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Nitrogen addition has divergent effects on phosphorus fractions in four types of soils

Ping Zeng¹, Qiong Zhao^{1*}, Jia-yu Hu¹, Xiang Zhang¹, Bing Mao², Qing-ye Sun¹ and Wen-ge Wu^{1,3,4*}

Abstract

Background Globally increasing atmospheric nitrogen (N) deposition has altered soil phosphorus (P) transformations and availability, and thereby influenced structure and function of terrestrial ecosystems. Edaphic characteristics and chemical form of deposited N could be important factors determining impacts of N deposition on soil P transformations, yet the underlying mechanisms remain largely unknown. Objectives of this study were to examine how mineral-N and amino N differently affect P fractions, and identify key soil properties determining N addition impacts on soil P transformations. Considering that amino N is an important component of deposited N and forest soils vary greatly in different regions, the results of present study can guide the management of forests across different soils under ongoing N deposition scenarios.

Methods We conducted a 60-day laboratory experiment to investigate the effects of N addition (NH_4NO_3 and glycine) on soil P fractions and related biochemical properties in four representative forest soils (brown, yellow brown, aeolian sandy, and red soils) in China. Glycine and NH_4NO_3 were separately added at three rates (5, 10 and 20 g N m⁻² yr⁻¹).

Results Firstly, the percent changes in organic P fractions with N addition were significantly greater than changes in inorganic P fractions across all soils. Secondly, the percent changes in P fractions with glycine and NH_4NO_3 additions were significantly correlated across all soils and treatments. However, glycine addition had significantly greater impacts on organic P fractions than NH_4NO_3 addition in the aeolian sandy and red soils with low organic carbon content. Thirdly, P fractions responded differently to N addition among the four soils. N-induced changes in microbial biomass and phosphatase activities, pH, exchangeable Ca^{2+} and Mg^{2+} contributed differently to the changes in P fractions with N addition in the four soils.

Conclusions The different responses of P fractions to N addition in the four soils were mainly generated by the differences in extent of microbial N limitation, acid buffering capacity, and cation exchange capacity among the soils. The different impacts of mineral and amino N on soil P fractions can be ascribed to their divergent effects on soil pH, microbial biomass and activities.

Keywords Acid buffering capacity, Glycine addition, Microbial activities, Phosphorus fraction, Soil type

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Background

Nitrogen (N) and phosphorus (P) are the primary limiting nutrient elements for plant growth, structure and function of terrestrial ecosystems (Deng et al. 2017). Human activities have resulted in a four-fold increase in global atmospheric N deposition since the industrial revolution, and the rate is expected to reach 195 Tg N yr⁻¹ by 2050 (Galloway et al. 2004). Impacts of chronic N input on the primary productivity of terrestrial ecosystems are quite uncertain, partially due to the complexity and uncertainty of the influences of N input on soil P availability (Deng et al. 2017; Zhou et al. 2018). Although chronic N deposition would aggravate P limitation in terrestrial ecosystems by supplying more N (Deng et al. 2017), it can also greatly change soil biochemical properties, and thereby influence soil P transformations and availability (Tian et al. 2018). Recent studies have revealed that impacts of N addition on soil P transformations depended mainly on N-induced changes in soil organic carbon (C), microbial biomass, pH, and base cations (Li et al. 2021; Zhang et al. 2022; Zheng et al. 2023). Responses of soil biochemical properties, P fractions and P availability to N addition have been widely observed, but varied greatly with edaphic characteristics, tree species, as well as the duration, form and rate of N addition (Lu et al. 2012; Deng et al. 2017). Therefore, further studies are needed to elucidate how edaphic characteristics, form and rate of N addition influence the impacts of N addition on soil P transformations and availability.

Differences in edaphic characteristics could be an important factor responsible for the inconsistent N addition impacts on soil biochemical properties and P fractions across different studies. For example, N addition inhibited soil microbial biomass and C mineralization and raised soil organic C in many N-rich forests, due to increased litterfall (Lu et al. 2021), while the opposite impacts occurred in some N-limited forests (Boot et al. 2016). N-induced soil acidification has been widely observed worldwide, the degree of acidification being correlated with the buffering capacity of metal cations (Ca²⁺, Mg²⁺ and K⁺) against soil acidification (Lu et al. 2015; Tian and Niu 2015).

