Introducing N2-fixing tree species into Eucalyptus plantations increases organic phosphorus transformation but decreases its accumulation within aggregates in subtropical China

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Abstract

Background and aims

Soil organic phosphorus (Po) fractions were deemed as potentially significant reservoirs of plant-available phosphorus, profoundly influenced by the physiochemical and biological characteristics of soil. Here we clarify how soil Po fractions and transformation in topsoil aggregates after 15 years of introducing N_2-fixing tree species into *Eucalyptus* plantation.

Methods

We measured different Po fractions and used phospholipid fatty acids (PLFAs) and four extracellular enzymes activities as bioindicators of soil microbiota and function, respectively. The research was carried out within a 15-years of monoculture *Eucalyptus urophylla* plantation (PP) and mixed plantation (MP) of *Eucalyptus urophylla* × *Acacia mangium*.

Results

The mean weight diameter (MWD) was 19.28% greater ($P < 0.05$) in MP than PP. Soil organic matter (SOM), total nitrogen (TN), NO_3^--N, C:P and N:P ratios were notably increased but Po content decreased significantly in bulk soil and most of the aggregates in MP than those in PP. Furthermore, the PLFA contents of total microbes, bacteria, and fungi were more abundant in bulk and aggregate soils in MP than PP. Enzyme activities related to N and P cycles showed significant improvement in bulk and most aggregate soils in MP than PP.

Conclusions

Our findings extend the evidence that promoting soil Po transformation may be related to the increasing of N availability, SOC, pH, fungi, and AMF colonization. Taken together, our results highlighted the soil Po fractions response to long term N_2-fixing tree species application which might be a suitable strategy through efficient management of P in subtropical *Eucalyptus* plantations.

Introduction

Soil phosphorus (P) exists as organic (Po) and inorganic (Pi) varieties, it has long been considered a key limiting nutrient in tropical and subtropical forest soils (Vincent et al., 2012). Yet, the available P in soil, which is the fraction readily accessible to plants, remains a small, usually < 5% of the total P (Cleveland et al., 2011; Turner et al., 2018; Brucker et al., 2020). Hence, the majority of soil P is bound to primary minerals in insoluble forms, making it less available (Hinsinger, 2001). Furthermore, the soil P cycle is extremely complex and is primarily influenced by the synergy of abiotic and biological factors (e.g., vegetation type, soil properties, litter production and decomposition, and soil microorganisms) (Hou et al., 2018; Zheng et al., 2023). Thus, information regarding distinct soil P fractions is essential for adequate assessment of P bioavailability in soil (McDowell et al., 2006). Soil aggregates are considered the functional unit of soil structure which could affect the soil's physical and chemical characteristics (Zhang et al., 2023). Aggregate stability can be used as an indicator of vital soil functions during assessment of soil nutrient quality and protection (Erktan et al., 2016). Additionally, the size of aggregate fractions can impact P availability through their involvement in soil P sorption and desorption. (Li et al., 2016). Prior studies have shown that P availability correlates with soil aggregate size (Wright, 2009; Li et al., 2016). Nevertheless, it remains unclear how the distribution of the microbial community in different aggregates regulates soil P availability and transformation at the aggregate-size scale.

The biogeochemical cycling of nitrogen (N) and P is tightly coupled, and one area of research interest comprises the potential for N enrichment to broadly induce ecosystem P limitation (Crowley et al., 2012). However, N and P substantially differ in terms

of sources and dynamics, such that either element may be or may become scarce, compared to the other (Vitousek et al., 2010). N addition may affect soil P fractions through various mechanisms. For example, it may alter soil microbial community function, thereby affecting the mineralization of Po to Pi (Kritzler and Johnson, 2010). Furthermore, soil phosphatases, including phosphomonoesterase (ACP) and phosphodiesterase (PDE), are crucial for hydrolyzing organic and condensed P, regulating the provision of available P to crops in forest ecosystems (Chen, 2003). Therefore, phosphatases can convert phosphomonoester- and phosphodiester-bound Po into biologically available forms (Nasto et al., 2014). Houlton et al. (2008) hypothesized that N_2-fixing tree species can supply fixed N to extracellular phosphatase production and activity, thereby providing greater capacity for acquisition of soil P (Nasto et al., 2017; Batterman et al., 2018). Other investigations have indicated that a mutually beneficial relationship exists between N_2-fixing tree species and arbuscular mycorrhizal fungi (AMF), which may exploit either the soil Pi pool by exploiting free Pi or the soil Po pool by producing extracellular acid phosphatases (i.e., ACP) (Koide and Kabir, 2000; Nasto et al., 2017). Overall, the dynamics of soil P status and the underlying mechanisms in response to N enrichment require clarification.

In 2015, *Eucalyptus* plantations in China covered 4.5 million hectares, constituting approximately 2% of the national forest area (Engler et al., 2016). Guangxi is the primary region for Eucalyptus, representing over 45% of the planted area, which totals 2.05 million hectares. (Yang et al., 2019). Nevertheless, the extensive use of *Eucalyptus* in short rotation cycles has resulted in soil nutrient depletion and reduced soil productivity. (Asante et al., 2011; González-García et al., 2012). Prior research has shown that blending N_2-fixing species with *Eucalyptus* in plantations can enhance litter input and quality (Huang et al., 2014; 2017). This alteration affects the soil microbial community composition and function, leading to improved soil C stability and N efficiency. Yet, prior studies have not adequately established the connections between N, P, and phosphatases in various biomes, despite robust theoretical expectations of their presence (Chen et al., 2003; Zheng et al., 2023).

To address this research gap, we examined soil organic P fractions and transformations (i.e., microbial biomass P, microbial community, and phosphatase activity), following the 15-year introduction of N_2-fixing tree species into a subtropical *E. urophylla* plantation in China. The primary aims of this study were to. (1) investigate the response of Po fractions in soil aggregates to the introduction of *A. mangium*, and (2) identify the underlying drivers of changes in Po fractions and transformation after the introduction of *A. mangium* into an *E. urophylla* plantation.

Materials and methods

Site description

The study was conducted in Pingxiang City, Guangxi Zhuang Autonomous Region, China (22°10’N, 106°50’E). The region experiences an average annual precipitation of approximately 1400 millimeters and an average annual temperature of ~ 21.1°C (Wang et al., 2010). The predominant soil type in this region is red soil.

The study site comprises a 49-ha 15-years pure *E. urophylla* plantation (PP) and an adjacent 59-ha 15-years mixed plantation of *E. urophylla* and *Acacia mangium* plantation (MP). PP and MP were established in 2004 on a site previously occupied by a 27-year-old *Pinus massoniana* plantation. The experimental design is outlined in the work by Huang et al. (2017). In August 2019, considering the variations in artificial forest layout and terrain, five 20 x 20 m sample plots were randomly chosen from two artificial forests. The basic characteristics of the sample plots are shown in Table S1.

Soil sampling and aggregate isolation

Soil samples were obtained at six systematically selected sampling locations within each plot, positioned 5 m away from the plot's centre. Sampling locations were arranged in a radial pattern around the plot's centre at intervals of 0°, 60°, 120°, 180°, 240°, and 300°. Soil was gathered up to a depth of 0–10 cm, which amounted to approximately 1,500 cm^3, after removing the soil surface debris. Combine all plot samples to create a single composite sample, which should be transported to the laboratory in a sterile plastic container with ice packs. Each soil sample is delicately disaggregated to remove stones and plant roots. The soil was divided into aggregates employing a dry-sieving method. Compared with the wet-sieving method, the dry-

sieving method incurs less disruption to the environmental conditions of the soil microbial community (Schutter and Dick, 2002; Sainju et al., 2003). Soil separation is performed in low-temperature environments using the “optimal moisture” method to standardise soil moisture content and minimise disruption to microbial communities. (Bach and Hofmockel, 2014). The soil samples were laid out on brown paper and left to air dry until their gravimetric water content reached around 100 g kg^{-1} soil (Wei et al., 2014; Bach et al., 2018). Soil was sieved to create four aggregate size categories: large (> 2 mm), medium (1–2 mm), small (0.25-1 mm), and very small (< 0.25 mm). Finally, the bulk soil and all soil samples with different particle sizes were preserved in a -20°C environment, then subjected to analysis of their chemical and biological characteristics.

Soil physical and chemical properties

The gravimetric sample H_2O level was established by measuring the mass loss as soil were dried to a stable weight at 105°C. The sample pH was determined in a 1:2.5 soil-to-H_2O suspension. Soil organic carbon (SOC) was determined using the $K_2Cr_2O_7$-H_2SO_4 calefaction technique. The soil's total nitrogen (TN) content was initially assessed using the semimicro Kjeldahl digestion technique, and then ammonium detection was carried out with a continuous flow analyzer (SEAL AA3, Norderstedt, Germany) (Bremner and Mulvaney, 1982). Similarly, soil NH_4^+-N and NO_3^--N were measured using 0.01 mol L^{-1} $CaCl_2$ and colorimetrically with a continuous flow analyzer (SEAL AA3, Norderstedt, Germany) (Murphy and Riley, 1962). Soil total P (TP) was quantified through ammonium molybdate spectrophotometric method after acid wet digestion (Murphy and Riley, 1962).

