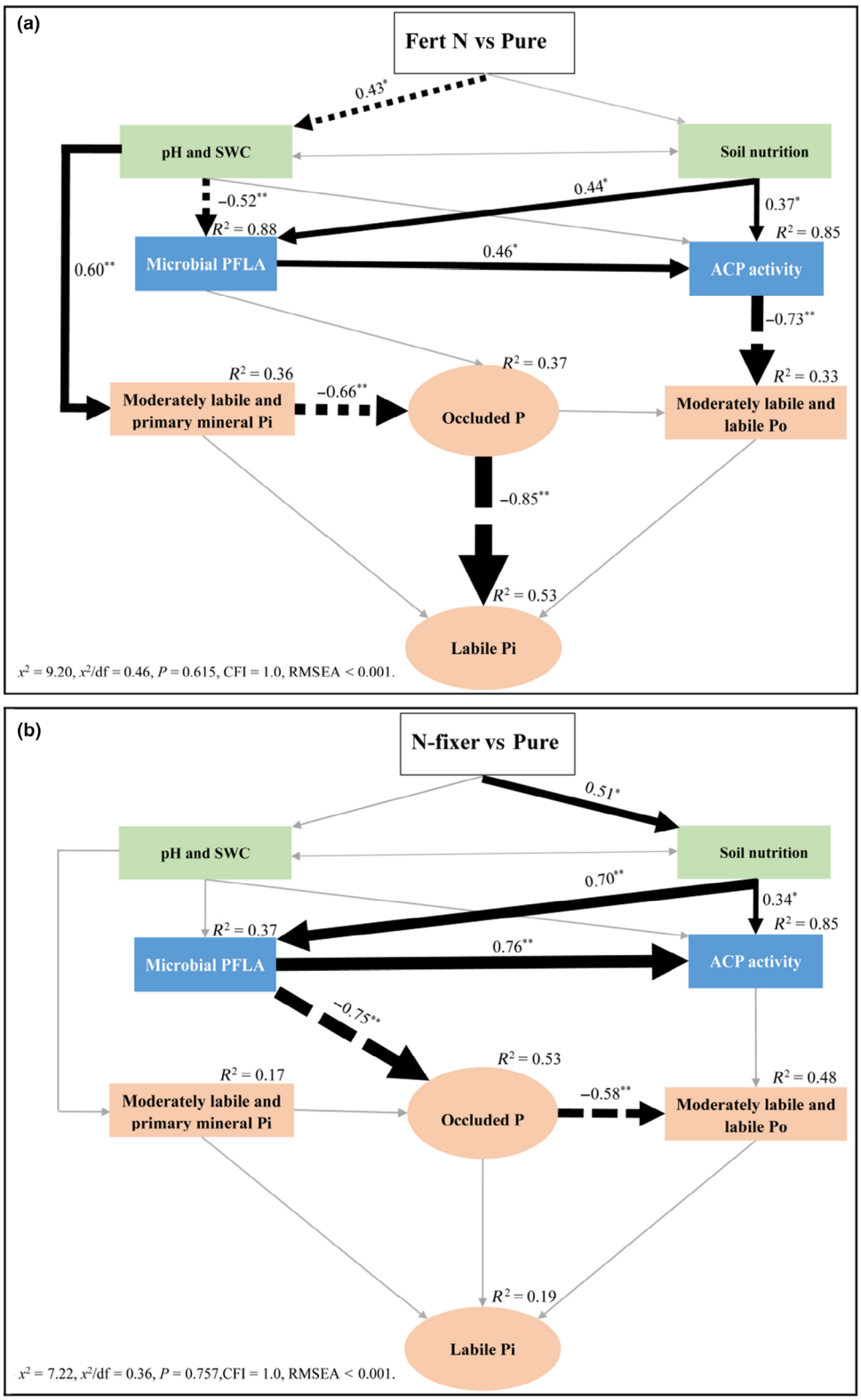

Fig. 3 Path model depicting the regulatory pathway of the controls of arbuscular mycorrhizal fungal biomass and soil acid phosphatase activity by the structural attributes to involve proportion of organic P. (a) N fertilization vs pure *Eucalyptus* plantations, (b) mixed with N_2-fixing species and *Eucalyptus* plantations vs pure *Eucalyptus* plantations. The black solid lines and dotted lines indicate significant positive and negative relationships, respectively; the thickness of the arrows reflect the degree of relationships, numbers at arrows are standardized path coefficients. Paths with non-significant coefficients are presented as grey lines. ACP, acid phosphatase; AMF, arbuscular mycorrhizal fungal biomass; Fert N, N fertilization in the pure *Eucalyptus* plantations; N-fixer, mixed with N_2-fixing species and *Eucalyptus* plantations; P, phosphorus; Pi, inorganic phosphorus; Po, organic phosphorus; Pure, pure *Eucalyptus* plantations.