Mechanisms and main pathways by which N addition influences soil P transformations could be varied in different soils (Li et al. 2021; Zhang et al. 2022; Zheng et al. 2023). For example, N deposition reduced soil labile P concentration in Hap-Boric Luvisol in a temperate *Larix gmelinii* plantation, owing to reduced soil microbial activities and increased plant uptake of P (Yang et al. 2015). In contrast, N addition did not affect or even increased labile P concentration in oxisols and ferrosols in tropical forests, due to reduced contents of Fe and Al oxides, increased soil phosphatase activities,

plant roots and litterfall production (Lu et al. 2012; Fan et al. 2019; Li et al. 2021). Previously, tests of N addition impacts on soil P fractions were mainly conducted at single site with contrasting climatic and edaphic conditions and vegetation. It is hard to separate the influences of these multiple factors on the impacts of N addition. To eliminate the interference of other factors and clarify how edaphic characteristics interact with N addition to affect soil P, N addition experiments across multiple sites or on different types of soils are required.

Chemical form and rate of N addition may also be important factors influencing N addition impacts on soil P transformations, but knowledge on this issue is scarce. Atmospheric deposited N consisted of approximately 70% inorganic N (nitrate and ammonium) and 30% organic N (urea, peptides, free amino acids and amines) (Cornell 2011). Organic N sometimes can make up to 50% of total deposited N in some regions of China (Zhang et al. 2012). Plants and microorganisms can directly absorb low-molecular-weight amino acids, such as glycine (Näsholm et al. 1998; Yang et al. 2021). In previous N deposition simulation experiments, N was mainly added as nitrate (NO₃⁻-N), ammonium (NH₄⁺-N), or urea, and glycine was seldom considered. Glycine differed from inorganic N and urea in affecting soil NO₃⁻-N production and N immobilization, and thus in affecting soil pH, microbial activities, and other biochemical properties related to P transformations (Inagaki and Miura 2002; McFarland et al. 2010). Compared to inorganic N, glycine can be more rapidly assimilated by microorganisms, and thus glycine addition has more positive effects on microbial biomass and enzyme activities than inorganic N addition (Lv et al. 2013; Yang et al. 2021).

To examine how mineral-N and amino N differently affect soil P fractions, and identify the main soil properties determining the N addition impacts on soil P transformations, a 60-day laboratory N addition experiment was conducted. Four representative forest soils with a gradient of fertility and acid buffering capacity were used. N was added as NH₄NO₃ and glycine with three rates. Field N addition experiments have been conducted previously at all these sites, and responses of soil P fractions have been examined (Yang et al. 2015; Zhang et al. 2022; Zheng et al. 2023). The present laboratory study eliminated the litterfall and root activities, thus can clearly reveal how N addition affect P transformations via its direct influences on soil biochemical properties. Furthermore, laboratory study on different soils eliminated the disturbance of vegetation and climate, which can clearly reveal the role of edaphic characteristics in mediating the N addition impacts. Comparison between the results of present laboratory study with previous field work can

help us deeply understand the N addition impacts on soil P transformations.

Soil P in different chemical fractions, and related soil microbial and physicochemical properties were examined at the end of the incubation. We hypothesized that: (1) N addition impacts on organic P fractions would be different in N-rich and N-poor soils, because its impacts on soil microbial biomass and activities differed in these soils as discussed above (Boot et al. 2016; Lu et al. 2021); (2) N addition impacts on inorganic P fractions would be greater in soils with lower acid buffering capacity, since soil pH is the predominant factor controlling soil inorganic P transformations and its response to N addition is highly dependent on soil acid buffering capacity (Lu et al. 2015; Tian and Niu 2015); and (3) glycine addition would have greater impacts on soil organic P fractions than NH_4NO_3 addition, since glycine addition had already been found to influence soil microbial activities to a larger degree (Lv et al. 2013).

Materials and methods

Site description and soil sampling

Soils from four forest sites in China were collected for laboratory N addition experiment. Reasons for selecting these sites are that soils at these sites are representative forest soils in China, and have contrasting fertility, acid buffering capacities and microbial activities (Table 1). Also, all the study sites had been exposed to severe ambient atmospheric N deposition since 1980s, and were free

of fertilization (Tian et al. 2017; Xu et al. 2018; Zhao and Zeng 2019; Huang et al. 2020). The sites are located across temperate to subtropical climate zones. The soils at the four sites are brown soil (Udalfs), yellow brown soil (Udalfs), aeolian sandy soil (Entisol), and red soil (Ultisol, U.S. soil taxonomy).

