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Responses of soil organic carbon compounds to phosphorus addition between tropical monoculture and multispecies forests

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Abstract

Tropical forests are sensitive to nitrogen (N) and phosphorus (P) availability, and under nutrient application the variation of soil organic carbon (SOC) preserving mechanism remains to be explored. To reveal the forest-specific SOC preservation via biochemical selection in response to nutrient application, we investigated a monoculture (Acacia plantation) and a multispecies forest both with chronic fertilization in subtropical regions, and measured specific fingerprints of plant- and microbial-derived C compounds. In addition, to quantify the effect of P application on SOC content among tropical forests, we conducted a meta-analysis by compiling 125 paired measurements in field experiments from 62 studies. In our field experiment, microbial community composition and activity mediated forest-specific responses of SOC compounds to P addition. The shift of community composition from fungi towards Gram-positive bacteria in the Acacia plantation by P addition led to the consumption of microbial residual C (MRC) as C source; in comparison, P addition increased plant species with less complex lignin substrates and induced microbial acquisition for N sources, thus stimulated the decomposition of both plant- and microbial-derived C. Same with our field experiment, bulk SOC content had neutral response to P addition among tropical forests in the meta-analysis, although divergences could happen among experimental durations and secondary tree species. Close associations among SOC compounds with biotic origins and mineral associated organic C (MAOC) in the multispecies forest suggested contributions of both plant- and microbial-derive C to SOC stability. Regarding that fungal MRC closely associated with MAOC and consisted of soil N pool which tightly coupled to SOC pool, the reduce of fungal MRC by chronic P addition was detrimental to SOC accumulation and stability in tropical forests.

Keywords: Nutrient addition, soil carbon compound, plant substrate, soil microbial activity, tropical forest

1. Introduction

Nitrogen (N) and phosphorus (P) are major limiting nutrients in terrestrial ecosystems (Reay et al., 2008; Vitousek et al., 2010). Tropical regions often exhibit low soil P availability due to the old soil age with extensive weathering (Walker and Syers, 1976), and imbalanced application of N and P may exacerbate P deficiency for plant growth and microbial metabolism (Cui et al., 2022; Peñuelas and Sardans, 2022). The relative availability of P compared to N thus plays a vital role in the tropical C budget, yet how nutrient availability regulates the conversion between sink and source in tropical forest soil organic carbon (SOC) remains uncertain. Neutral or negative responses of specific SOC compounds to nutrient addition are frequently reported (Chen et al., 2020; Li et al., 2023; Zhang et al., 2023), despite increased bulk SOC content with N and P application in tropical forests in previous meta-analyses (Lu et al., 2021; Feng et al., 2023). Caring for the diversity and heterogeneity of C components instead of locking away bulk C in soil is preferred for SOC management nowadays (Lehmann et al., 2020; Lavallee et al., 2020), thus studying how SOC composition and stability respond to nutrient input should be emphasized for better soil management in tropical forests.

Biochemical selection is recognized as one of the mechanisms underlying SOC preservation other than mineral protection (Hemingway et al., 2019), as both plant-derived complex compounds and microbial turnover products can be sustained in the soil and contribute to SOC preservation. When plant-derived organic matter decomposes in the soil, compounds such as lignin, cutin and suberin, and waxes are physically transferred into the soil and preferentially preserved due to their chemical complexity (Cotrufo et al., 2015); meanwhile, labile components of plant origin are quickly utilized by soil microorganisms and form microbial-derived C compounds such as necromass and other large molecules (Cotrufo et al., 2015; Roth et al., 2019). Microbial residual C (MRC) is the product of microbial turnover and can make up more than half of SOC content (Liang et al. 2019), it can be further preserved in soil through mineral protection or aggregation (Cao et al., 2023). Soil microorganisms actively participate in the biochemical selection processes, and soil N and P availability can regulate SOC composition through the impact of microbial decomposers. The addition of N could stimulate soil labile C consumption due to higher microbial C acquisition (Xu et al., 2022), but preserved more lignin compounds because of suppressed oxidative enzyme activity (Xiao et al., 2018). In contrast, P application in tropical forests could alter the limiting nutrient for microorganisms that utilize MRC as a source (Yuan et al., 2021), and enhance oxidative enzyme activity for faster lignin decomposition (Lin et al., 2019; H. Li et al., 2023). Previous

meta-analyses found that increased Gram-positive bacteria: Gram-negative bacteria (bacterial G^+:G^-) ratio and decreased proportion of arbuscular mycorrhizal fungi (AMF) were caused by N addition (Zhang et al., 2018), and decreased fungi: bacteria (F: B) ratio and increased bacterial G^+:G^- ratio were caused by P addition (Wu et al., 2022). Altered microbial biomass and community composition by nutrient application can thus change MRC content and its composition. Physically separating SOC into particulate organic C (POC) and mineral-associated C (MAOC) fractions allows for the investigation of SOC stability. Although both fractions contain plant- and microbial-derived C (Angst et al., 2021; Ludwig et al., 2015), C compounds in POC are more accessible to roots and microbes than in MAOC and thus considered easier to be decomposed (Cotrufo et al., 2019). A relatively larger amount of MAOC in SOC thus suggests higher stability of SOC. Moreover, associations between specific C compounds and soil C fractions suggest the linkages of these C compounds to SOC stability, which awaits to be explored under the P application scenario.

Reforestation helps to recover SOC content in tropic regions (Don et al., 2011; Veldkamp et al., 2020), as the accumulation of debris and transformation of C associated with plant and microbial metabolism provide inputs of organic matter into the soil C pool. Secondary forests have been widely established in tropical regions to mitigate forest degradation. Monoculture plantation is a major practice for reforestation, along with demand for timber products (Paquette and Messier, 2010). Having the advantages of fast growth and soil N enrichment by N_2 fixation, *Acacia* spp. are commonly found in tropical forests (Houlton et al., 2008), and are often selected as pioneer species for forest restoration (Zhang et al., 2012). In long-term forest management, however, high lignin content but low non-structural C components in the litter of *Acacia* spp. dampens litter turnover (Santos et al., 2017), and the fixation of P in plant biomass decreases soil P availability (Balieiro et al., 2020), leading to low plant substrate quality and P-poor condition. Plant diversity can be beneficial to various ecosystem functions including soil carbon sequestration (Chen et al., 2018). Furthermore, increasing soil P availability (Chen et al., 2022) and balancing nutrient stoichiometry (Chen and Chen, 2021) highlight the advantages of plant mixing management. Divergent responses of plant- and microbial-derived C to forest succession and management regimes have been reported recently (T. Li et al., 2023; Ma et al., 2018; Ye et al., 2019). Therefore, comparing the variations in SOC composition and stability in response to nutrient application between monoculture and multispecies forests can shed light on the forest-specific preserving mechanism of SOC compounds in tropical reforestation planning.

To reveal the forest-specific properties of microbial composition and activity mediating biochemical selection for SOC preservation in response to P application, we investigated both a monoculture forest (*Acacia* plantation) and a multispecies forest with four nutrient addition treatments, including control, N addition (100 kg N ha^{-1} a^{-1}), P addition (100 kg P ha^{-1} a^{-1}) and combined N and P addition (100 kg N and P ha^{-1} a^{-1}), for more than a decade were investigated. In addition, to quantify the effect of P application on SOC content among tropical forest types, we conducted a meta-analysis compiling 125 paired measurements in field experiments with control and a corresponding P treatment from 62 studies covering the major monoculture and multispecies forest types in tropical and subtropical regions. We hypothesized that: (1) biochemical selection could be more sensitive to P addition in the multispecies forest, since larger diversity and higher substrate availability in the multispecies forest compared to the monoculture (Chen et al., 2022; Wang et al., 2023) could benefit microbial activity; (2) compared to plant-derived C compounds, microbial-derived C could be more involved in biochemical selection due to its nature of both C and N sources (Liang et al., 2019; Wang et al., 2021) under P addition; and (3) P addition could enhance SOC content in tropical forests considering the P-poor soil condition (Walker and Syers, 1976), and especially for N_2-fixing tree species with relatively high soil N availability (Feng et al., 2023; Luo et al., 2023).