Soil Po fractionation was sequentially extracted in accordance with the modified Bowman-Cole method (Bowman and Cole, 1978). Briefly, an air-dried sample was sequentially extracted with 0.5 mol L^{-1} $NaHCO_3$ (pH 8.5) (labile P), 0.1 mol L^{-1} NaOH (recalcitrant P), and 1 mol L^{-1} H_2SO_4 (moderately labile P) after $CHCl_3$ had been added to the sample for pretreatment. Adjust the pH of the NaOH extract to 3 (moderately recalcitrant P); highly recalcitrant P was determined by subtracting moderately recalcitrant P from recalcitrant P.

The soil microbial biomass N (MBN) and microbial biomass P (MBP) were assessed employing the chloroform fumigation extraction approach (Brookes et al., 1982; Vance et al., 1987). Briefly, fresh soil was fumigated with alcohol-free chloroform for 24 h, and MBN and MBP were extracted from treated and untreated soil samples using 0.5 mol L^{-1} K_2SO_4 and 0.5 mol L^{-1} $NaHCO_3$, respectively. MBN and MBP are determined by subtracting the extractable N and P in the fumigated sample from the control sample and then dividing the result by the respective factors, 0.54 for MBN (K_{EN} factor) and 0.40 for MBP (K_{EP} factor). The K_{EN} and K_{EP} represent the extraction efficiency of MBN and MBP, respectively.

Soil microbial communities and extracellular enzyme activities

Soil microbial community structure was ascertained via phospholipid fatty acid (PLFA) analysis following the procedure outlined by Bossio and Scow (1998). Indicator PLFAs were used for microbial community classification (Zelles, 1999; Huang et al., 2014; 2017; Myers et al., 2001). Specific classifications of microbial community structure are shown in Table S2.

We several enzyme activities related to N and P conversion. Specifically, β-1,4-N-acetylglucosaminidase (NAG; EC:3.2.1.30) catalyzes the cleavage of β-1,4 glycosidic bonds present in N-acetylglucosamine-containing polymers, resulting in the release of N. The N-terminal leucine and other hydrophobic amino acids within the peptide were subjected to hydrolysis catalyzed by leucine aminopeptidase (LAP; EC: 3.4.11.1) (Saiya-Cork et al., 2002; Allison et al., 2007). Briefly, we measured NAG and LAP activities using 4-MUB-N-acetyl-β-D-glucosaminide and L-leucine-7-amino-4-methylcoumarin as the reagent, with the buffer adjusted to pH 4.5 (Saiya-Cork et al., 2002). Phosphatases can be categorized into two main groups: phosphodiesterases, which are responsible for the hydrolysis of phosphodiesters, and phosphomonoesterases, which target the hydrolysis of phosphomonoesters (Allison et al., 2007). Acid phosphatase (ACP; EC:3.1.3.2) was analyzed by employing 4-MUB-phosphate, while ensuring the pH of the buffer was set at 4.5. Phosphodiesterase (PDE; EC:3.1.4.1) was evaluated with bis-p-nitrophenyl phosphate (Saiya-Cork et al., 2002; Wei et al., 2014). The *p*-nitrophenol generated because of phosphodiesterase activity was

extracted employing an alkaline Tris solution and quantified through colorimetric analysis at 400 nm. Each sample was analyzed using eight replicates on each substrate, and enzyme activity units were expressed as nmol g^{-1} soil h-1.

Statistical analyses

Mean weight diameter (mm) was used to evaluate the cohesion of soil aggregates as calculated by the following formula (Castro Filho et al., 2002):

$$MWD = \sum_{i=1}^{n} X_i W_i$$

here, X_i represents the average diameter of size fraction i (mm), and W_i signifies the percentage of each size fraction within the entire soil composition.

To comprehend the impact of different planting types on soil physicochemical parameters, Po components, soil microbial biomass and community structure, as well as P transformation, independent sample t-tests were carried out using SPSS software (version 20.0; SPSS Inc., Chicago, IL, USA) to assess the distinctions between PP and MP.

Conduct Redundancy Analysis (RDA) to assess the relationship between site biotic and abiotic factors and Po components. The forward selection process in RDA, with 499 Monte Carlo permutation iterations, was used to identify the statistically meaningful variables related to environmental factors and specific soil phosphatase activities. Statistically significant variables ($P < 0.05$) were utilised in the ultimate analyses. Significance testing of RDA results using CANOCO software (version 4.5; Biometris-Plant Research International, Wageningen, The Netherlands).

Structural equation modeling (SEM) analysis was conducted to identify theoretical pathways that could elucidate the direct and indirect effects of introducing N2-fixing tree species on soil P alteration (ACP activity). SEM models (i.e., path models) were constructed based on the RDA results. Utilizing the maximum likelihood estimation feature in Amos software for model fitting (version 24.0; SPSS Inc., Chicago, IL, USA). We assessed model fitting adequacy through several tests, such as the χ^2 test ($P > 0.05$, CMIN/df < 2), goodness of fit (values > 0.8 and < 1), root square means error of approximation (values < 0.05), and comparative fit index (values > 0.9) (Zhang et al., 2013). To obtain a more streamlined model, we removed irrelevant variables and routes from the ultimate path structure. Before conducting SEM analysis, we checked data for normal distributions to identify heteroscedasticity and examined all bivariate relationships for nonlinearity. (You et al., 2014; Huang et al., 2017).

Results

Soil physicochemical characteristics in bulk soil and aggregate size fractions

The distribution of aggregates in PP and MP indicated that the dominant fractions were large macroaggregates that constituted 38.65–50.96% of whole soil, followed by small macroaggregates at 20.51–27.15% (Table 1). Microaggregates constituted 10.44–12.95% and comprised the smallest proportion of the whole soil. Furthermore, the mean weight diameter was about 19.28% higher ($P < 0.05$) in MP compared to PP. (Table 1).

Table 1
Distribution and stability of soil aggregate factions in PP and MP. Data are mean ± standard error (n = 5).

Plant type	Distribution of soil aggregate fractions (%)				MWD (mm)
	> 2 mm	1–2 mm	0.25-1 mm	< 0.25 mm	
PP	38.65 ± 0.61b	21.25 ± 0.53a	27.15 ± 0.64a	12.95 ± 0.43a	1.66 ± 0.01b
MP	50.96 ± 2.49a	18.09 ± 0.31b	20.51 ± 0.84b	10.44 ± 1.03a	1.98 ± 0.04a

Different lowercase letters in the same column indicate significant difference between PP and MP ($P < 0.05$).

The existence of N_2-fixing tree species significantly affected the concentrations of SOC, TN, and NO_3^--N. The content of SOC in MP was notably elevated in bulk soil (22.33%) ($P < 0.01$) and in the > 2 mm (29.89%) ($P < 0.01$), 1–2 mm (41.98%) ($P < 0.05$), 0.25–1 mm (33.07%) ($P < 0.01$), and < 0.25 mm (33.32%) ($P < 0.05$) size fractions, compared with PP (Table 2). The TN levels in MP significantly surpassed those in PP, with increases of 39.84% in bulk soil and 30.00%, 50.41%, 52.00%, and 67.30% in all aggregate size fractions, respectively ($P < 0.01$) (Table 2). The NO_3^--N content in MP exhibited a substantial increase compared to PP, with significant increments of 122.51%, 145.26%, 171.07%, 191.90%, and 170.48% in bulk soil and all aggregate size fractions, respectively ($P < 0.001$) (Table 2). However, the Po content in MP was markedly lower in bulk soil (17.76%) ($P < 0.05$) and in the > 2 mm (26.86%) ($P < 0.05$), 1–2 mm (33.94%) ($P < 0.05$), and 0.25–1 mm (29.66%) ($P < 0.05$) size fractions, respectively, compared with PP (Table 2). Conversely, soil N:P and C:P ratios were notably greater in MP compared to PP ($P < 0.05$). The C:P ratio was significantly higher by 32.48% ($P < 0.01$), 70.24% ($P < 0.01$), 49.23% ($P < 0.01$), 36.17% ($P < 0.05$), and 40.98% ($P < 0.01$), while the N:P ratio displayed a significant rise of 49.25% ($P < 0.01$), 71.73% ($P < 0.001$), 58.16% ($P < 0.01$), 56.78% ($P < 0.01$), and 76.03% ($P < 0.001$) in bulk soil and all aggregate size fractions, respectively, in MP compared to PP (Table 2). Finally, the pH in MP was remarkably higher ($P < 0.01$) by 11.78% and 10.52% in bulk soil and the large macroaggregates, respectively, compared with PP (Table 2).