In May 2022, 30 soil cores (0–20 cm) were taken with a 10 cm diameter soil corer and homogenized into one sample in each forest stand. At the same time, field soil bulk density was measured using the cutting ring method (Lu 2000). Field soil samples were transported to the laboratory in a cooler box, and sieved to pass through a 2 mm mesh to remove roots and other debris. Soil biochemical properties, acid-base properties and P fractions were determined prior to the laboratory N addition experiment. Every soil sample was divided into two subsamples, one subsample was kept at 4 °C for the measurement of acid phosphatase activities (APase), microbial biomass C and P (C_{mic} and P_{mic}), ammonium ($\text{NH}_4^+\text{-N}$), and nitrate ($\text{NO}_3^-\text{-N}$) within 7 days. Another subsample was air-dried for the determination of P fractions, organic C, total N, total P, pH, exchangeable base cations (K^+ , Na^+ , Ca^{2+} , Mg^{2+}), cation exchange capacity (CEC) and acid buffering capacity.

Laboratory N addition experiment

A laboratory N addition experiment was conducted to examine impacts of chemical form and rate of N addition on soil P transformations in different forest soils.

Table 1 Characteristics of the four forest stands used for the sampling of four types of soils

| Soil type | Brown soil | Yellow brown soil | Aeolian sandy soil | Red soil |
|---|-----------------------|--------------------------------|---|--------------------------------|
| Site physiography | | | | |
| Tree species | <i>Larix olgensis</i> | <i>Cunninghamia lanceolata</i> | <i>Pinus sylvestris</i> var. <i>mongolica</i> | <i>Cunninghamia lanceolata</i> |
| Location | 41°51'N, 124°54'E | 30°08'N, 117°02'E | 42°54'N, 122°25'E | 30°16'N, 116°14'E |
| Annual average temperature (°C) | 3.9–5.4 | 17.4 | 6.0 | 16.8 |
| Mean annual precipitation (mm) | 700–850 | 1600 | 450 | 1398 |
| Soil properties | | | | |
| Clay:silt:sand ratio | 23:51:26 | 21:45:34 | 4:5:91 | 7:29:64 |
| Organic C (g kg^{-1}) | 46.62 | 28.74 | 18.11 | 4.64 |
| Total N (g kg^{-1}) | 2.72 | 1.56 | 0.83 | 0.23 |
| Total P (g kg^{-1}) | 3.44 | 0.31 | 0.20 | 0.18 |
| C:N ratio | 17.17 | 18.39 | 21.87 | 20.48 |
| pH | 5.45 | 4.38 | 5.40 | 4.90 |
| Acid buffering capacity (mmol kg^{-1}) | 45.45 | 24.33 | 14.58 | 11.29 |
| N deposition rate ($\text{kg N ha}^{-1} \text{yr}^{-1}$) ^a | 17.0–21.4 | 10.6–18 | 18.8 | 33.2 |

^a Ambient atmospheric N deposition rate of the four study sites are derived from (Tian et al. 2017; Xu et al. 2018; Zhao and Zeng 2019; Huang et al. 2020)

Two forms of N (NH_4NO_3 and glycine) were added separately at three rates (corresponding to $5 \text{ g N m}^{-2} \text{ yr}^{-1}$; $10 \text{ g N m}^{-2} \text{ yr}^{-1}$; $20 \text{ g N m}^{-2} \text{ yr}^{-1}$ in the field) to the four soils. Each treatment was triplicated. Amount of N (g N kg^{-1} dry soil) added to the incubated soil was calculated according to the following equation.

$$N(\text{gNkg}^{-1}\text{drysoil}) = \frac{N_{\text{rate}}}{\text{BD} \times \text{Depth}} \times 100 \quad (1)$$

where N_{rate} is the corresponding filed N addition rate ($\text{g N m}^{-2} \text{ yr}^{-1}$); BD is soil bulk density (g m^{-3}). The bulk density of the four types of soils was 1.31 g cm^{-3} in the brown soil, 1.29 g cm^{-3} in the yellow brown soil, 1.50 g cm^{-3} in the aeolian sandy soil, and 1.28 g cm^{-3} in the red soil. Depth is the depth of the soil (cm), which was assumed as 20 cm in the present study.

Within 3 days of soil collection, field moist soil sample equivalent to 100 g of oven-dried soil was preincubated for 5 days, and then incubated under each treatment for 60 days at 60% of water-holding capacity at 25°C . Solution of NH_4NO_3 or glycine was sprayed to the soil every 5 days during the incubation period. At the end of incubation, soils were harvested, homogenized, and measured for soil P fractions, microbial and biochemical properties.