2. Materials and Methods

2.1 Field experimental design

This study was conducted in two subtropical secondary forests in Guangdong, China. The monoculture plantation of *Acacia auriculiformis* was at the Heshan National Field Research Station of Forest Ecosystems (22°34′N; 112°50′E). The study site at the Heshan station has a typical monsoon climate with an obvious wet season (April- September). The mean annual temperature is 22.5°C, and the mean annual precipitation is 1543 mm. The soil type is Ultisols developed from sandstone. The *Acacia* plantation was established on a grassland after clear-cutting in 1984 and has been undisturbed since the establishment and the plantation remained to be legume-dominated. More detailed information on the site can be found in Zhang et al., (2012). The multispecies forest with broadleaved trees was at the Xiaoliang Research Station of Tropical Coastal Ecosystems, the Chinese Academy of Sciences (21°27′N, 110°54′E). The study site at the Xiaoliang station is also characterized by a typical monsoon climate with an obvious wet season (April- September). The mean annual temperature of 23 °C, and the mean annual

precipitation ranges from 1400 to 1700 mm. The soil type is Ultisols developed from granite. The establishment of the multispecies forest on a former monoculture plantation was completed in 1975. Broadleaved tree species were introduced after land clearing, and the forest succeeded naturally afterward. Dominant tree species are *Cinnamomum camphora*, *Aphanamixis polystachya*, *Castanopsis fissa*, *Aquilaria sinensis*, *Acacia auriculaiformis*, *Cassia siamea*, *Carallia brachiata*, and *Ternstroemia pseudoverticillata*. More detailed information on the site can be found in Li et al., (2015). Background N application is similar in two forests (approximately 40 kg N ha^{-1} a^{-1}, Huang et al., 2015; Mo et al., 2015).

The fertilization experiments were established with 3 blocks (n = 3) in the *Acacia* plantation in July 2010, and with 5 blocks (n = 5) in the multispecies forest in September 2009. Both experiments adopted the same randomized block design and fertilization protocol. Within each block, four 10 m × 10 m plots were established. In the *Acacia* plantation, each plot was separated by a 10 m wide buffer zone with the neighboring plot in one block. In the multispecies forest, PVC platers (40 cm depth) with a 1 m-width buffer area surrounding each plot were inserted into the soil to prevent interference among treatments. Four treatments were applied as control, N addition (100 kg N ha^{-1} a^{-1}), P addition (100 kg P ha^{-1} a^{-1}), and N + P addition (100 kg N and P ha^{-1} a^{-1}) in each block. Ammonium nitrate (NH_4NO_3) and sodium biphosphate (NaH_2PO_4) solution were sprayed into the soil on the corresponding plot bimonthly. The same amount of groundwater without any fertilizer was added to the control plot. Soils were sampled in December 2020 (dry season) and July 2021 (wet season) in both forests. In each plot, five soil cores (0 - 20 cm) were collected randomly and mixed homogeneously to form one composite sample for laboratory investigation.

2.2 Soil biochemical properties

Plant-derived C compounds investigated in this study included fatty acids, cutin suberin, and lignin. Based on standard protocols (Otto and Simpson, 2007; Tamura and Tharayil, 2014), a soil sample was sequentially extracted by solvent extraction, base hydrolysis, and CuO oxidation, to determine long-chain fatty acids, cutin-and suberin-derived compounds and lignin-derived phenols, respectively. Vanillyls (V-phenol), syringyls (S-phenol) and cinnamyls (C-phenol) compounds isolated from CuO oxidation represented lignin. Lignin S:V ratio, lignin C:V ratio, and ratios of acid (Ad) to aldehyde (Al) in V- and S-phenol were calculated. Cutin, suberin, and long-chain lipids are also common in vascular plants and frequently preserved in soil (Otto et al., 2005), thus are included as plant-derived C.

Microbial-derived C components in this study were represented by amino sugars, including glucosamine (GluN), galactosamine (GalN), and muramic acid (MurN). Amino sugars were extracted according to Indorf et al. (2011) and Mou et al. (2021). The identification and quantification of three amino sugars were performed using High-performance liquid chromatography (Dionex Ultimate 3000, Thermo Fisher Scientific, Waltham, USA). The concentrations of amino sugars were expressed as μg g^{-1} dry soil. MurN and GluN are assumed to simultaneously exist in the bacterial cell walls at a 1~2 M ratio based on Engelking et al. (2007). Therefore, MRC was calculated as:

F-GluN = total GluN – 2 × MurN × (179.2/ 251.2)

where 179.2 and 251.2 are the molecular weights of GluN and MurN, respectively (Shao et al., 2017).

Fungal MRC = F-GluN × 9

Bacterial MRC = MurN× 45

where 9 and 45 are conversion factors, respectively (Appuhn and Joergensen, 2006).

Soil fractionation of particulate organic matter (POM) and mineral-associated organic matter (MAOM) was performed based on Marriott and Wander (2006) with a minor modification. Briefly, 20 g soil was added to a 50 mL plastic bottle with 5% (w:v) sodium hexametaphosphate as a dispersant. The slurry was shaken for 1 h at 180 rpm before being transferred and spread on a reciprocal sieve shaker (AS200 control, Retsch, Germany) with a 53 μm sieve. The shaker performed with the sieve up and down 3 cm with 100 repetitions. Deionized water steadily rinsed the sieve to facilitate the dispersion of the organic matter protected in aggregates. After drying at 60 ℃ for 24 h, POM (> 53 μm) and MAOM (< 53 μm) were obtained. Organic C content in bulk soil, POM, and MAOM was determined as SOC, POC, and MAOC, respectively, by the $K_2Cr_2O_7$ titration method.

The activities of β-1,4-glucosidase (BG), β-*N*-acetyl-glucosaminidase (NAG), and acid phosphatase (AP) enzymes were measured according to German et al. (2011) with a minor modification. The substrates for BG, NAG, and AP were *p*-nitrophenyl-β-d-glucopyranoside, *p*-nitrophenyl-*N*-acetyl-β-d-glucosaminide and *p*nitrophenyl phosphate tetrahydrate, respectively. The pH of the modified universal buffer (MUB) was adjusted to 6, 5.5, and 6.5 for BG, NAG, and AP activity, respectively. Then soil slurry was incubated at 37°C for 1 h, 1 h, and 0.5 h for BG, NAG, and AP activity, respectively. Enzyme activities were measured using a Microplate Photometer (the Multiskan™ FC Thermo, Fisher Scientific) at a wavelength of 405 nm. All enzyme activities were expressed as μmol g^{-1} dry soil h^{-1}. Microbial acquisition for soil nutrients was defined by calculating the vector lengths and angles of proportional activities among BG,

NAG, and AP based on Moorhead et al. (2016). Therefore, microbial nutrient acquisition was calculated as:

$X = BG/NAG$

$Y = BG/AP$

Vector length = $sqrt(X^2 + Y^2)$

Vector angle = $atan2(X,Y) \times 180/\pi$

Phospholipid fatty acid (PLFA) was used to characterize microbial community composition. The extraction protocol was based on Bossio and Scow, (1998). Briefly, lipids were extracted from the samples using a chloroform: methanol: phosphate buffer (0.8:1:2 v:v:v). Fatty acids were analyzed using gas chromatography (6890N, Agilent Technologies, Santa Clara, USA) with MIDI peak identification software (MIDI Inc., Newark, USA). Biomarkers were i14:0, i15:0, a15:0, i16:0, i17:0 and a17:0 for Gram-positive bacteria (G^+); 16:1ω7c, 16:1ω9c, 18:1ω7c, cy17:0 and cy19:0 for Gram-negative bacteria (G^-); 18:1ω9c, 18:2ω6c and 18:3ω6c for saprophytic fungi; 16:1ω5c for AMF and 10Me16:0, 10Me17:0 and 10Me18:0 for actinomycetes. Biomarkers of 14:0, 15:0, 16:0, 17:0, and 18:0 indicated general bacteria, thus the sum of general, bacterial G^+, and G^- biomarkers represent the total bacterial community. All PLFAs were expressed as nmol g^{-1} dry soil. F:B ratio and bacterial G^+:G^- ratio were calculated accordingly in this study. Background soil physiochemical properties in the two forests are shown in Table 1. Methods and procedures to determine these soil traits were provided in the Supplementary file.