Table 2
Soil chemical properties in bulk soil and all aggregate-sized fractions in PP and MP. Data are mean ± standard error (n = 5).

	Plantation type	SOC ($g \cdot kg^{-1}$)	TN ($g \cdot kg^{-1}$)	TP ($g \cdot kg^{-1}$)	C/N_{soil}	C/P_{soil}	N/P_{soil}	Po ($mg \cdot kg^{-1}$)	NH_4^+-N ($mg \cdot kg^{-1}$)	NO_3^--N ($mg \cdot kg^{-1}$)	pH
Bulk soil	PP	15.54 ± 0.36b	1.23 ± 0.06b	0.23 ± 0.01a	12.74 ± 0.70a	67.21 ± 3.65b	5.36 ± 0.47b	50.12 ± 3.67a	28.80 ± 1.43a	5.51 ± 0.13b	4.67 ± 0.06b
	MP	19.01 ± 0.79a	1.72 ± 0.09a	0.22 ± 0.01a	11.16 ± 0.63a	89.04 ± 4.75a	8.00 ± 0.29a	41.22 ± 0.81b	24.82 ± 0.94a	12.26 ± 0.65a	5.22 ± 0.12a
> 2 mm	PP	14.49 ± 0.91b	1.30 ± 0.03b	0.29 ± 0.04a	11.12 ± 0.68a	53.86 ± 7.24b	4.81 ± 0.47b	48.43 ± 3.38a	23.29 ± 0.83a	5.48 ± 0.15b	4.66 ± 0.06b
	MP	18.82 ± 0.76a	1.69 ± 0.07a	0.21 ± 0.01a	11.12 ± 0.30a	91.68 ± 1.32a	8.26 ± 0.14a	35.42 ± 3.03b	21.31 ± 1.11a	13.44 ± 0.69a	5.15 ± 0.13a
1–2 mm	PP	13.77 ± 1.42b	1.23 ± 0.06b	0.22 ± 0.01a	11.21 ± 1.14a	64.59 ± 6.93b	5.76 ± 0.35b	55.75 ± 5.88a	24.77 ± 1.26a	5.53 ± 0.18b	4.69 ± 0.06a
	MP	19.55 ± 1.11a	1.85 ± 0.10a	0.20 ± 0.01a	10.67 ± 0.78a	96.39 ± 6.22a	9.11 ± 0.52a	36.83 ± 2.02b	21.38 ± 1.67a	14.99 ± 0.86a	5.07 ± 0.16a
0.25-1 mm	PP	17.99 ± 1.17b	1.50 ± 0.08b	0.26 ± 0.01a	12.08 ± 0.95a	71.19 ± 6.74b	5.90 ± 0.36b	56.91 ± 6.09a	29.88 ± 3.22a	5.68 ± 0.17b	4.65 ± 0.06a
	MP	23.94 ± 0.78a	2.28 ± 0.14a	0.25 ± 0.01a	10.56 ± 0.36a	96.94 ± 4.24a	9.25 ± 0.60a	40.03 ± 1.57b	28.12 ± 2.02a	16.58 ± 1.0a	5.02 ± 0.17a
< 0.25 mm	PP	22.81 ± 0.50b	1.59 ± 0.11b	0.26 ± 0.01a	14.57 ± 0.84a	89.96 ± 4.32b	6.30 ± 0.58b	59.74 ± 2.87a	44.04 ± 4.14a	6.03 ± 0.13b	4.69 ± 0.13a
	MP	30.41 ± 2.15a	2.66 ± 0.19a	0.24 ± 0.01a	11.50 ± 0.80a	126.83 ± 7.83a	11.09 ± 0.55a	55.22 ± 4.31a	35.61 ± 3.73a	16.31 ± 0.98a	5.04 ± 0.17a

SOC, soil organic carbon; TN, total nitrogen; TP, total phosphorus; C/N_{soil}, C/N ratio of soil; C/P_{soil}, C/P ratio of soil; N/P_{soil}, N/P ratio of soil; Po, soil organic phosphorus, $NH4^+$-N, ammonium nitrogen; NO_3^--N, nitrate nitrogen; pH, soil pH value. Values designated by different lowercase letters in a column indicate significant differences ($P < 0.05$) between PP and MP within each aggregate fraction. PP: pure plantation of *Eucalyptus*; MP: mixed plantation of *Eucalyptus* and *Acacia mangium*

Soil microbial biomass and community composition in bulk soil and aggregate size fractions

The content of soil MBN in MP was significantly higher by 137.89% ($P < 0.001$), 210.65% ($P < 0.01$), 449.08% ($P < 0.001$), 154.16% ($P < 0.05$), and 105.35% ($P < 0.01$) in bulk soil and all aggregate size fractions, respectively than those in PP (Fig. 1a). Similarly, the content of soil microbial biomass P in MP was significantly higher by 94.74% ($P < 0.01$), 58.20% ($P < 0.01$), 80.16% ($P < 0.05$), 111.09% ($P < 0.01$), and 76.34% ($P < 0.001$) in bulk soil and all aggregates, respectively (Fig. 1b).

The total microbial biomass (the cumulative extracted PLFAs) exhibited a significant increase in MP relative to PP ($P < 0.05$). The total amount of PLFAs was notable higher in MP by 65.78% ($P < 0.01$), 54.78% ($P < 0.001$), 48.32% ($P < 0.01$), 21.09% ($P < 0.05$), and 26.49% ($P < 0.05$) in bulk soil and all aggregate size fractions, respectively (Fig. 2a). In comparison to PP, the

bacterial PLFA levels in MP were significantly higher by 59.67% ($P < 0.01$), 65.85% ($P < 0.01$), 45.02% ($P < 0.05$), 20.08% ($P < 0.05$), and 33.13% ($P < 0.01$) in bulk soil and all aggregates, respectively (Fig. 2b). The contents of fungal PLFAs were markedly greater by 104.55% ($P < 0.01$), 100.67% ($P < 0.01$), and 70.40% ($P < 0.05$) in bulk soil, large and medium macroaggregates in MP, compared with PP (Fig. 2c). Furthermore, the contents of AMF PLFAs in MP were significantly higher by 53.44% ($P < 0.05$), 54.00% ($P < 0.01$) and 54.79% ($P < 0.05$) in bulk soil and in the > 2 mm and 1–2 mm size fractions, respectively (Fig. 2d). Actinobacterial PLFA content in MP was significantly higher by 69.22% ($P < 0.01$), 72.73% ($P < 0.001$), 52.83% ($P < 0.01$), 21.25% ($P < 0.05$), and 30.92% ($P < 0.05$) in bulk soil and four aggregates, respectively, in comparison to PP (Fig. 2e). However, the F:B ratio showed no noteworthy disparity between PP and MP ($P > 0.05$) (Fig. 3f). Overall, the overall quantities of PLFAs, bacterial PLFAs, fungal PLFAs, AMF PLFAs, and actinobacterial PLFAs in PP and MP were highest in microaggregates.

Soil Po fractions and soil enzymes in bulk soil and aggregate size fractions

The labile P level in MP significantly exceeded that in PP, with increases of 121.72% in bulk soil ($P < 0.001$), 80.20% in large macroaggregates ($P < 0.05$), 43.31% in small macroaggregates ($P < 0.05$), and 57.08% in microaggregates ($P < 0.05$) (Fig. 3a). In contrast, the content of highly recalcitrant P in MP was markedly lower in bulk soil and all aggregates by 41.99% ($P < 0.05$), 55.27% ($P < 0.05$), 51.45% ($P < 0.01$), 57.19% ($P < 0.05$), and 43.65% ($P < 0.05$), respectively, compared with PP (Fig. 3d). Thus, the presence of N_2-fixing tree species did not significantly affect either moderately labile P or moderately recalcitrant P (Fig. 3b, c). Labile Po in bulk soil, > 2mm, and < 0.25 mm aggregates showed a strong positive correlation with MWD values (Fig. 4a, $P < 0.01$). Meanwhile, there was an obviously positive correlation between moderately recalcitrant Po in < 0.25mm aggregate and MWD value (Fig. 4c, $P < 0.05$). However, the moderately Po and highly recalcitrant Po were not significantly correlated with the MWD values (Fig. 4b, d, $P > 0.05$).