Soil P fractions

Soil P fractions were measured according to a modified Hedley's sequential extraction procedure (Tiessen and Moir 1993). Soil P in different chemical forms and with decreasing availability were removed sequentially with 0.5 M NaHCO_3 , 0.1 M NaOH , 1.0 M HCl , concentrated HCl , and the residue was digested with $\text{H}_2\text{SO}_4\text{-HClO}_4$. Inorganic and total P concentrations in the extracts were determined spectrophotometrically by the molybdenum antimony method directly and after acid persulfate digestion, respectively (Murphy and Riley 1962; EPA 1971; Tiessen and Moir 1993). Organic P concentrations in the extracts were calculated as the difference between total and inorganic P concentrations. A solution of 1.0 M HCl is inefficient in extracting organic matter from soils, and therefore, does not extract much organic phosphorus (Po). Total inorganic P (TPi) was calculated as the sum of inorganic P extracted in all steps and digested by $\text{H}_2\text{SO}_4\text{-HClO}_4$. Total organic P (TPo) was calculated as the sum of organic P extracted by NaHCO_3 , NaOH , and concentrated HCl .

Analyses of soil biochemical properties

Soil APase were measured using the method described by Tabatabai (1994) at $\text{pH}=6.5$, with the disodium *p*-nitrophenyl phosphate (*p*-NPP) as the substrate. Soil microbial biomass C and P were determined using the chloroform fumigation extraction procedure, with the

correction factors of 0.38 and 0.40 used to estimate the recoveries of C_{mic} and P_{mic} during fumigation (Brookes et al. 1982; Vance et al. 1987).

Soil pH was determined by a glass electrode with a soil/water ratio of 1/2.5 (*w/v*). Soil $\text{NH}_4^+\text{-N}$ was analyzed by phenol-sodium hypochlorite-indophenol blue method, and $\text{NO}_3^-\text{-N}$ was determined by spectrophotometry at dual-wavelength (220 nm and 275 nm), after the soil was extracted with 2.0 M KCl solution (Weatherburn 1967; Doane and Horwath 2003). Soil organic C was determined by the $\text{K}_2\text{Cr}_2\text{O}_7\text{-H}_2\text{SO}_4$ oxidation method (Nelson and Sommers 1996). Total N and total P were determined using Kjeldahl method and molybdate blue method following $\text{H}_2\text{SO}_4\text{-HClO}_4$ digestion, respectively (Olsen and Sommers 1983; Bremner 1996). Soil cation exchange capacity (CEC) and exchangeable base cations were measured after the soil was extracted with 1.0 M ammonium acetate. K^+ and Na^+ concentrations in the ammonium acetate extracts were measured on a flame photometer and Ca^{2+} and Mg^{2+} were determined on an atomic absorption spectrophotometer (Robertson et al. 1999). Total exchangeable base cations (TEB) is the sum of exchangeable K^+ , Na^+ , Ca^{2+} and Mg^{2+} . Base saturation (BS) was calculated as the percentage of CEC occupied by the TEB. Soil acid buffering capacity ($\text{mmol H}^+ \text{ kg}^{-1} / \text{pH unit}$) was the quantity of acidity needed to reduce pH by one unit, which was determined using titration techniques (Aitken and Moody 1994).

Statistical analyses

One-way analysis of variance (ANOVA) with Tukey's HSD multiple comparison was performed to assess the differences in soil properties among the four types of soils. The percent changes in P fraction with N addition were calculated as the difference between the ambient (no N added) treatment and the N addition treatment, to indicate the magnitude of N addition impact. Then, a three-way ANOVA was conducted to test for the effects of soil type, form and rate of N addition on the percent changes in soil variables. To compare the overall differences between N addition impacts on inorganic and organic P fractions, and between NH_4NO_3 and glycine addition impacts, independent sample *t*-test was performed on percent changes in P fractions after the data were gathered accordingly. Finally, means of soil variables were compared between each N addition treatment and the control by Dunnett's *t*-test, in each soil type separately. Levene's test was used to test for homogeneity of error variances prior to ANOVA, and non-parametric test was used when the data did not meet ANOVA assumptions. Statistical significance was set at $p < 0.05$.