2.3 Meta-analysis

Peer-reviewed literature was searched from the ISI Web of Science and China National Knowledge Infrastructure (https://www.cnki.net) databases before March 15, 2024, using specific search terms (Table S1). Papers that met the following criteria were selected: (1) the climate zone was reported or could be estimated by latitudes, mean annual temperature, and mean annual precipitation; (2) the paper presented forest type, plantation species, and load and duration of P addition; (3) the control and the corresponding P addition treatment were conducted as field experiment with same climatic, soil, and vegetation conditions; (4) the means and sample sizes of bulk SOC content (g kg^{-1}) in uppermost mineral soils must be explicitly reported or could be calculated; (5) only data from control and the corresponding P addition plots were collected when the experiment includes other treatments. We finally obtained 62 papers following these criteria and a PRISMA flow diagram for meta-analysis (Fig. S1). References of 62 papers were provided in the Supplementary file.

The analyses were carried out using R studio with packages of "meta", "metafor" and "dmetar". Log response ratio (RR) was used as the measure of effect size to quantify the effect of P addition on SOC in tropical forest ecosystems (Hedges et al., 1999), detailed calculations of RR and sampling variance for each observation were provided in the Supplementary file. Mult-level mixed effect models were conducted to determine the effect of P addition on variables using the "rma.mv" function. Duration of P addition (year), load of P fertilizer (kg P ha^{-1} a^{-1}), forest type (primary or secondary), secondary tree species (multispecies, *Acacia* spp., *Cunninghamia* spp., *Eucalyptus* spp., and others) and age of secondary forest (year) were included as categorical moderators. The response of soil total nitrogen (TN) concentration was included as a continuous explanatory variable. The restricted maximum likelihood (REML) method was used for between-study variance. The effects of P additions on SOC were considered significant if the 95% confidence intervals (CIs) did not overlap with zero. Publication bias was tested by creating funnel plots using the "funnel" function, Egger's test using "res.et" (Fig. S2, Egger's test: z = -1.446, p = 0.150), and Rosenthal's fail-safe number using "fsn" function (fail-safe N: 590, $p < 0.0001$).

2.4 Data analyses

R packages of "Matrix", "lme4", "emmeans", "rfPermute" and "A3" were used for data analyses in the field experiment. The relative change (%) of soil C compounds and abiotic and biotic properties in nutrient treatment was calculated as $(content_{treatment} - content_{control})/content_{control} \times 100$. Data were log or square root transformed accordingly to meet the assumption of normality. Linear mixed-effect models were used to assess the effects of nutrient addition and seasonal variation on soil and plant properties using "lmer" functions, and the plot was treated as a random factor. ANOVAs were performed to investigate the effects of N addition, P addition, season, and their interactions on soil C compounds and plant substrate chemistry, and pairwise comparisons were conducted using the "emmeans" function. One sample t-test was performed for the relative changes in soil and microbial properties to assess the significance of the difference in nutrient treatment. Linear regression models were performed to determine the correlations among C compounds, and microbial and plant community composition. Random forest models were used to assess the average importance of soil C compounds, and abiotic and biotic properties for the change of SOC content. The percentage increase of mean squared error (InMSE%) of the variable and its significance was calculated using the "rfPermute" function, higher InMSE% of the variable suggested its greater importance for SOC content. Cross-validated R^2 and the significance of the

model was estimated with 1000 permutations using the "a3" function. Graphs were created using GraphPad Prism 7 (GraphPad Software, San Diego, USA).

3. Results

3.1 Responses of SOC compounds to P addition

Three-way ANOVA with factors of N addition, P addition, and season showed that neither SOC, POC nor MAOC content in the *Acacia* plantation exerted a significant response to nutrient addition, regardless of the season (Fig. 1; Table S2). Soil C compounds with diverse biotic origin did not show seasonal variation either (Table S2), but fungal MRC content was significantly decreased by N+P addition (Fig. 1e). POC and MAOC were tightly linked to SOC content in the *Acacia* plantation (Fig. S4a). Regarding the relationships between SOC compounds and C fractions, only bacterial and fungal MRC contents had positive correlations with POC and MAOC (Fig. 2a,c).

SOC fractions and compounds with biotic origins in the multispecies forest did not differ between the wet and dry seasons (Fig. 1, Table S2). SOC compounds were generally sensitive to P addition, and significant reduction occurred in fungal MRC and lignin responding to P treatment (Fig. 1e,g, Table S2). Same with the *Acacia* plantation, both POC and MAOC had significant correlations with SOC content in the multispecies forest (Fig. S4b). In contrast to the *Acacia* plantation, all C compounds with biotic origins were positively correlated with MAOC in the multispecies forest (Fig. 2 b,d,f,h,j).

3.2 Responses of plant and microbial traits to P addition

For the *Acacia* plantation, the ratios among V-, S-, and C-phenol compounds did not differ among nutrient treatments, but (Ad:Al)v ratio was inhibited by P addition in the dry season (Fig. S3a, Table S2). Regarding microbial activity, soil AP activity was inhibited by both P and N+P addition in the *Acacia* plantation (Fig. 3c). Microbial C acquisition was dampened by N addition but stimulated by P addition (Fig. 3d). In comparison, microbial P acquisition was generally reduced by P addition (P addition: p = 0.078, Fig. 3e). P addition (including N + P) decreased F:B ratio but increased bacterial G^+:G^- ratio in the *Acacia* plantation (P addition on G^+:G^- ratio: p = 0.095, Fig. 3g, i). Inorganic N was increased by N addition (excluding N + P); meanwhile, soil total P and soluble P were enhanced by both P addition (including N +P, Fig. 4b,f,g). Based on the random forest model, soil TN content ranked as the most important abiotic driver of SOC content, lignin oxidation indicated by Ad:Al ratio and microbial community composition were biotic drivers significantly associated with SOC content in the *Acacia*

plantation (Fig. 5a).

In comparison, no significant change of lignin oxidation existed in the multispecies forest, but the proportion of C-phenol was significantly increased by P addition (Fig. S3d, Table S2). Soil NAG activity was enhanced by P addition, but both microbial C and P acquisition were reduced with P addition (Fig. 3d,e). PLFA concentration was decreased by N addition and P addition, bacterial G^+:G^- ratio decreased but the proportion of AMF increased with N addition (Fig. 3f,g,h). Inorganic N, total P, and soluble P with nutrient addition were in line with trends observed in the *Acacia* plantation (Fig. 4b, f, g). However, unlike the *Acacia* plantation, soil TN content was reduced by P addition, and soil pH was decreased by N addition but increased by P addition in the multispecies forest (Fig. 4a,c). Soil TN and enzyme activity were the most important abiotic and biotic drivers regulating SOC content in the multispecies forest (Fig. 5b). Compared to the *Acacia* plantation, SOC compounds (except for cutin and suberin) and C fractions were all significantly associated with SOC content in the multispecies forest based on random forest model (Fig. 5b).