The higher soil extracellular enzyme activities, which reflect N transformation, were observed in MP compared with PP (Fig. 5). Specifically, NAG in MP was significantly higher by 80.83% ($P < 0.001$), 87.27% ($P < 0.001$), 101.81% ($P < 0.001$), 82.54% ($P < 0.001$), and 64.89% ($P < 0.001$) in bulk soil and four aggregates, respectively, compared with PP (Fig. 5a). Moreover, LAP in MP soil exhibited a substantial increase of 122.11% ($P < 0.05$), 91.47% ($P < 0.05$), and 116.87% ($P < 0.05$) in bulk soil, < 0.25 mm and small macroaggregates, respectively, compared with PP (Fig. 5b). In terms of the P cycle, the activities of phosphatases (ACP and PDE) were markedly elevated in MP compared to PP. The ACP in MP was significantly greater by 107.55% ($P < 0.001$), 91.02% ($P < 0.01$), 52.23% ($P < 0.01$), 102.15% ($P < 0.01$), and 56.55% ($P < 0.01$) in bulk soil and four aggregates, respectively, compared with PP (Fig. 5c). in comparison to PP, PDE activity in MP showed significant increases of 20.73% ($P < 0.05$), 35.56% ($P < 0.01$), 65.33% ($P < 0.05$), 68.12% ($P < 0.01$), and 166.16% ($P < 0.01$) across the five soil samples (Fig. 5d).

Linear regression results indicated that the P-hydrolyzing enzymes [ln (ACP)] and [ln (PDE)] were significantly positively correlated with the N-hydrolyzing enzymes [ln (LAP + NAG)] ($r = 0.85$ and 0.60, respectively, $P < 0.001$; Fig. 6).

Identification of influential factors on soil Po fractions and transformation induced by A. mangium

In the RDA ordination biplot, axis 1 and axis 2 explained 56.2% and 30.5% of the variance in the relationship between specific Po fractions and the corresponding abiotic factors (Fig. 7). More than half (59%) of the eighteen factors (the total of Lambda-β in Table 3). During the initial screening of the eighteen factors in RDA ordinations, it became apparent that the Po fractions were predominantly influenced by NO_3^--N, AMF, pH, and ACP (Table 3).

Table 3
Marginal and conditional effects of site factors on soil Po fractions obtained from the summary of forward selection in Redundancy analysis (RDA).

Variable	Lambda-α[1]	Lambda-β[2]	*P*[3]	*F*-ratio[4]
NO_3^--N	0.239	0.24	**0.002**	**15.10**
AMF	0.159	0.09	**0.002**	**6.72**
pH	0.178	0.05	**0.020**	**3.60**
MBP	0.127	0.03	0.080	2.47
ACP	0.174	0.04	**0.034**	**3.15**
Fungi	0.105	0.03	0.070	2.57
SOC	0.166	0.02	0.190	1.65
N/P_{soil}	0.174	0.02	0.178	1.69
Actinomycetes	0.150	0.02	0.416	0.97
Total PLFAs	0.155	0.01	0.366	1.00
PDE	0.189	0.01	0.436	0.93
C/P_{soil}	0.210	0.01	0.556	0.74
MBN	0.228	0	0.630	0.64
MWD	0.196	0.01	0.572	0.62
TN	0.153	0.01	0.766	0.36
Bacteria	0.153	0	0.776	0.32
NAG	0.235	0	0.992	0.06
LAP	0.055	0	0.998	0.01

[1]Marginal effects, indicating the variance explained by each variable in isolation. [2]Conditional effects, indicating the additional variance explained by each variable on inclusion in the model. [3]Level of significance for Lambda-β (Monte Carlo test). [4]Monte Carlo test statistic for Lambda-β at the 0.05 significance level. NO_3^--N: nitrate nitrogen; AMF: arbuscular mycorrhizal fungi; MBP: microbial biomass phosphorus; ACP: acid phosphatase; SOC: soil organic carbon; N/P_{soil}: N/P ratio of soil; PDE, phosphodiesterase; C/P_{soil}: C/P ratio of soil; MBN, microbial biomass nitrogen; MWD: mean weight diameter; TN: total nitrogen; NAG: β-N-acetylglucosaminidase; LAP: leucine aminopeptidase. Bold values indicate significant effects at $P < 0.05$.

The regulatory SEM model of soil P transformation (ACP activity) associated with N_2-fixing tree species was strongly supported by all statistical tests ($\chi^2 = 9.0$, $P = 0.436$; CMIN/df = 1.002; goodness of fit = 0.954; comparative fit index = 1.000; root square mean error of approximation = 0.006) (Fig. 8a). The SEM model explained 75.5% of the variance in the rate of P transformation. As per the structural equation modeling (SEM) path analysis, the pace of P transformation is directly and positively regulated by the presence of *A. mangium* plantation and AMF (Fig. 8a). Apart from direct influences, path analysis also revealed significant indirect cascading impacts of *A. mangium* and site-specific factors via their correlations on the rate of P transformation (Fig. 8a). When direct and indirect effects were considered together, N_2-fixing tree species exhibited the strongest positive control on the rate of P transformation, followed by a direct or indirect positive effect of AMF, SOC, and pH in descending order (Fig. 8b).

Discussion

Correlations of soil Po fractions and soil properties with the introduction of N_2-fixing tree species

Following the incorporation of *A. mangium* into *Eucalyptus* plantations for 15 years, the C:P and N:P ratios in the soil saw a substantial increase in both bulk soil and all aggregate fractions when compared to plantations lacking N_2-fixing tree species (Table 2). In this study, the introduction of an *A. mangium* enhanced the fixation and accessibility of soil N, while increasing soil C sequestration. These findings align with those of prior studies (Galiana et al., 2002; Huang et al., 2014; 2017). Soil C:P ratio is an index used for assessment of soil P availability (He et al., 2008); the soil N:P ratio can reflect N pool capacity and mineralization rates of N and P, indirectly predicting the baseline conditions of the community and nutrient supply levels (Inagaki et al., 2011; Heuck et al., 2015). Moreover, our results are supported by the findings of Smal and Olszewska (2008), who reported that the introduction of N_2-fixing tree species did not significantly change soil TP content. These results indicate that the output and input of TP via below- and above-ground may be in balance (Zhao et al., 2007; Deng et al., 2017). However, in the current study, 15 years of introducing *A. mangium* into *Eucalyptus* plantations resulted in significant decrease of the Po concentration in the bulk soil and most of aggregates (Table 2). Such results could be explained by at least three factors. First, several soil physicochemical properties (e.g., SOC, TN, C:P ratio, and N:P ratio) induced by the *A. mangium* were substantially increase in the bulk soil and all aggregates (Table 2). These properties could be responsible for P adsorption-desorption, precipitation-dissolution, and oxidation-reduction reactions and resulted in decreasing Po accumulation (Kwak et al., 2018; Sun et al., 2020). Furthermore, higher SOC and TN contents provide available nutrients for microorganisms, increasing the rate of SOC decomposition (Teng et al., 2018); this promotes Po mineralization (Maharjan et al., 2018). In addition, the increase of N availability may stimulate biotic P demand and ultimately enhanced P uptake by trees and might account for the decease of soil Po concentration (Hu et al., 2010).

Our experimental findings confirm our initial hypothesis and underscore the influence of *A. mangium* on soil Po fractions in *Eucalyptus* plantations. The increases in the labile Po fraction in bulk soils and most aggregate fractions, following the introduction of an *A. mangium*, may be linked to decreases in the highly recalcitrant Po fraction (Fig. 3a, d). This process may be caused by greater nutrient acquisition by soil microorganisms when N and SOC are sufficiently abundant, which promotes the biological processes of microorganisms and their coupling with plant root systems; such changes allow the transformation of highly recalcitrant P into active organic phosphorus (Zhang et al., 2013; Hu et al., 2016). Furthermore, a positive relationship existed between pH and labile P, while a negative correlation was observed with highly recalcitrant P (Fig. 7). This suggests that elevated pH levels in acidic soil enhance microorganism viability, contribute to the decomposition of highly recalcitrant P, and foster the formation of labile P. Because of sharp decreases in pH, free phosphate is easily adsorbed onto the surfaces of Fe and Al hydroxides (Moore, 2002; Lu et al., 2016; Zhou et al., 2016). In addition, the < 0.25 mm size fraction exhibited the highest concentration of labile P in aggregates for both MP and PP, potentially due to microbial activity and enzymatic hydrolysis (Tabatabai,1994).