Pearson's correlation and redundancy analysis (RDA) were conducted to analyze the relationships between soil P fractions and related biochemical properties, and between their percent changes with N addition. Pearson's correlations were conducted between inorganic P fractions and acid-base properties, and between organic P fractions and microbial properties, respectively. RDA with scaling II was conducted, with biochemical properties as explanatory variables, and P fractions without transformation as response variables. Monte Carlo permutation test (999 unrestricted permutations) and multicollinearity test were used for pre-selection of explanatory variables. The ANOVA and correlation analyses were performed using SPSS 24.0 (IBM, Chicago, USA), and RDA was performed using CANOCO 5.0 software.

Results

Differences in soil properties among the four types of soils

Soil biochemical properties differed significantly among the four types of soils (supplementary Table S1 and S2). Soil acid buffering capacity was highest in the brown soil (45.45 mmol kg⁻¹), and then decreased in the order of yellow brown soil (24.33 mmol kg⁻¹) > aeolian sandy soil (14.58 mmol kg⁻¹) > red soil (11.29 mmol kg⁻¹). Similarly, the brown soil had the highest and the red soil had the lowest concentrations of soil organic C, total N and P, NO₃⁻-N, APase, microbial biomass C and P, as well as acid-base properties (pH, exchangeable Ca²⁺, Mg²⁺ and K⁺, TEB, and CEC). In contrast, soil C:N ratio was lower in the brown and the yellow brown soils than in the aeolian sandy and red soils.

Distribution pattern of soil P in different fractions varied greatly among the four types of soils (Supplementary Fig. S1). Organic P dominated total P (57.37%) in the yellow brown soil, while inorganic P dominated in all the other soils (66.58–91.21% of total P). For inorganic P, HCl_{1M}-Pi was predominant in the brown and aeolian sandy soils (72.71% and 29.30% of total P), residual-P was predominant in the yellow brown soil, and HCl_{conc.}-Pi was predominant in the red soil. For organic P, NaOH-Po was predominant in all the soils except the red soil, where HCl_{conc.}-Po predominated.

Effects of N addition on soil P fractions

Three-way ANOVA showed the significant main effects and interactions of soil type, chemical form and rate of N addition on the percent changes in P fractions with N addition (Supplementary Table S3). Soil type had the greatest main effects, as it contributed much more (23.39–96.51%) to the sums of squares of the three-way ANOVA than the rate and form of N addition (0.32–67.30% and 0.50–30.02%). No general pattern was

observed for the changes in P fractions with increasing rate of N addition.

Addition of NH₄NO₃ and glycine had similar impacts on soil P fractions, as the percent changes in soil P fractions with NH₄NO₃ and glycine additions were significantly and positively correlated across all the soil types ($r=0.564$, $p=0.000$) (Fig. 1A). However, the effect of glycine addition on organic P fractions was significantly greater than that of NH₄NO₃ addition in the aeolian sandy and red soils, but not in the two other soils (Fig. 1C). Glycine addition also had greater impacts on inorganic P fractions than NH₄NO₃ addition in the red soil (Fig. 1B). Also, soil organic P fractions generally responded to N addition to a greater extent than inorganic P fractions in all soil types (Fig. 2). Not only the means of percent changes in all organic P fractions (20.81–32.29%) were significantly higher than inorganic P fractions (2.61–10.50%) in most of soils, the variation range of percent changes in organic P fractions (from -95.24% to 2.2 times) was much wider than inorganic P fractions (from 80.94% to 100.27%) in all soil types (Fig. 2). The overall responses of soil P fractions to N addition also significant differently in four soil types. Percent changes in inorganic P fractions were much larger in the yellow brown and red soils than in two other soil types, while percent changes in organic P fractions were obviously larger in the brown and red soils (Fig. 2).

Specifically, responses of soil P fractions to N addition differed greatly in the four types of soils (Figs. 3 and 4). For inorganic P fractions, NaHCO₃-Pi concentration reduced with N addition slightly in some treatments of the brown and aeolian sandy soils (by 9.33–34.96%) and reduced greatly in the yellow brown soil (39.08–56.49%), but did not change in the red soil (Fig. 3A). NaOH-Pi concentration changed slightly (<19.14%) with N addition in all the soils, with the slight increases in some treatments of the brown and aeolian sandy soils, and decreases in the yellow brown and red soils (Fig. 3B). HCl_{1M}-Pi concentration did not change with N addition in the brown soil, it was significantly raised in some treatments of yellow brown (by 33.38–100.13%) and aeolian sandy soils (by 8.94%), while it was reduced in the red soil (by 66.64–77.35%) (Fig. 3C). HCl_{conc.}-Pi tended to decrease with N addition in almost all the soils (by 4.29–13.88%) (Fig. 3D). Residual-P concentration did not change with N addition in most cases (Fig. 3E). Total inorganic P concentration significantly decreased with N addition in some treatments of the brown, yellow brown and red soils, but increased slightly in the aeolian sandy soil (Fig. 3F).