3.3 Response of SOC content to P addition among tropical forests

Both field study and meta-analysis presented a neutral response of SOC content to P addition in tropical and subtropical forests (Fig. 1a, 6a), suggesting a balance between C input and output in soil C cycling with P application. Nonetheless, in terms of forest type and duration of P addition, SOC content exerted different responses to P addition in the meta-analysis. Longer duration of P addition (> 5 years) had a significant and negative effect on SOC content (effect size = -8.54%, p = 0.003), but shorter-term P addition (< 5 years) tended to increase SOC content (effect size = 2.97%, p = 0.059). SOC content had different responses to P addition among secondary forest types, it was increased in *Acacia* plantations (effect size = 8.02%, p = 0.044) but decreased in *Cunninghamia* plantations (effect size = -5.65%, p = 0.049). The age of the secondary forest did not show any significant effects on the response of SOC content to P addition. The dose of P addition did not impact the response of SOC content, except that there was a marginal increase of SOC content at higher doses of P addition (> 50 kg P ha^{-1} a^{-1}, effect size = 3.91%, p = 0.057). As an environmental factor, the response of TN content to P addition had a significant and positive correlation with the response of SOC content to P addition (effect size = 7.56%, p < 0.001, Fig. 6b).

4 Discussion

4.1 Plant preferential preservation in response to P addition

Through interaction with nutrient availability, microbial regulation on SOC preservation was highly context-dependent in tropical forests (J. Li et al., 2023; Mou et al., 2023). We thus performed a conceptual framework depicting microbial-mediated mechanisms of biochemical selection in response to P application between the monoculture and the multispecies forests (Fig. 7).

Decreased lignin S:V and C:V ratios suggested changes in vascular plant species towards the ones with higher complexity of lignin compounds (Thevenot, 2010), as units of S- and C-phenol are preferentially degraded (Otto and Simpson, 2006). Ad:Al ratios in V- and S-phenol indicate the extent of lignin transformation (Thevenot et al., 2010), and higher ratios suggest a higher degree of lignin degradation. Smaller C:V ratio and bigger Ad:Al ratios suggested a plant community with higher complexity of lignin compounds and higher extent of lignin decomposition in the monoculture forest than the multispecies forest (Table 1). Plant community composition did not shift in the monoculture forest, in regard to stable C:V ratio and S:V ratio among nutrient addition (Fig. S3b); meanwhile, (Ad:Al)v ratio was decreased with P addition (Fig. S3a), likely via the change of microbial community composition.

Since fungi are major lignin degraders and only white-rot fungi can completely mineralize lignin (Thevenot, 2010), a reduced F:B ratio due to P addition might lead to the reduction of the (Ad:Al)v ratio in the monoculture forest (Fig. S3a). Plant-derived C compounds were unresponsive to nutrient addition in the *Acacia* plantation soil (Fig. 1f,g,h), despite previous studies indicating that plant debris decomposition could be either inhibited by N addition or stimulated by P addition in the studied forests (Chen et al., 2016; Zhang et al., 2020; Zhu et al., 2016).

The lignin C:V ratio was significantly enhanced by P addition in the multispecies forest (Fig. S3d), suggesting that the plant community composition shifted towards species that preferentially degrade lignin compounds (Otto and Simpson, 2006). Lignin content was negatively correlated with the lignin C:V ratio in this multispecies forest (Fig. S7), suggesting that less lignin content was preserved in the soil with reduced chemical complexity of lignin compounds.

Older stands might take much longer to adapt to new fertility status (Wright et al., 2018), and size-dependent nutrient limitation reveals a larger dependence on external P input for small trees than for large trees (Liu et al., 2018). Therefore, P addition might mainly modify the plant traits of small trees in the forest. This is partially supported by a sharp decrease in the foliar N:P ratio in understory species, rather than overstory species, after P addition in the studied multispecies forests (Mo et al., 2020).

Improved leaf nutrient concentration in understory species might contribute to the stimulated decomposition of plant-derived C compounds in the multispecies forest, which is consistent with reduced lignin content under nutrient addition (Fig. 1f,g,h). Previous studies found increased peroxidase and phenol oxidase by Improved leaf nutrient concentration in understory species might contribute to the stimulated decomposition of plant-derived C compounds in the multispecies forest, which was in accordance with reduced lignin content under nutrient addition (Fig. 1f,g,h). Previous studies found increased peroxidase and phenol oxidase by P addition in the studied multispecies forests (H. Li et al., 2023). Peroxidase and phenol oxidase are known for their roles in decomposing lignin and phenol compounds (Sinsabaugh, 2010). Therefore, lignin content was decreased by P addition in the multispecies forest (Fig. 1g).

4.2 Microbial activity and turnover in response to P addition

Indicated by initial higher microbial C acquisition compared to the multispecies forest, C substrates were in high demand for soil microbial activity in the *Acacia* plantation, likely because biosynthetic N_2 fixation is energy-consuming (Crews, 1999). N addition decreased and P addition increased soil N_2 fixation in the studied *Acacia* plantation (Zheng et al., 2016a, 2016b), thus might explain increased microbial C acquisition with P addition (Fig. 3d). The contribution of fungi to biogeochemical process decreases as the plant-soil system becomes static and productive (Orwin et al., 2018; Yu et al., 2022), the significant reduce of F:B ratio by P addition was likely due to improved soil nutrient condition (Fig. 3i). G^+ bacteria can utilize more complex C sources than r-strategist G^- bacteria (Fanin et al., 2019), and microbial necromass could be C sources for microorganisms (Wang et al., 2021). The addition of P significantly increased bacterial G^+:G^- ratio in the monoculture forest, and bacterial G^+:G^- ratio was negatively correlated with fungal MRC content, suggesting a larger consumption of fungal MRC as C substrate with a higher proportion of G^+ bacteria by P addition (Fig. 3g, S6).

Initial lower microbial P acquisition echoed higher total P content and lower soil N:P ratio In the multispecies forest (Table 1), suggesting higher soil P availability for plants and microbes compared to the monoculture forest. The change in plant substrate composition altered microbial utilization of sources in the multispecies forest. In contrast to no impact of P addition on NAG activity on a global scale (Xiao et al., 2018), P addition increased NAG activity in the multispecies forest (Fig. 3b). NAG enzyme can hydrolyze chitin and glucosamine (Sinsabaugh et al., 2008), and a substrate shift from cellulose to chitin and peptidoglycan in soil microbial community was reported after P addition (Mori and Aoyagi, 2019),

in line with decreased microbial C acquisition and increased N-acquiring enzymatic activity in this multispecies forest (Fig. 3b,d). Decreased fungal MRC under P addition could indicate that MRC was consumed as an N source by microorganisms (Fig. 1e, Liang et al., 2019).

Consistent with our first hypothesis, biochemical selection was more sensitive to P addition in the multispecies forest than in the monoculture forest. In the monoculture forest, where plant substrates contain a high complexity of lignin compounds and a high extent of lignin decomposition, better soil nutrient conditions for microbial communities might occur with P addition. Microbial communities mediated biochemical selection through the shift of community composition from fungi towards bacteria (especially G^+ bacteria), and mainly decreased microbial-derived C content by utilizing fungal MRC as a C source (Fig. 7). In comparison, P addition modified plant community composition in the multispecies forest, where soil P availability was higher and plant substrates were more diverse than the monoculture forest. Lower complexity of plant substrate compounds existed in the multispecies forest soil and the shift of microbial nutrient acquisition towards N source was driven by P addition, thus leading to microbial decomposition of both plant- and microbial-derived C compounds in the multispecies forest (Fig. 7).