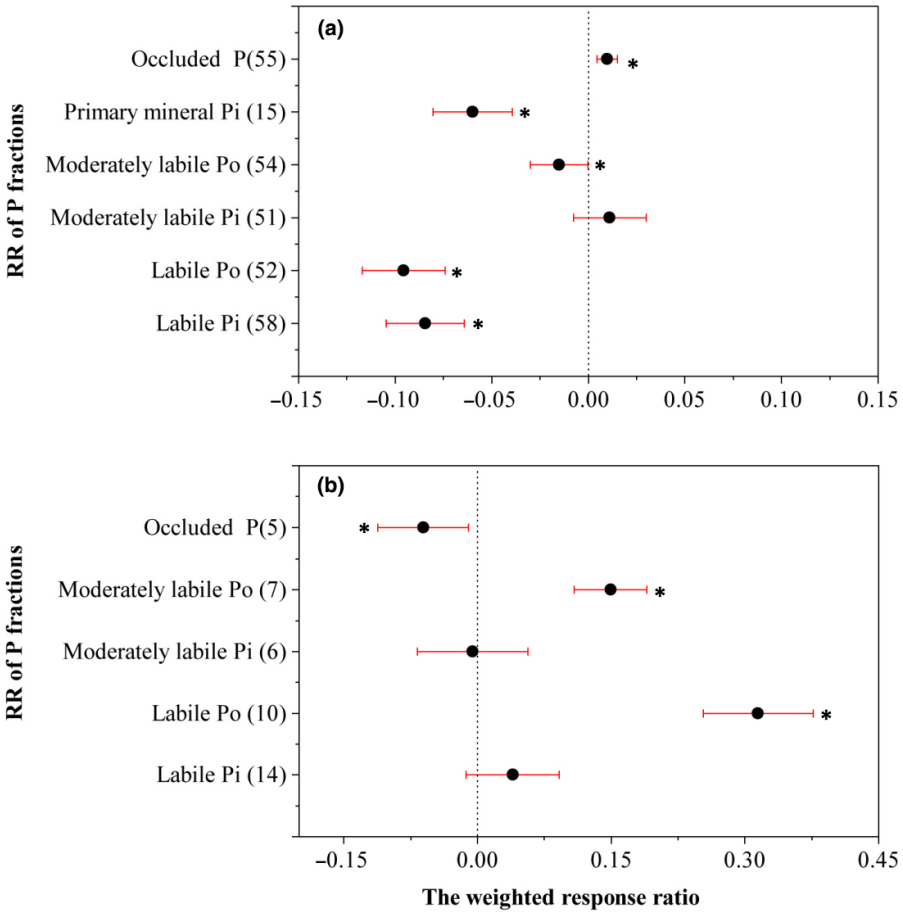

Fig. 4 Effects of N fertilization on phosphorus fractions transformation in tropical and subtropical soils (a), and effects of introducing N_2-fixing tree species on phosphorus fractions transformation in the *Eucalyptus* plantation soil (b). Dots with error bars denote the overall mean values and the 95% confidence interval (CI), and the sample sizes for each variable are shown next to the variable. The vertical dash line indicates $x = 0$; *, significant effects of Fert N or N-fixer treatments on phosphorus fractions (significant difference from zero response ratio). P, phosphorus; Pi, inorganic phosphorus; Po, organic phosphorus.

14.92%, respectively (Fig. 4b). The occluded P significantly decreased by 6.04% (Fig. 4b), demonstrating that introducing N_2-fixing tree species increased the Po pool but decreased the occluded P, consistent with our experiment results.