Correlations of soil Po transformation and soil microbial communities with the introduction of N_2-fixing tree species

Given the fast turnover of soil microbial biomass, it is believed that the regulation of P immobilization and release by soil microbes is closely linked to the production and buildup of soil Po fractions (Richardson and Simpson, 2011; Brucker et al., 2020). This study found various degrees of increases in the soil bacterial, fungi, actinobacterial, AMF, and total PLFAs in all soil samples following the introduction of an N2-fixing tree species into a *Eucalyptus* plantation for 15 years. Huang et al. (2014) have reported similar findings. Our results may be explained by the ability of plant communities affect soil microbial community structure and composition directly or indirectly by influencing of litterfall, and soil physicochemical characteristics (Buyer et al., 2016; Huang et al., 2017). Furthermore, microbial community structure is significantly affected by the size of soil aggregates because of the higher rate of nutrient utilization by microorganisms in smaller aggregates (Sessitsch et al., 2001; Wei et al., 2014). There are several potential mechanisms for the higher levels of Po availability in MP soils in relation to the improved soil biological activity associated with increased soil microbial biomass and ACP activity. First, the observed enhancements in soil microbial biomass and ACP activity in MP might have accelerated the rate of soil Po mineralization, thus

mobilizing greater amounts of Pi from both insoluble and Po sources (Richardson and Simpson, 2011; Spohn and Kuzyakov, 2013) and resulting in increased labile P pools (Nannipieri et al., 2011). Second, several investigations have documented that increases in the biomass and diversity of soil microbes typically lead to enhanced biosynthesis and activity of associated phosphate-solubilizing soil enzymes (e.g., acid phosphatase) (Wei et al., 2014; Heuck et al., 2015), ultimately increasing P mobilization into the labile P pool. In temperate forests, previous studies have found that a substantial proportion, ranging from 68–78%, of the total biomass P can be located within the soil microbial biomass (Turner et al., 2013; Rosling et al., 2016), suggesting that soil MBP is important in soil Po dynamics. In this study, the notably elevated levels of soil MBN and MBP in MP (Fig. 1) were linked to the presence of increased soluble N and P; this availability presumably resulted from increases in plant residues after the introduction of the N_2-fixing tree species (Elgharably and Marschner, 2011).

The importance of phosphatase activity in P cycling has been widely acknowledged because such enzymes hydrolyze C-O-P ester bonds in Po compounds in soil, leading to the release of Pi (Chen, 2003; LeBrun et al., 2018). Furthermore, phosphatase can be actively released through excretion by living roots and fungi; it can be passively released from ruptured cells (Wei et al., 2014). In this study, we observed changes in phosphatase activity in MP. Particularly, ACP and PDE activities were elevated in MP compared to PP. The increase in phosphatase activity provoked by an *A. mangium* can be explained by two potential mechanisms. First, the introduction of an N_2-fixing tree species could induce the production of phosphatase enzymes by alleviating N limitation (Nasto et al., 2014; Batterman et al., 2018; Zheng et al., 2023). Alternatively, the introduction of *A. mangium* may alter the abundances of microbes, especially fungi and AMF (Fig. 2c, d). Moreover, the significant positive correlations between NAG + LAP enzymes and phosphatase (i.e., ACP and PDE) activities support this hypothesis (Fig. 6a, b).

Both RDA and SEM analyses strongly indicate that Po fractions and transformation result from the intricate interactions among abiotic and biotic factors associated with ecological processes influenced by the presence of an *A. mangium*. The significantly greater net impacts indicate that changes in soil NO_3^--N, AMF, fungi, pH, and ACP are the primary factors that control the Po fractions (Fig. 7). We employed soil ACP activity to gauge the dynamics of P conversion or turnover in the SEM path analysis. The changes of SOC, NO_3^--N, and pH cause by direct impacts of *A. mangium* affect soil fungi and AMF variation and ultimately driving Po transformation. These six key drivers explained most of the variability in Po transformation (Fig. 8). Our findings align with the results of previous studies. For example, González-Ramírez and Eisenhauer (2018) reported that changes in abiotic soil properties affected biodiversity-ecosystem function relationships through changes in certain soil biological variables. In addition, N_2-fixing tree species reportedly enhance the photosynthetic rate of some plants, possibly allowing greater production in AMF through C allocation (Jia et al., 2004). Therefore, high densities of N_2-fixing tree species involve widespread mutualism between plants and AMF; N_2-fixing tree species harbor much greater AMF colonization than do non-N_2-fixing tree species (Nasto et al., 2014). Furthermore, Sato et al. (2015) tested the hypothesis that AMF exude soluble ACP from extraradical hyphae. Our findings imply that the introduction of an N_2-fixing tree species (i.e., *A. mangium*) may stimulate Po transformation through the mycobiome, increasing the rate at which Po becomes available to associated plants. These results highlight the importance of AMF for improving Po transformation in eucalyptus plantation systems (Pereira et al., 2018; Santana et al., 2020).

Conclusions

The current investigation disclosed how the incorporation of N_2-fixing tree species into *Eucalyptus* plantation decreased Po accumulation. It also showed how promoting Po transformation was controlled by complex interactions between abiotic and biotic variables play crucial roles in soil biota-driven Po transformation processes. The changes of SOC, NO_3^--N, and pH cause by direct impacts of *A. mangium* affect soil fungi and AMF variation, thereby driving Po transformation. Nevertheless, our findings demonstrate that the extended use of N_2-fixing species enhances the accumulation of SOM, increases nutrient availability, supports a larger microbial biomass community, and consequently alters overall rates of P cycling. These results are important for forest management, by reinforcing the merits of mixed N_2-fixing species, but also by combining application of organic and inorganic fertilizer could be considered in fertilization strategies. It could be a beneficial effect of soils on the

growth and accumulation of P, together with N, for *Eucalyptus* plantations. Our findings will help to facilitate the development of sustainable P management strategies for *Eucalyptus* plantations in subtropical China.

Declarations

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Author contributions

Haocheng Xu: Investigation, Formal analysis, Visualization, Methodology, Writing –original draft. Yeming You: Conceptualization, Writing – review & editing. Yi Wang: Investigation, Formal analysis. Guannv Gao: Data curation, Methodology. Angang Ming: Resources, Investigation. Xueman Huang: Conceptualization, Funding acquisition, Supervision, Writing – review & editing. All authors read and approved the final manuscript.

Declarations

The authors declared that they have no conflicts of interest to this work. We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.

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Figures

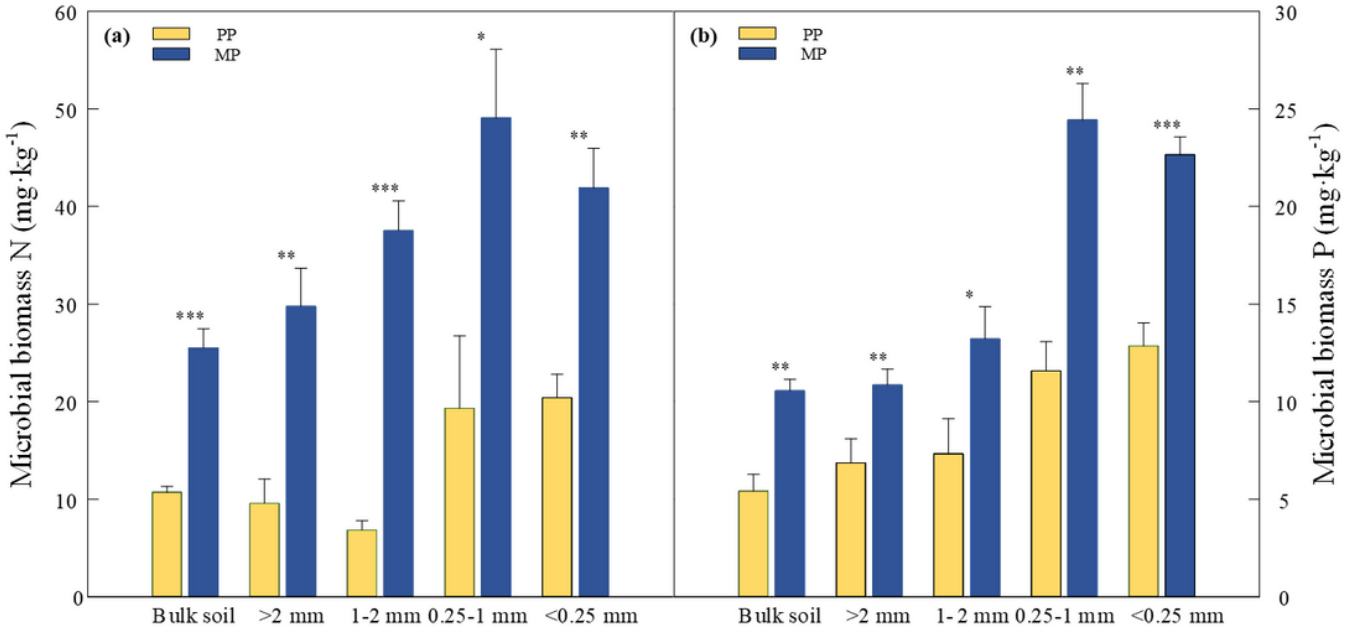

Figure 1

Soil microbial biomass N and P in bulk soil and all aggregate-sized fractions in PP and MP. Error bars represent ± *SE* (*n* = 5). Asterisks indicate significant differences between PP and MP values (NS $P>0.05$, * $P<0.05$, ** $P<0.01$, *** $P<0.001$).