Microbial-derived C played an active role in biochemical selection, aligning with our second hypothesis. Fungal MRC was consumed as either a C or N source in the monoculture and multispecies forest, respectively. Regarding the contribution of fungal residues to soil aggregate stability (Cao et al., 2023), the close association between fungal MRC and MAOC underscored the significance of fungal MRC content in maintaining SOC stability in tropical forests (Fig. 2c,d). Soil TN content exhibited a strong correlation with SOC content in tropical forests (Fig. 5,6). In the multispecies forest, the addition of P accelerated the decomposition of soil organic nitrogen, such as fungal MRC, and mobilized N was prone to leaching, leading to significant soil N loss in the studied area (Mao et al., 2022). The observed soil N loss with P addition in the multispecies forest suggested a potential negative impact of chronic application on SOC content and stability in tropical forests.

4.2 SOC content and stability in response to P addition in tropical forests

Partially inconsistent with our third hypothesis, both the meta-analysis and the field experiment in this study found a neutral response of SOC content to P application in tropical forests. A latest meta-analysis reported that tropical forest soil C sink had positive feedback to increased P application (Feng et al., 2023). The discrepancy between the previous meta-analysis and this study might come from different

405 data pools, especially data with chronic nutrient addition scenarios. First, latitudes of 30°S - 30°N for
406 tropical and subtropical forests were considered in our study compared to 23.5°S - 23.5°N in Feng's work.
407 Second, our study focused on bulk SOC content (n = 125) and Feng's work included SOC components
408 (n = 56) as target data. SOC content in the plantation of N_2-fixing species (*Acacia* spp.) indeed had a
409 positive response to P addition in our meta-analysis (Fig. 6a), but it was in contrast to our field experiment.
410 The divergent responses of SOC content to P addition between shorter- and longer-term P addition
411 experiments might explain the contrasting results. Our meta-analysis found a significant and negative
412 response of SOC content to longer duration (>5 years) of P addition in tropical forests (Fig. 6a),
413 suggesting that soil C loss might outpace C input in tropical forest soils with chronic P addition. Out of
414 12 observations for *Acacia* forests there was only one observation (8-year addition) considered with
415 longer duration (>5 years, Fig. 6a). Meanwhile, the *Acacia* plantation in our field experiment has been
416 fertilized for 10 years. Contrasting to higher SOC accumulation with a longer duration of P input on the
417 global scale (Luo et al., 2023), the result of this study highlighted the ecosystem-specific response of soil
418 C pool and suggested the susceptibility of SOC preservation to chronic P application in tropical forests.
419 The neutral response of SOC content to P application in our study suggested that C input was offset by
420 C loss with P addition in tropical forests. SOC accumulation by P addition in P-poor tropical forests is
421 normally due to increased plant production and growth (Cunha et al., 2022; Hou et al., 2020). Activated
422 plant-soil-microbial interactions achieved a net C flow from plant to soil and thus SOC accumulation.
423 However, broad generalization of P benefit on plant productivity in tropical forests is challenging, owing
424 to confounding variations in tree species composition and both climatic and edaphic factors (Waring et
425 al., 2019; Wright et al., 2018). Neither aboveground nor belowground biomass was increased by P
426 addition in two forests (Huang et al., 2023; Fig. S5), and no responses of leaf photosynthetic capacity to
427 P addition in the multispecies forest (Mo et al., 2019) suggested stable leaf C fixation into the system.
428 Mineralization of SOC drives the outflow of soil C, it can be enhanced by P addition in tropical forests
429 associated with increased soil respiration (Johnston et al., 2019; Liu et al., 2019). In the current field
430 experiment, soil microbial respiration might be stimulated, since microbial acquisition for C and N
431 sources were activated by P addition in the monoculture and the multispecies forest, respectively (Fig.
432 3).
433 Regarding SOC fractions, no correlations between plant-derived C compounds and POC in the *Acacia*
434 plantation seemed to contradict previous notions that POC mainly consists of plant-derived C (Cotrufo

et al., 2019). Disproportionate preservation of plant-derived C compounds into POC and MAOC (Angst et al., 2021) is a plausible reason for the poor association between plant-derived C and POC in the *Acacia* plantation. Loose linkages among plant-derived C compounds and MAOC in the *Acacia* plantation indicated that biochemical selection of plant-derive C had less contribution to SOC stability (Fig. 7). MAOC is considered more stable than POC because it is formed with microbial-transformed organic matter and protected by mineral association (Cotrufo et al., 2019). In comparison, plant-derived C compounds were major parts of MAOC in the multispecies forest, as they could be preserved in stable C pools after microbial transformation (Angst et al., 2021). Significant associations among C compounds with biotic origin and MAOC indicated that biochemical selection of both plant- and microbial-derive C contributed to SOC stability in the multispecies forest (Fig. 7).

This study primarily examines the impact of P addition on plant substrates, microbial community composition, and soil enzymatic activity in tropical forests. However, we acknowledge N as a crucial driver of microbial processes and biochemical selection for SOC preservation, despite the lack of significant effects of N addition on SOC compounds in field experiments. Previous studies in multispecies forests have shown increased fungal microbial residues (MRC) and glomalin-related soil protein (Zhang et al., 2023)with N addition, alongside reduced soil phenol oxidative enzyme activities (Li et al., 2023) , suggesting enhanced surface SOC accumulation. Similarly, in *Acacia* plantations, depth-dependent responses of MRC (Fan et al., 2024) and delayed litter biomass decomposition under N addition (Zhu et al., 2016), imply a regulatory role of N application in SOC preservation.

It is important to note limitations in quantitatively estimating plant- and microbial-derived carbon. Certain proteinaceous and aromatic compounds, such as proteins and root exudates, which may be preferentially preserved in the soil, were not measured in this study (Kögel-Knabner, 2002), potentially leading to an underestimation of their contribution to SOC preservation. Additionally, the conversion factor for microbial necromass C calculation varies among microbial communities (Joergensen, 2018), , possibly affecting the response of microbial necromass to nutrient addition. Future studies should adopt combined approaches, such as novel molecular fingerprinting techniques with isotopic tracers, across various soil contexts (Whalen et al., 2022).

5 Conclusion

Soil, composed of carbon fractions with diverse biotic and abiotic origins, exhibits forest-specific

responses of SOC compounds to P application in monoculture and multispecies tropical forests. Microbial composition and activity play a key role in biochemical selection for SOC preservation. In *Acacia* plantations with low-quality plant substrates, P addition shifts soil microbial community composition towards bacteria, particularly Gram-positive bacteria, while utilizing fungal MRC as a C source. In contrast, the multispecies forest, with lower plant substrate complexity, shows a shift in microbial nutrient acquisition towards N sources under P addition. The presence of microbial communities consuming both plant- and microbial-derived carbon compounds in the multispecies forest suggests greater sensitivity to P addition compared to the monoculture forest. Close associations among POC, MAOC, and fungal MRC highlight the significance of fungal MRC content in SOC stability. The neutral response of bulk SOC content to P addition across tropical forests suggests a balance between soil C input and output. However, continuous P application may potentially convert the tropical forest SOC pool into a carbon source. These findings offer valuable insights into forest-specific mechanisms of SOC preservation under P application in tropical forests, emphasizing the need for comprehensive investigations of ecosystem C cycling to inform tropical reforestation planning.

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Competing Interests

The authors declare that they have no competing interests.

Supplementary Information

Supplementary information is available online for this paper.

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Table 1 Soil and plant properties and their significance of the difference between control treatments in the *Acacia* plantation and the multispecies forest. SOC, soil organic carbon; POC, particulate organic carbon; MAOC, mineral-associated organic carbon; TN, total nitrogen; TP, total phosphorus; lignin S:V, ratio of syringyls to vanillyls in lignin; lignin C:V, ratio of cinnamyls to vanillyls in lignin; (Ad:Al)v, ratio of acid to aldehyde in vanillyls; (Ad:Al)s, ratio of acid to aldehyde in syringyls; G+:G-, ratio of Gram-positive bacteria to Gram-negative bacteria; AMF proportion, proportion of arbuscular mycorrhizal fungi.