Discussion

Impacts of nitrogen fertilization on soil phosphorus transformation

We showed that N fertilization significantly enhanced the occluded P pool and reduced the other P pools in *Eucalyptus* plantations (Fig. 1). Despite stimulating acid phosphatase activity, N fertilization significantly reduced *Eucalyptus* leaf P concentration, leading to a higher leaf N : P ratio (Table 2). In agreement with our first hypothesis, these findings suggest that N fertilization could reduce soil P availability and probably exacerbate *Eucalyptus* P limitation. The reduced soil P availability reported here was further verified by a mini meta-analysis with and without N addition (Fig. 4a). Previous meta-analysis studies also showed that N addition could show a exacerbated ecosystem P limitation particularly in tropical forests (Li *et al.*, 2016; Deng *et al.*, 2017). Collectively, the *Eucalyptus* productivity was likely enhanced by N fertilization in short term only, and over long term it could be limited by N-induced lower bioavailable P.

Based on the SEM derived from our data, we showed that changes in different P fractions under N fertilization were primarily driven by N-induced soil acidification (Fig. 3a). The relationships of pH and different P fractions (positive for different Pi pools and negative for occluded P pool) are observed here in line with pH-sensitive geochemical processes (Table 1). Continuous input of exogenous N generally causes soil acidification (Lu *et al.*, 2014; Zhang *et al.*, 2020), due to enhanced nitrification and associated proton (H^+) production accompanied by the leaching of base cations with nitrate (NO_3^-), as well as the biological uptake of ammonium (NH_4^+), which, in turn, releases H^+ (Matson *et al.*, 1999; Guo *et al.*, 2010). In acidic soils where the exchangeable base cation concentrations are typically low, the acidification is mainly buffered by the dissolution of Al- or Fe-bearing minerals (Gu *et al.*, 1994; Jiang *et al.*, 2015). The dissolution of mineral phases would cause the enrichment of highly charged exchangeable cations (Al^{3+} and Fe^{3+}) and the activation of Fe/Al oxides (Shang *et al.*, 1992; Wang *et al.*, 2013; Hu *et al.*, 2022), which enables more dissolved P to be bound on their surfaces (J. Ma *et al.*, 2021). Moreover, the P bound to Fe/Al oxides typically has low solubility, and can be further transformed to the occluded P pool through adsorption and co-precipitation during acidification (Jiang *et al.*, 2015; Helfenstein *et al.*, 2018; Hu *et al.*, 2022). These acidification-induced pH-sensitive geochemical processes have been revealed by many previous studies of N addition in acidic soils

(Jiang *et al.*, 2015; Helfenstein *et al.*, 2018; Hu *et al.*, 2022). Therefore, our results indicate that N-induced soil acidification likely promoted the transformation of relatively labile Pi forms into the occluded P pool.

Impacts of introduced N_2-fixing tree species on soil phosphorus transformation

Different from the impacts of N fertilization, introducing N_2-fixing tree species into *Eucalyptus* plantations did not change the labile Pi pool, even enhanced the Po pools, and reduced the occluded P pool (Fig. 1). These changes in different soil P pools were further verified by a mini meta-analysis of *Eucalyptus* plantations with and without introduced N_2-fixing tree species (Fig. 4b). Introduced N_2-fixing tree species also stimulated *Eucalyptus* growth and enhanced leaf P concentration without change in leaf N : P ratio (Table 2). These findings suggest that introduced N_2-fixing tree species could improve soil P availability and probably enhance *Eucalyptus* P absorption, consistent with our second hypothesis.