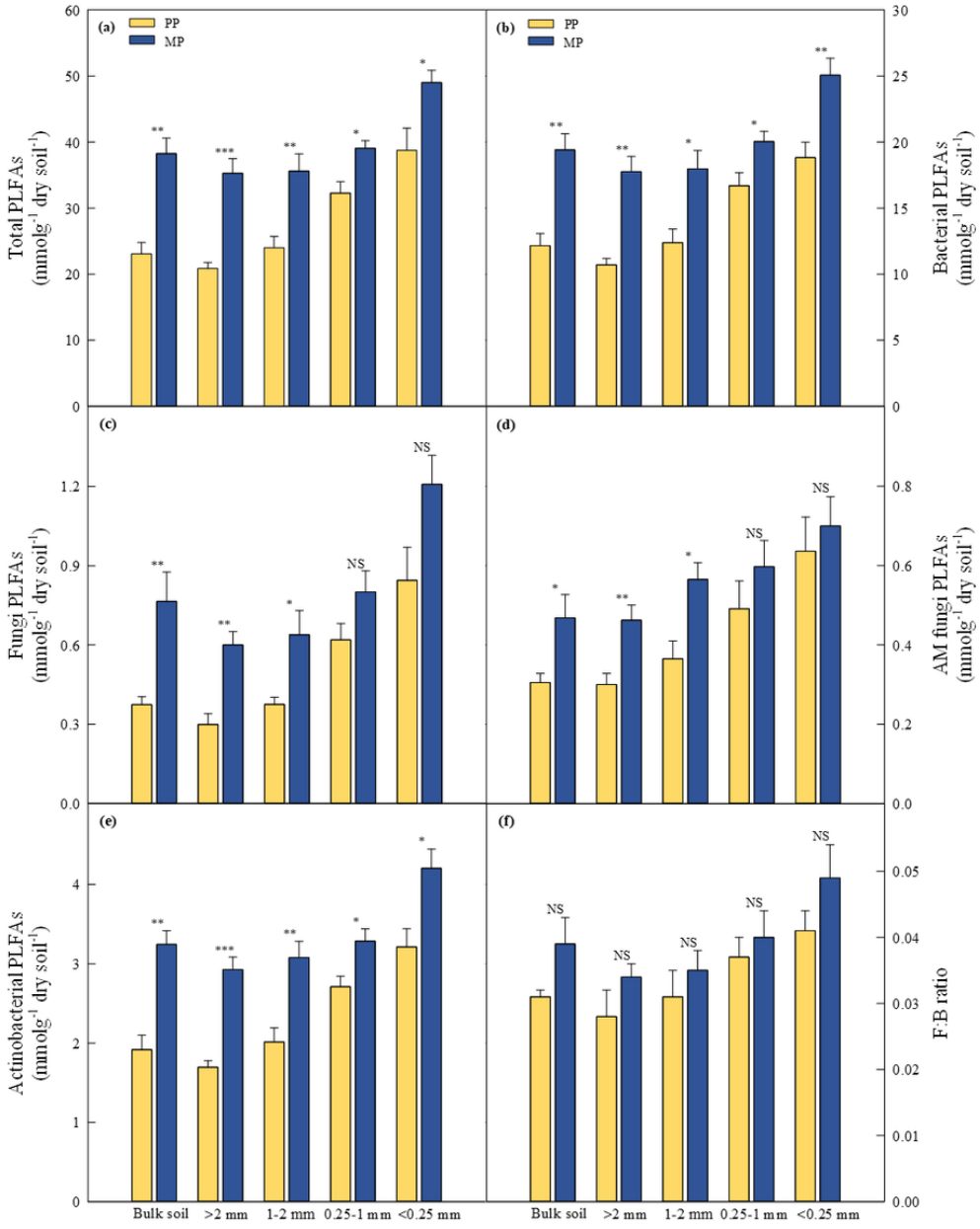

Figure 2

Soilmicrobial phospholipid fatty acids (PLFAs) in bulk soil and all aggregate-sized fractions in PP and MP. AMF, arbuscular mycorrhiza fungi; F:B ratio, ratio of soil fungal PLFAs to soil bacterial PLFAs. Asterisks indicate significant differences between PP and MP values ((NS $P>0.05$, * $P<0.05$, ** $P<0.01$, *** $P<0.001$).

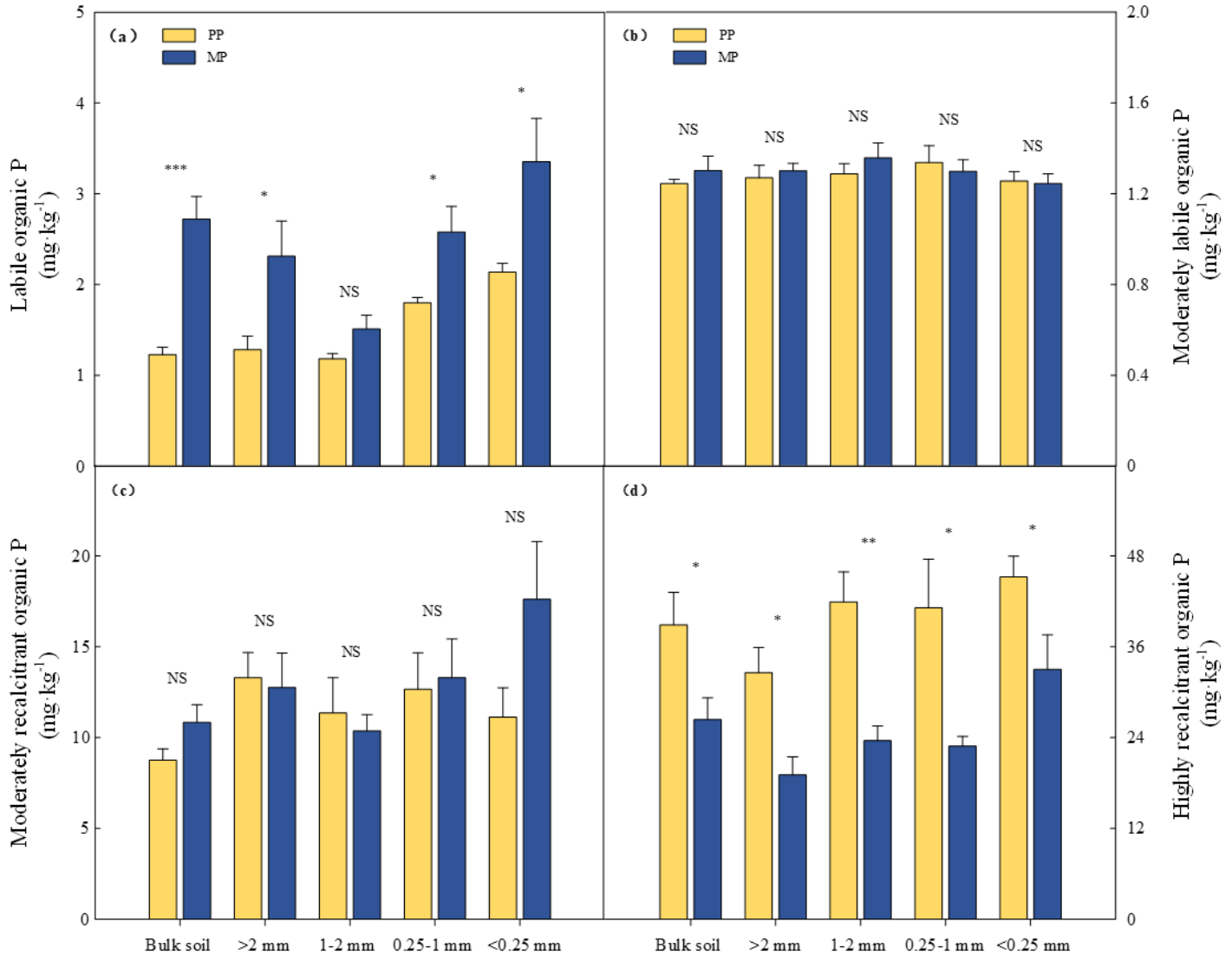

Figure 3

Soil organic P fractions in bulk soil and all aggregate-sized fractions in PP and MP. Error bars represent ± *SE* (*n* = 5). Asterisks indicate significant differences between PP and MP values (NS $P > 0.05$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