Soil characteristics	Forest		*P* value
	Acacia plantation	Multispecies forest	
log(SOC)	1.41(0.03)	1.18(0.01)	< 0.001
log(POC)	0.86(0.04)	0.45(0.04)	< 0.001
log(MAOC)	1.22(0.03)	1.11(0.03)	0.007
POC:MAOC	0.30(0.01)	0.19(0.01)	0.005
TN	1.78(0.18)	1.61(0.11)	0.368
log(TP)	2.26(0.01)	2.37(0.03)	0.023
Soil pH	3.72(0.03)	3.99(0.05)	< 0.001
SOC:TN	14.97(1.01)	9.86(0.79)	< 0.001
TN:TP	9.86(1.03)	6.94(0.63)	0.036
sqrt(lignin S:V)	0.73(0.03)	0.76(0.02)	0.515
sqrt(lignin C:V)	0.489(0.02)	0.762(0.02)	< 0.001
(Ad:Al)v	0.21(0.02)	0.13(0.01)	< 0.001
(Ad:Al)s	1.06(0.03)	0.88(0.04)	0.003
C acquisition	0.42(0.03)	0.29(0.03)	0.005
P acquisition	81.44(1.00)	76.18(1.65)	0.026
Fungi:Bacteria	0.10(0.01)	0.14(0.00)	< 0.001
G^+:G^-	1.80(0.09)	1.81(0.05)	0.893
AMF proportion	0.02(0.00)	0.03(0.00)	0.214

Fig. 1 Relative changes of soil organic carbon (a), mineral-associated organic carbon (b), particulate organic carbon (c), bacterial necromass (d), fungal necromass (e), cutin and suberin (f), lignin (g) and fatty acids (h) by nitrogen, phosphorus and both nutrient addition treatments in the *Acacia* plantation and the multispecies forest, respectively. The significance of the difference between the relative change by certain fertilization treatments and zero is analyzed and noted as "*": $p < 0.05$, "**": $p < 0.01$.

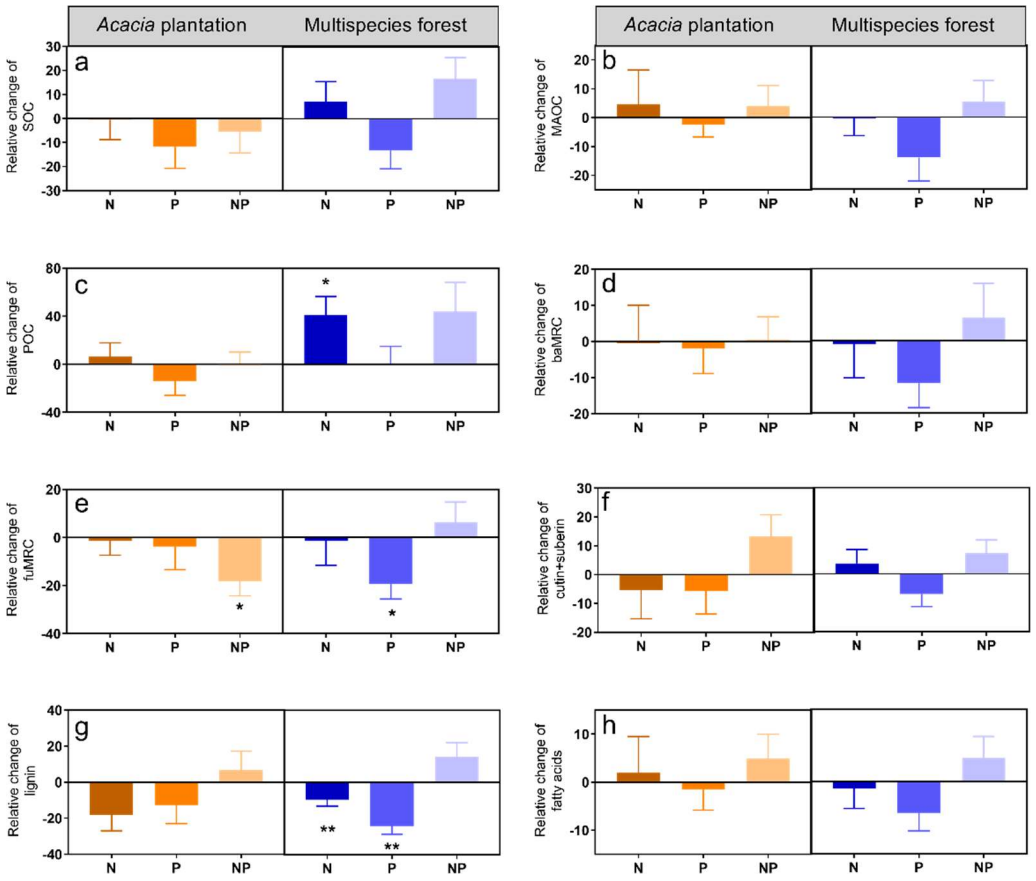


Fig. 2 Linear correlations between soil carbon components with different biotic origins and soil organic carbon, particulate organic carbon, and mineral-associated organic carbon in the Acacia plantation (a,c,e,g, i) and the multispecies forest (b,d,f,h,j), respectively. The straight lines and dashed lines indicate the mean and 95% confidence interval of the slope, respectively. SOC, soil organic carbon; MAOC, mineral-associated organic carbon; POC, particulate organic carbon; baMRC, bacterial necromass and fuMRC, fungal necromass.