Based on the SEM derived from our data, we showed that introduced N_2-fixing tree species significantly altered soil P fractions primarily by changing soil microbial community composition particularly stimulating AMF abundance (Fig. 3b). Consistent with previous studies (Huang *et al.*, 2014; Li *et al.*, 2022), the enhanced abundances of total microbes including bacteria, fungi, and AMF with introduced N_2-fixing tree species were closely relation to soil nutrient status and stoichiometry (Figs 2, 3, S4b). Generally, introduced N_2-fixing tree species favors bacterial growth because N-rich litterfall is easily decomposed by bacteria (Wardle *et al.*, 2003; Viketoft *et al.*, 2009). A diversity of litter inputs with introduced N_2-fixing tree species may also facilitate microbial diversity including the abundance of fungi and AMF (Li *et al.*, 2022). In addition, introduced N_2-fixing tree species could increase soil TN content by directly fixing atmospheric-N, leading to soil nutrient imbalance with higher N : P ratio (Table 1). Compared with bacteria, fungi generally possess a higher adaptability in P-limited environment and a stronger ability to obtain P (DeForest & Scott, 2010; Jones & Oburger, 2011). The negative relationship of soil N : P ratio with fungi or AMF abundances under N-fixer treatments (Fig. S4b) suggests that microbial community composition may shift toward higher abundance of fungi to produce more extracellular enzymes to cleave organic nutrients that are in relative excess to other nutrients in the soil (S. Ma *et al.*, 2021). The positive relationship between acid phosphatase activity and fungi abundance as observed under N-fixer treatments (Fig. S4b) further confirms that fungi rather than bacteria may produce more phosphatase enzymes to accelerate soil Po mineralization. Higher AMF abundance under N-fixer treatments may also reflect a greater P demand of N_2-fixing tree species than non-N_2-fixing tree species because N fixation requires large amounts of P (Sprent & Raven, 1985; Wang *et al.*, 2021). Previous studies also indicated that co-evolution of AM and rhizobial symbioses in N_2-fixing tree species is closely related to the availability of N and P (Afkhami & Stinchcombe, 2016; van der Heijden *et al.*, 2016; Primieri *et al.*, 2021a). Moreover, N_2-fixing tree species may be genetically more predisposed to colonization by another symbiont-AMF. Much of the genes that encode for signal transduction and regulate the symbiosis establishment of N_2 fixation bacteria and AMF are same, which may make the AMF symbiosis inherently more common in N_2-fixing tree species (Antunes *et al.*, 2005; Javaid, 2010; Primieri *et al.*, 2021b).

In combination of results from recent study by Epihov *et al.* (2021) that sampled the soils beneath N_2-fixing tree species, our results have provided direct evidence that N_2-fixing tree species could unlock the occluded P for uptake by themselves and adjacent non-N_2-fixing tree species. However, the mechanistic controls between ours and theirs may differ. In our study, introducing N_2-fixing tree species into *Eucalyptus* plantations did not change soil pH value. Moreover, N-induced soil acidification has shown to enhance rather than to reduce the occluded P pool. Intriguingly, we showed that the reduced occluded P pool was negatively correlated with the AMF abundance when introducing N_2-fixing tree species into *Eucalyptus* plantations (Fig. S5b). This may be not surprising. The occluded P pool is generally inaccessible to microbes, but may be solubilized by AMF through secreting organic acids, phenolic compounds, and protons (Hinsinger *et al.*, 2015; Rosling *et al.*, 2016). Although we did not measured those compounds or protons, a similar relationship between the occluded P and AMF abundance was revealed in a recent study to maintain the high productivity and biodiversity in P-limited subtropical forests of China (Liu *et al.*, 2021b). The roles of AMF in promoting the dissolution of occluded P pool as observed here based on the SEM and correlation analyses warrant further investigation and verification by directly isolating the mycorrhizal influence.

Several mechanisms could help to explain the enhanced the labile and moderately labile Po pools under the N-fixer treatment. The dissolution of occluded P pool likely released more labile Pi, which is taken up by *Eucalyptus* and N_2-fixing tree species and then recycled back in Po form though litterfall. Moreover, the dissolutions of occluded P pool under the N-fixer treatment may be directly transferred into the relatively labile Po pools (Liu *et al.*, 2021b). In addition, N_2-fixing tree species may absorb P from the deeper soils (> 20 cm), promoting Po accumulation in the surface soils (< 20 cm) through faster turnover of litterfall (Li *et al.*, 2022). N_2-fixing tree species may also reduce the leaching loss of Po through changing soil physical and chemical properties and stimulating microbial P assimilation (Nesper *et al.*, 2015). Finally, the stimulated acid phosphatase activity with introduced N_2-fixing tree species likely cleaved more Po forms into labile Pi for *Eucalyptus* uptake, which probably stimulated *Eucalyptus* growth and enhanced leaf P concentration without change in N : P ratio.