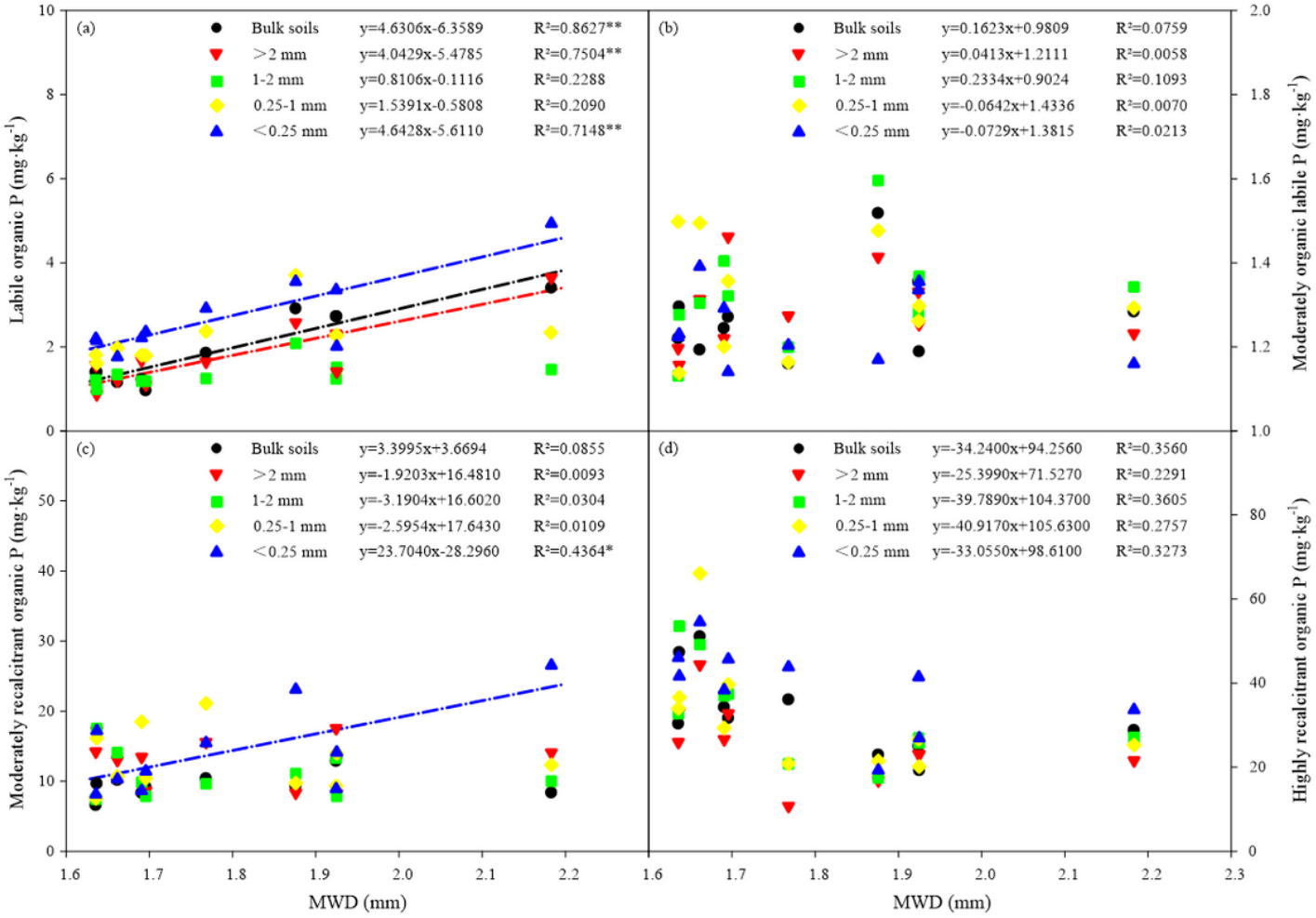

Figure 4

Correlations between MWD and organic P fraction in the bulk soil and within aggregate fractions. * *P*<0.05.

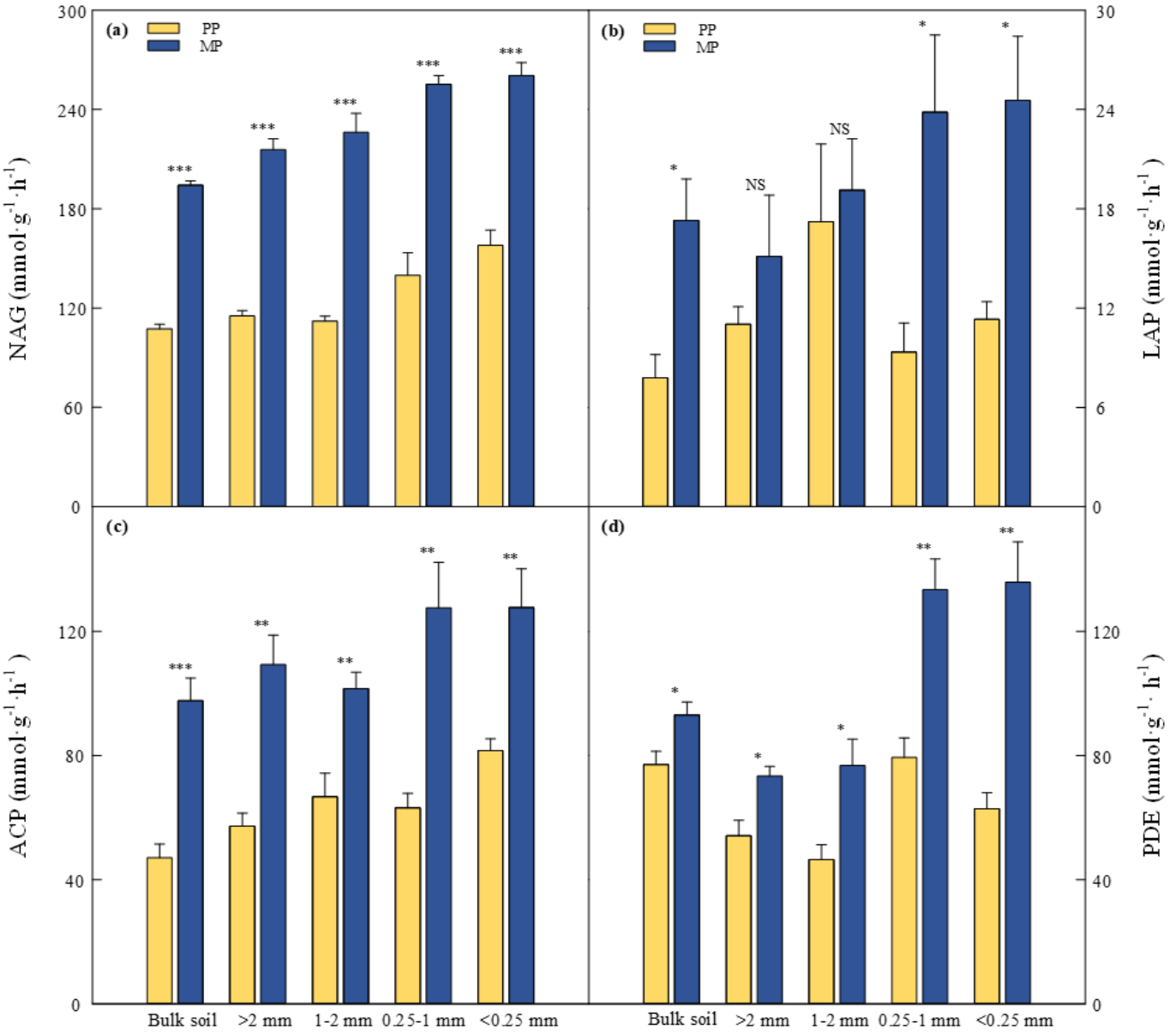

Figure 5

Soil extracellular enzyme (NAG, LAP, ACP and PDE) activities in bulk soil and all aggregate-sized fractions in PP and MP. NAG, β-N-acetylglucosaminidase; LAP, leucine aminopeptidase; ACP, acid phosphatase; PDE, phosphodiesterase.Asterisks indicate significant differences between PP and MP values (NS$P > 0.05$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