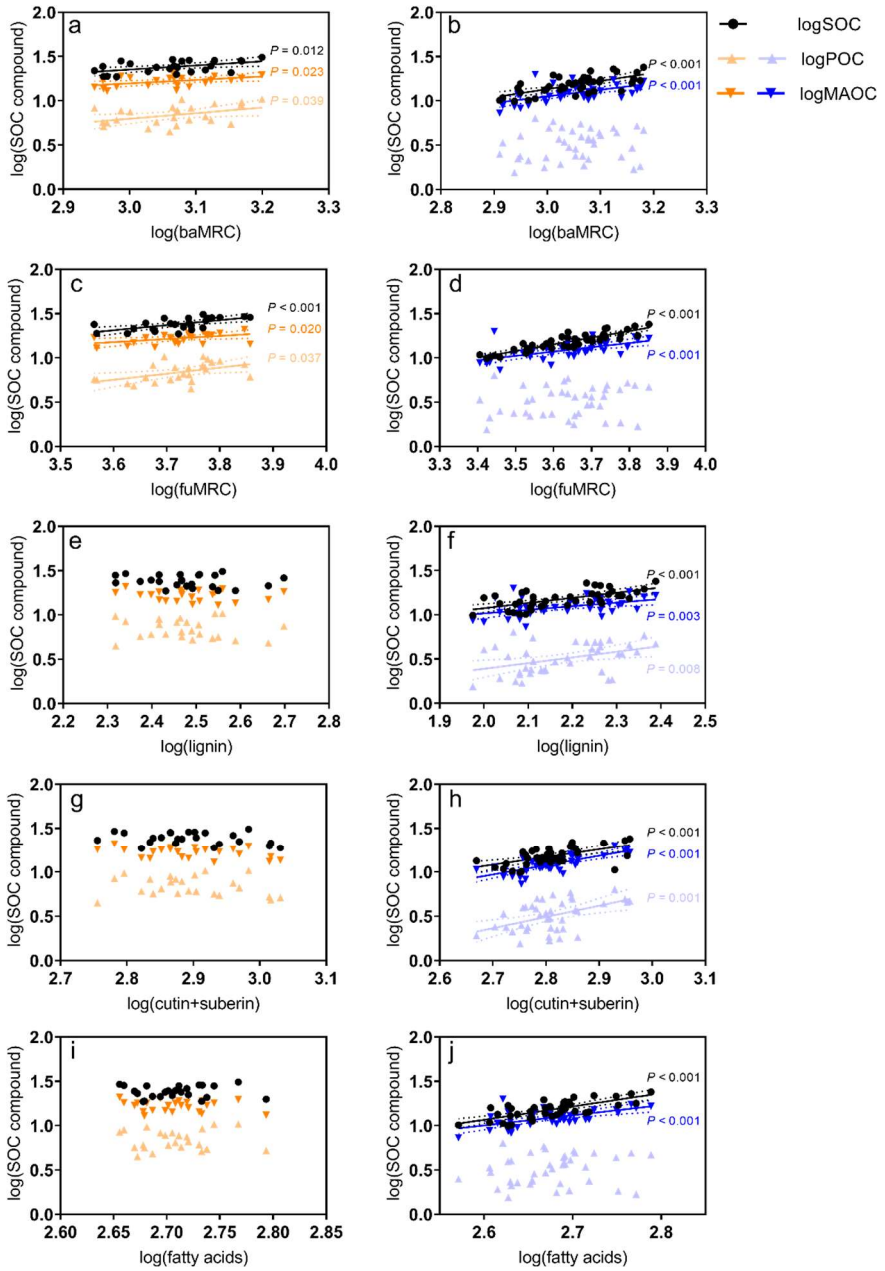


Fig.3 Relative changes (%) of β-1,4-glucosidase activity (a), β-*N*-acetyl-glucosaminidase activity (b), acid phosphatase (c), microbial carbon acquisition (d), microbial phosphorus acquisition (e), total phospholipid fatty acids (f), Gram-positive bacteria: Gram-negative bacteria ratio (g), arbuscular mycorrhizal fungi proportion (h) and fungi: bacteria ratio (i) by nitrogen, phosphorus and both nutrient addition in the *Acacia* plantation and the multispecies forest, respectively. The significance of the difference between the relative change by certain fertilization treatments and zero is analyzed and noted as "*": $p < 0.05$, "**": $p < 0.01$.

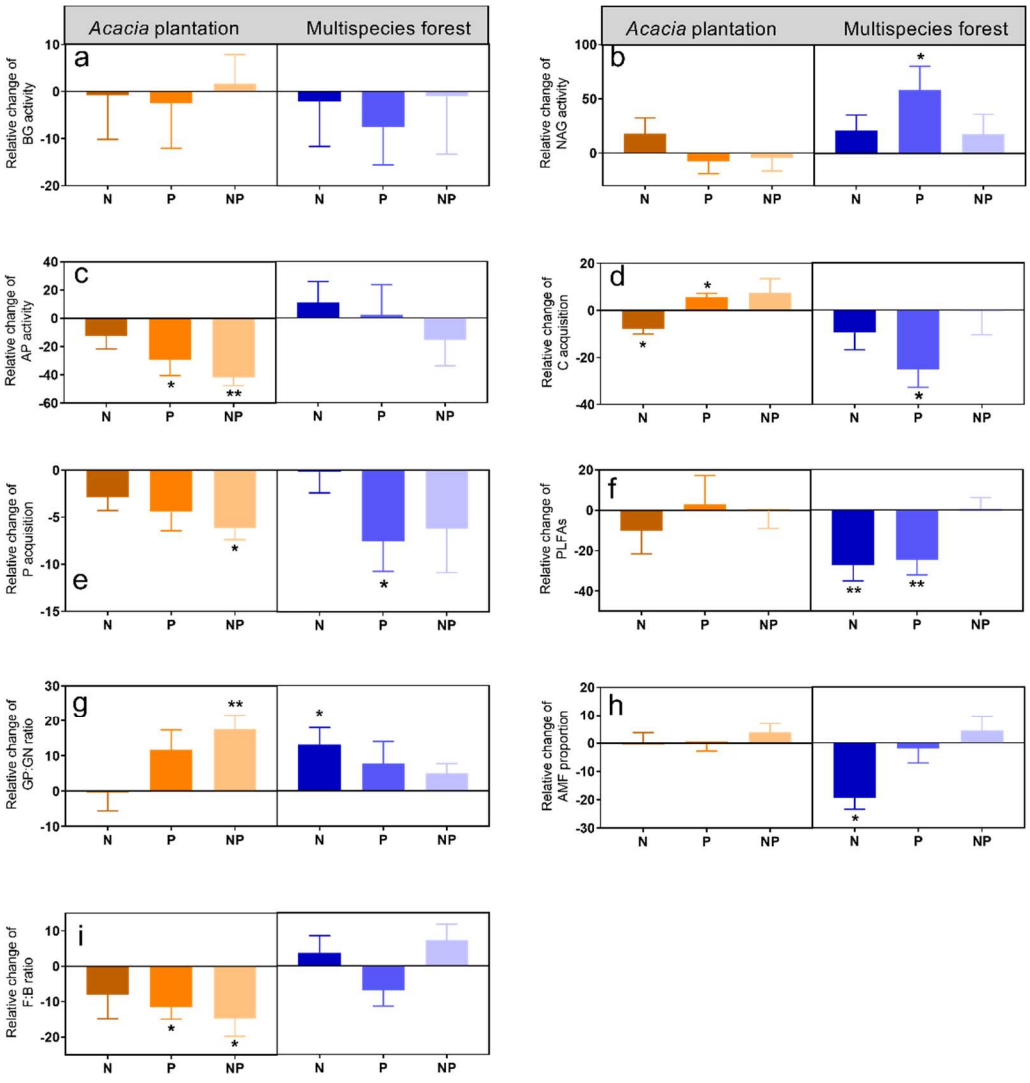


Fig. 4 Relative changes (%) of soil total nitrogen (a), total phosphorus (b), pH value (c), soil moisture (d), dissolved organic carbon (e), inorganic nitrogen (f), and soluble phosphorus (g) by nitrogen, phosphorus and both nutrient addition in the *Acacia* plantation and the multispecies forest, respectively. The significance of the difference between the relative change by certain fertilization treatments and zero is analyzed and noted as "*": $p < 0.05$, "**": $p < 0.01$.