As with other mixed plantations of N_2-fixing tree species and *Eucalyptus*, our results may reflect uncertainty in the factors that control *Eucalyptus* P absorption and biomass productivity. For example, introduced N_2-fixing tree species often increases light availability and reduces crowding or competition for belowground nutrient resources, which may directly facilitate *Eucalyptus* to grow faster and acquire more P from the soils. *Eucalyptus*

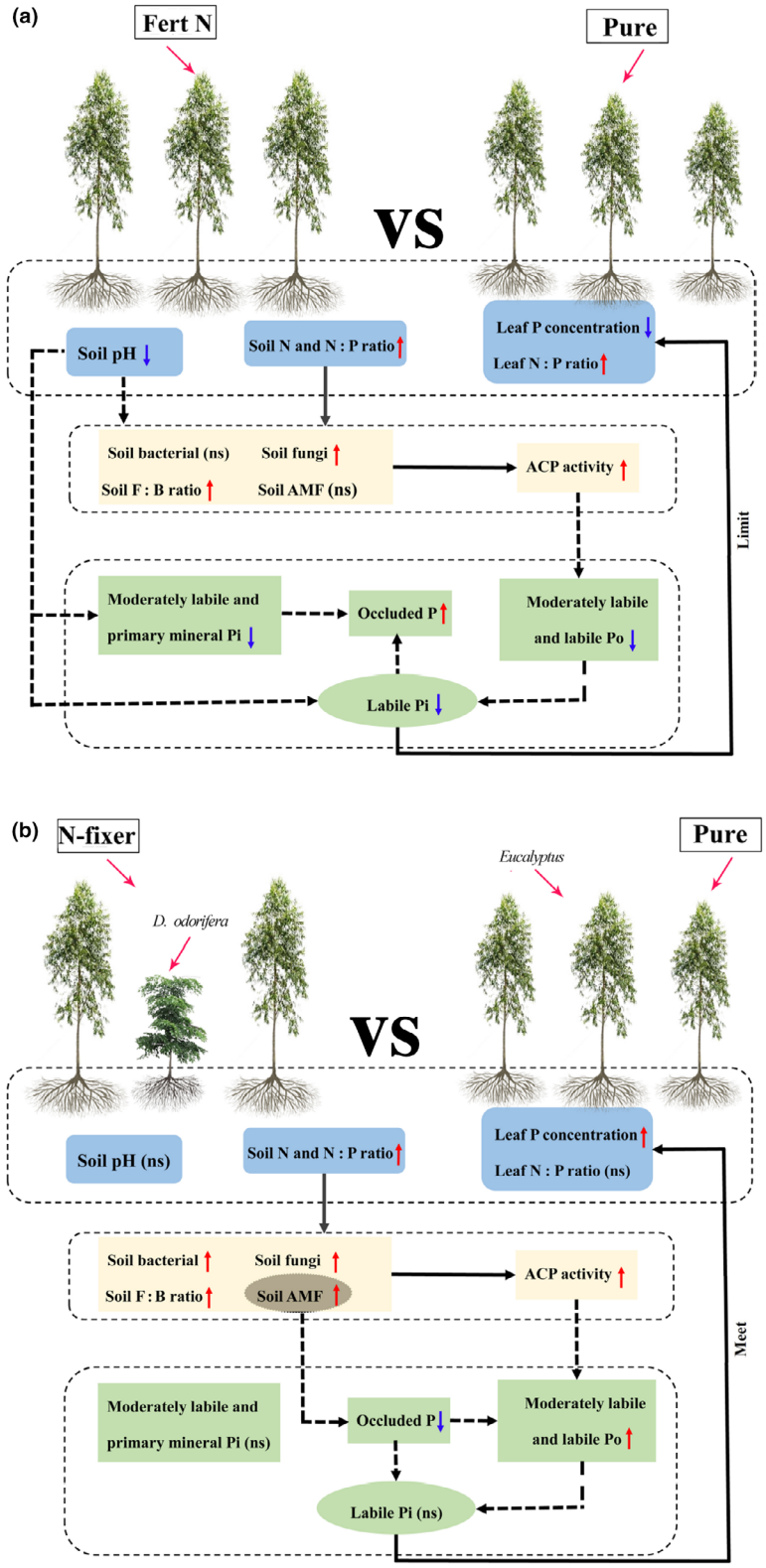

Fig. 5 A conceptual framework of how N fertilization (a) or *Dalbergia odorifera* (b) help *Eucalyptus* for increasing demand for phosphorus through microbial PLFA and ACP activity. The 'ns', red arrows and blue arrows indicate the variables no change, increase and decrease, respectively; the solid arrows and dashed arrows indicate the positive and negative effects, respectively. ACP, soil acid phosphatase; AMF, Arbuscular mycorrhizal fungal biomass; Fert N, N fertilization in the pure *Eucalyptus* plantations; N-fixer, mixed with N_2-fixing species and *Eucalyptus* plantations; P, phosphorus; Pi, inorganic phosphorus; Po, organic phosphorus; Pure, pure *Eucalyptus* plantations.

may also root deeply (up to 10 m) to absorb nutrients from the deeper soils, although their fine roots are typically concentrated in surface soils (Lambais *et al.*, 2017). Further studies on the possible benefits of *Eucalyptus* for P are warranted to conduct more long-term experiments that take better account of interspecific and intraspecific competition and the effects of root's vertical stratification.