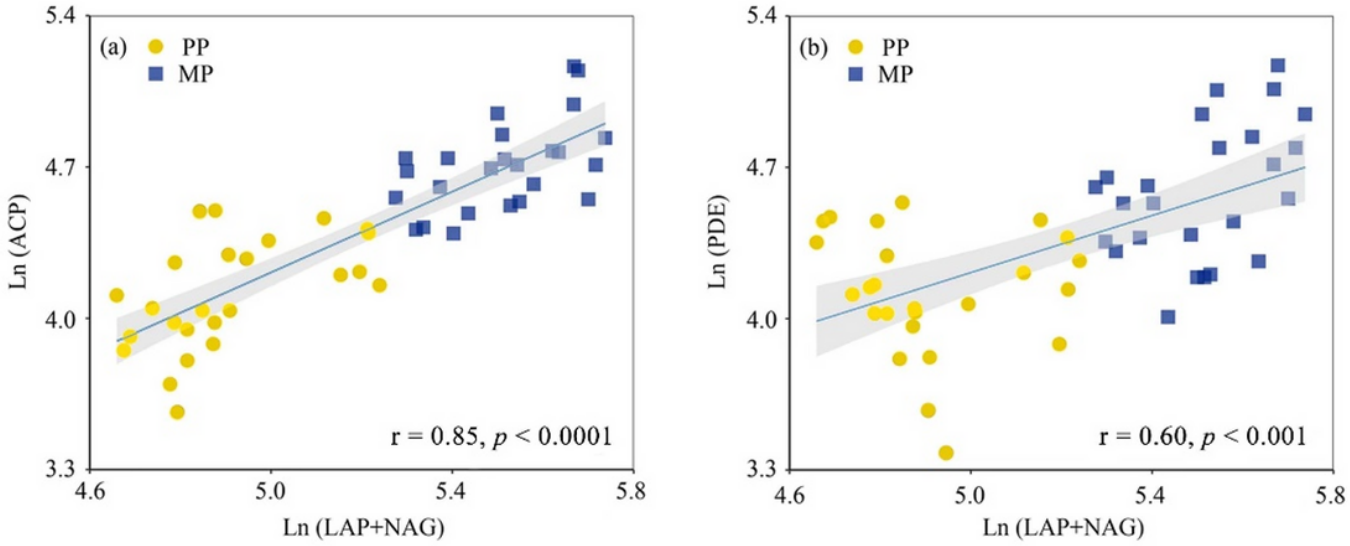

Figure 6

Relationships of soil N-, and P-related extracellular enzymes activities in PP and MP. LAP, leucine aminopeptidase; NAG, β-N-acetylglucosaminidase; ACP, acid phosphatase; PDE, phosphodiesterase.

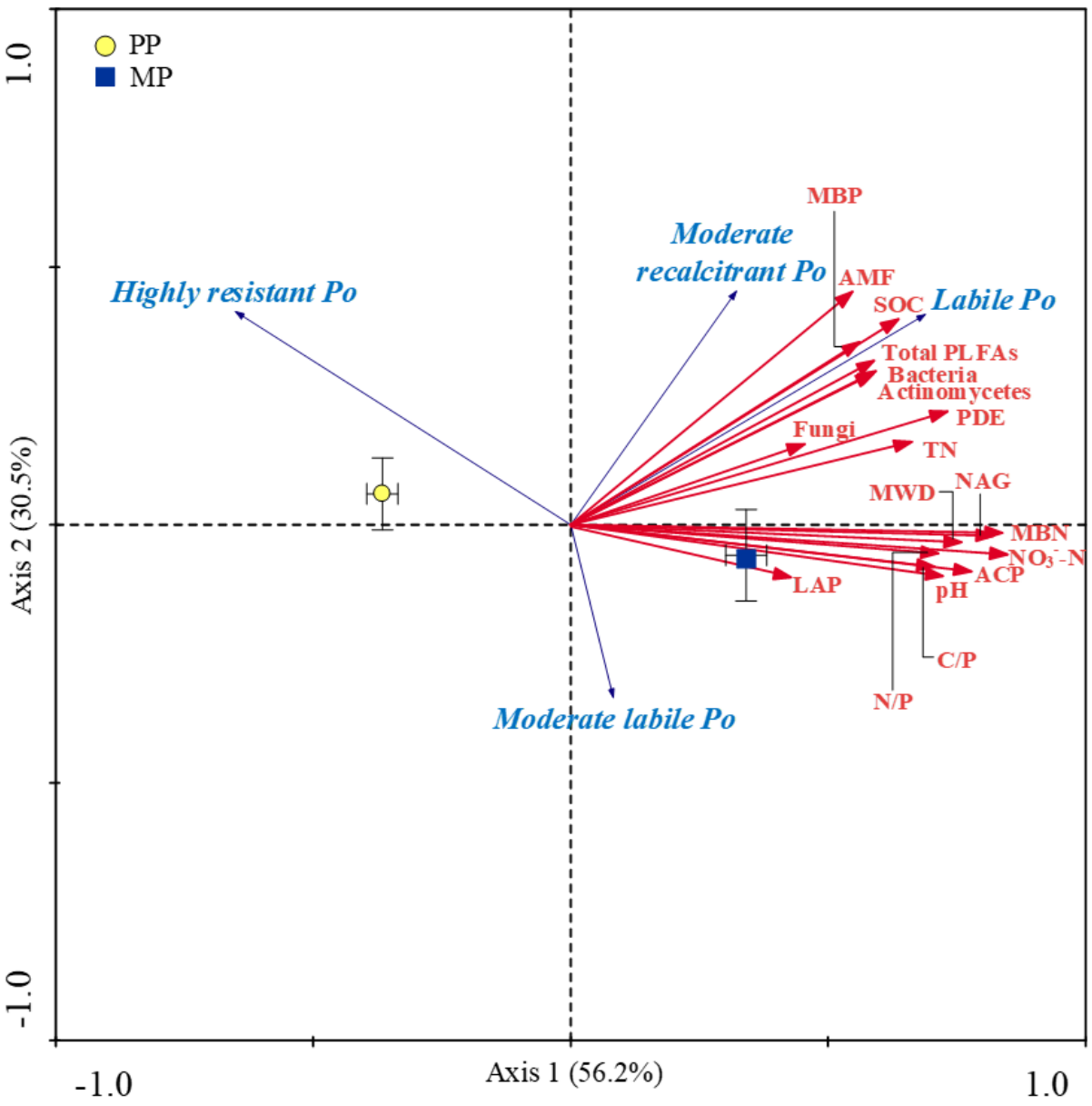

Figure 7

RDA plot showing the variance in Po fractions explained by soil and microbial properties. NO_3^--N: nitrate nitrogen; AMF: arbuscular mycorrhizal fungi; MBP: microbial biomass phosphorus; ACP: acid phosphatase; SOC: soil organic carbon; N/P_{soil}: N/P ratio of soil; PDE, phosphodiesterase; C/P_{soil}: C/P ratio of soil; MBN, microbial biomass nitrogen; MWD: mean weight diameter; TN: total nitrogen; NAG: β-N-acetylglucosaminidase; LAP: leucine aminopeptidase.

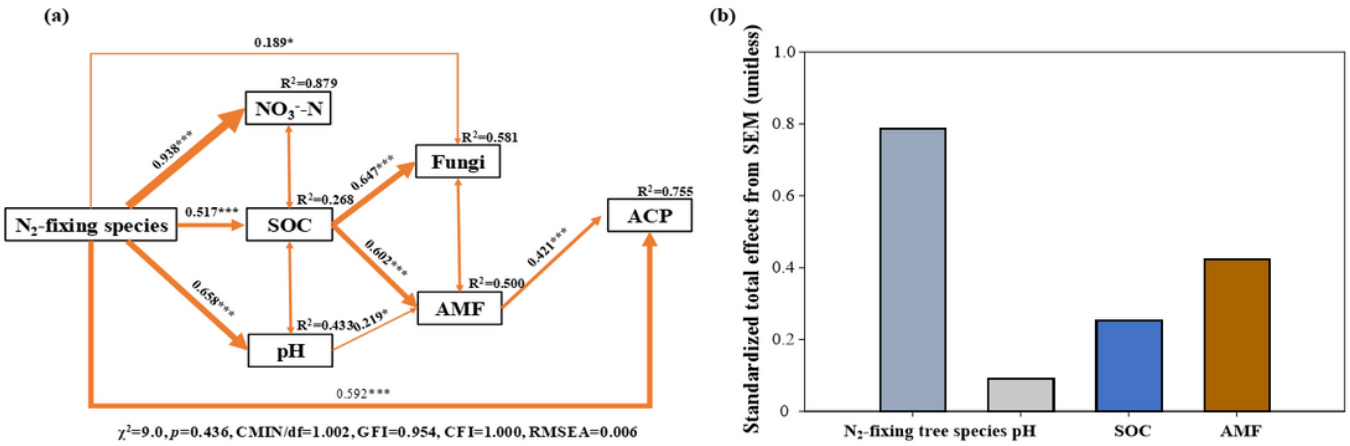

Figure 8

(a) Redundancy analysis (RDA) of soil extracellular phosphatases in PP and MP. AMF, arbuscular mycorrhiza fungi; Actino, actinomycetes. (b) Path analysis of the factors that influence the P transformation by introducing N_2-fixing tree species in Eucalyptus plantations. Numbers on arrows are standardized direct path coefficients ((direct effects). R^2 value represents the proportion of total variance explained for the specific dependent variable. Solid arrows represent positive effects. The thickness of the arrow and the associated value indicate the strength of the effect. Asterisks indicate significant differences between PP and MP values (* $P< 0.05$, ** $P< 0.01$, *** $P< 0.001$).

Supplementary Files

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