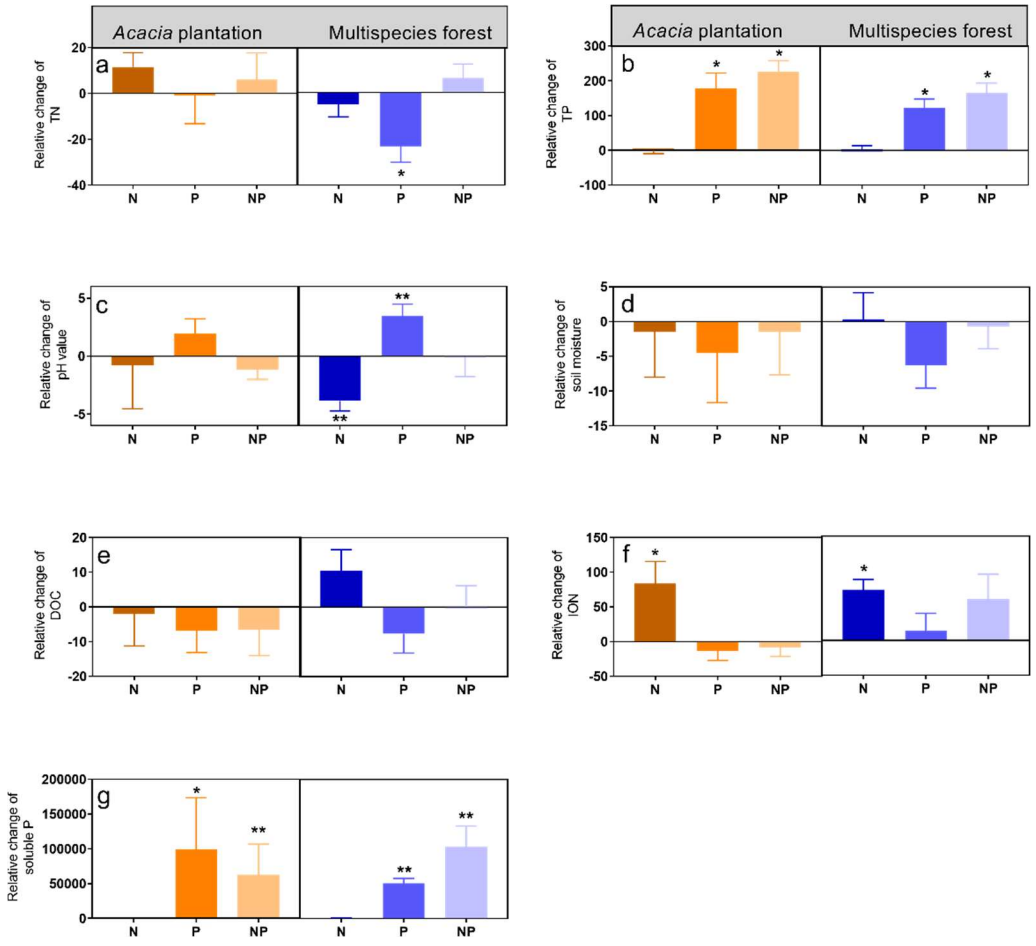


Fig. 5 The average predictive importance for soil carbon compounds, and abiotic and biotic properties for total soil organic carbon in the *Acacia* plantation (a) and the multispecies forest (b), respectively. InMSE%, the percentage increase of mean squared error; fuMRC, fungal necromass; baMRC, bacterial necromass; POC, particulate organic carbon; MAOC, mineral-associated organic carbon; TN, total nitrogen; TP, total phosphorus; DOC, dissolved organic carbon; ION, inorganic nitrogen; PLFA, phospholipid fatty acid; G^+:G^-, Gram-positive bacteria: Gram-negative bacteria ratio; lignin C:V, ratio of cinnamyls to vanillyls in lignin; (Ad:Al)v, ratio of acid to aldehyde in vanillyls; BG activity, β-1,4-glucosidase activity and NAG activity, β-*N*-acetyl-glucosaminidase activity. The brown bar is the soil carbon compound, the pink bar is the biotic property and the blue bar is the abiotic property. The significances are noted as "*": $p < 0.05$, "**": $p < 0.01$.

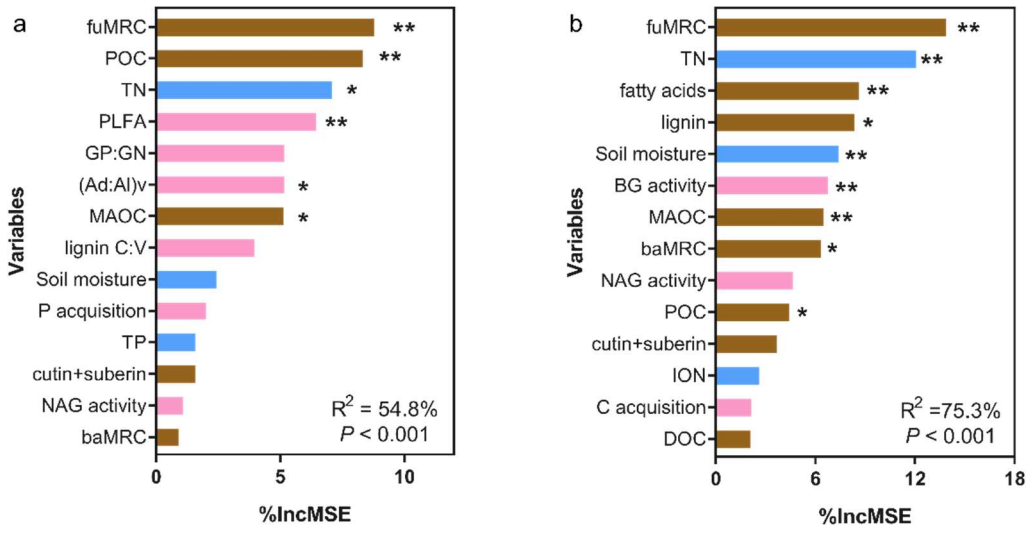


Fig. 6 Effects of phosphorus (P) addition on soil organic carbon (SOC) in relation to its moderators (a) and correlations among the responses of soil total nitrogen (TN, b) and the response of SOC. The dashed line represents the effect size = 0. Numbers in (a) indicate sample sizes of observations, the error bars represent 95% confidence intervals, and the significant effects of P addition are noted as "*": $p < 0.05$. The straight lines and shaded areas in (b) indicate the mean and 95% confidence interval of the slope, respectively.

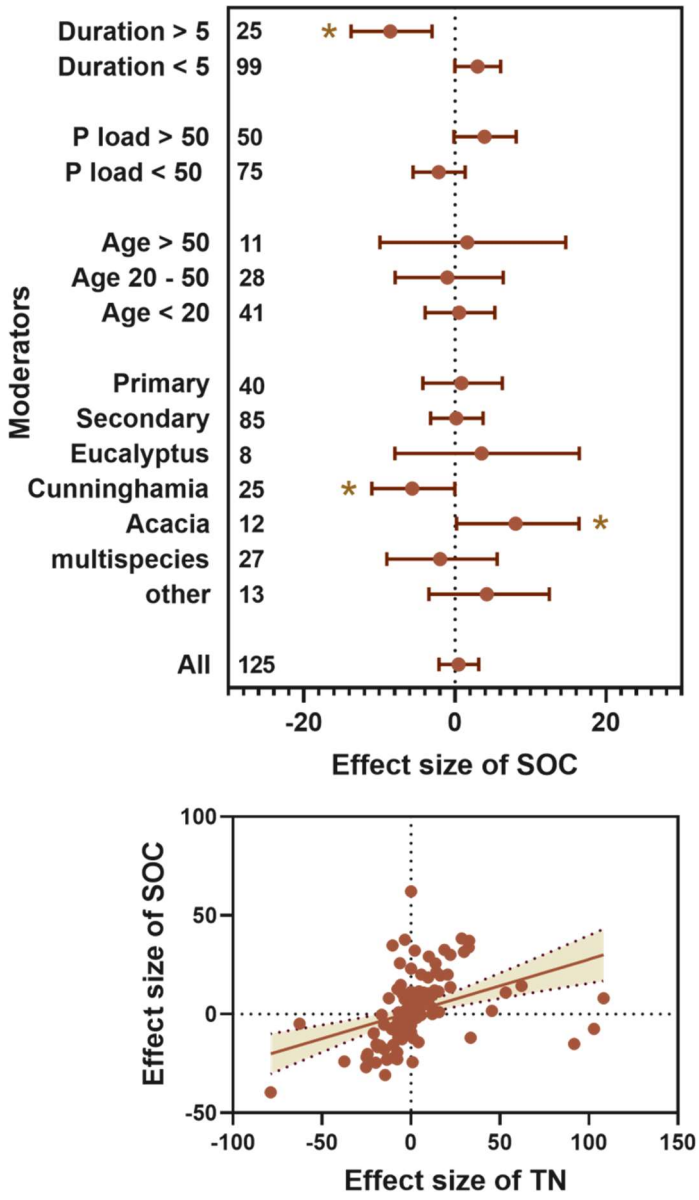


Fig. 7 A conceptual framework depicting the preserving mechanisms of soil organic carbon compounds via both plant selective preservation pathway and microbial turnover pathway under phosphorus application in the monoculture forest and the multispecies forest, respectively. (Ad:Al)v, ratio of acid to aldehyde in vanillyls; F:B ratio, ratio of fungi to bacteria; G^+:G^- ratio, ratio of Gram-positive bacteria to Gram-negative bacteria; NAG, β-N-acetyl-glucosaminidase; MAOC, mineral-associated organic carbon; POC, particulate organic carbon.