Implications for sustainable forestry

Understanding soil P transformation and the underlying mechanisms is critical for sustainable P management in agroforestry. This would be of particularly importance for the *Eucalyptus* plantations, as not only *Eucalyptus* experiences high nutrient assimilation during growth and substantial nutrients removal at the end of each rotation, but also *Eucalyptus* is extensively planted on P-poor subtropical and tropical soils, and in all sites there is potential supply for a high demand of P in *Eucalyptus* plantations (Laclau *et al.*, 2003). Phosphorus fertilization, to a certain extent, can alleviate the P demand of *Eucalyptus*. However, the fertilizer-P use efficiency remains quite low with only 10–25%, because the high concentrations of reactive Al/Fe phases in subtropical and tropical acidic soils favor the soluble P being bound to their surfaces (Carreira *et al.*, 2000; Wan *et al.*, 2021; Ma *et al.*, 2021). Here we showed differential responses and mechanistic controls of soil P transformation in *Eucalyptus* plantations between N fertilization and introduced N_2-fixing tree species (Fig. 5). While N fertilization generally exacerbates *Eucalyptus* P limitation due to acidification-induced pH-sensitive geochemical processes (Fig. 5a), introduced N_2-fixing tree species could improve P availability by microbially exploiting different P sources in soils (Fig. 5b). Given that the large amounts of P (generally > 50% of total soil P) are occluded in insoluble minerals in subtropical and tropical soils, the enhanced Po pools and reduced occluded P pool with introduced N_2-fixing tree species may have broad implications for sustainable P management in *Eucalyptus* plantations.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (nos. 31870461, 32201549), the China Postdoctoral Science Foundation (2022M713193), the National Key R&D Program of China (no. 2021YFC3100402), the 'Hundred Talent Program' of South China Botanical Garden at the Chinese Academy of Sciences (no. Y761031001) and the 'Young Top-notch Talent' in Pearl River talent plan of Guangdong Province and by the Science (no. 2019QN01L763), Guangdong Forestry Science and Technology Innovation Project (no. 2023KJCX001), Guangdong Basic and Applied Basic Research Foundation (no. 2022B1515020014).

Competing interests

None declared.

Author contributions

QD and XY conceived the study; XY, SX and JX collected the field data; QD and XY conducted the analysis; XY and QD wrote the first draft; QD, DH and EH contributed critically to the drafts. All authors edited the manuscript and gave final approval for submission.

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Data availability

The data generated or analyzed in this study are included in this article and its supplementary information files. Other materials that support the findings of this study are available from the corresponding author on reasonable request.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Dataset S1 Data of soil phosphorus fractions with nitrogen fertilization or introducing N fixers into *Eucalyptus* plantations.

Fig. S1 Schematic representation of the trial and the planting and sampling design showing the inner plot comprising 90 trees of mixed-species 67% *Eucalyptus* and 33% *Dalbergia odorifera* (67E : 33D), pure *Eucalyptus* (Pure and Fert N treatments) replicated in five blocks.

Fig. S2 Flow chart of the sequential P extraction.

Fig. S3 The proportions of phosphorus fractions as affected by introduction N_2-fixing tree species in *Eucalyptus* plantations or N fertilization treatment.

Fig. S4 The correlations between the changes in soil physico-chemical properties and soil microbial.

Fig. S5 The correlations between the changes in soil physico-chemical properties and soil P fractions.

Fig. S6 The correlations between the changes in soil microbial and soil P fractions.

Fig. S7 The correlations between the changes in soil Pi, Po, and occluded P fractions.

Notes S1 A list of 21 papers from which the data were extracted for a mini meta-analysis of nitrogen fertilization on soil phosphorus fractions.

Notes S2 A list of eight papers from which the data were extracted for a mini meta-analysis of N fixer on soil phosphorus fractions.

Table S1 Stand characteristics of the three experimental stands measured in April 2021.

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