For organic P fractions, NaHCO₃-Po concentration was significantly reduced by 15.34–46.77% with N addition in the brown soil, while raised in the other three

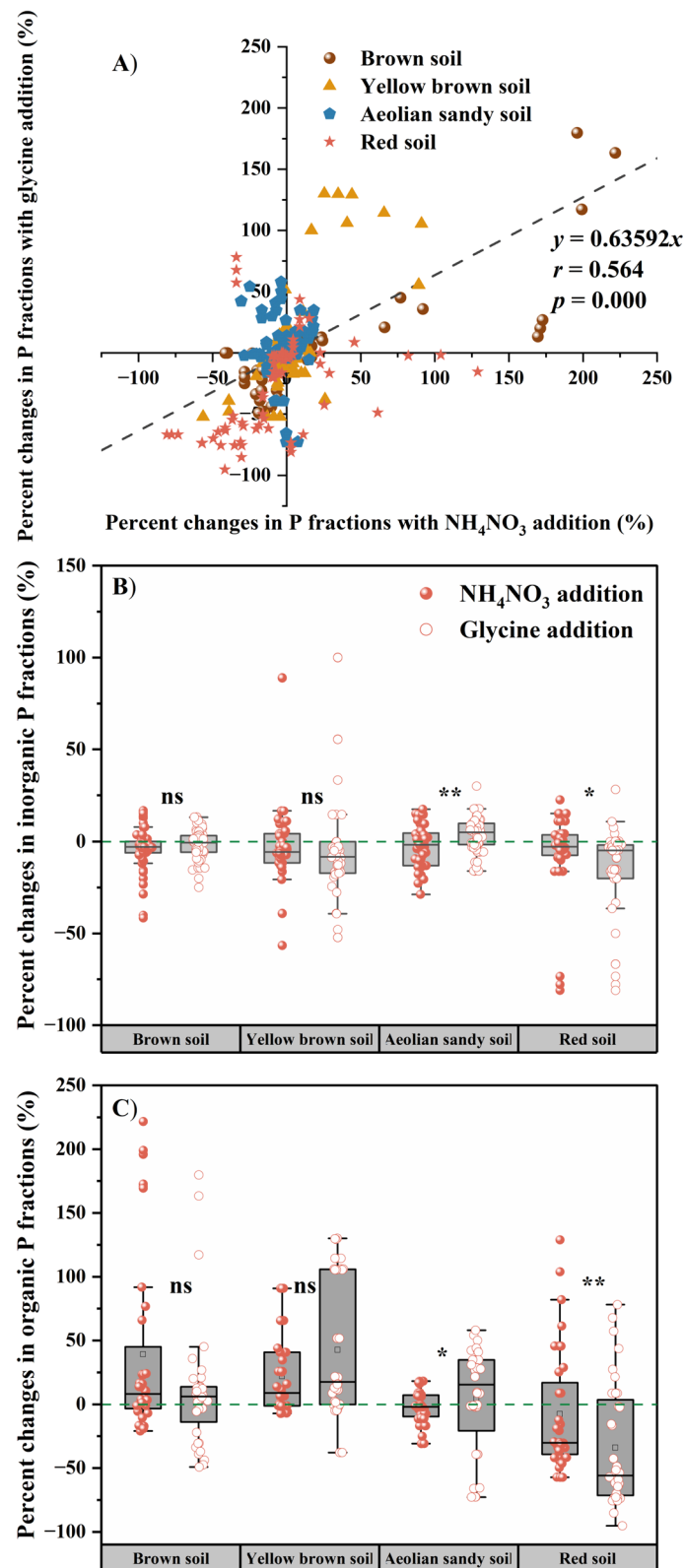


Fig. 1 Relationships and differences between NH_4NO_3 and glycine additions on percent changes in soil P fractions in the four types of soils (**A** $n=360$; **B** $n=54$; and **C** $n=36$). Asterisks * and ** indicate significant differences between NH_4NO_3 and glycine additions in each type of soils at the level of $p < 0.05$ and $p < 0.01$, ns indicates no significant effect ($p > 0.05$)

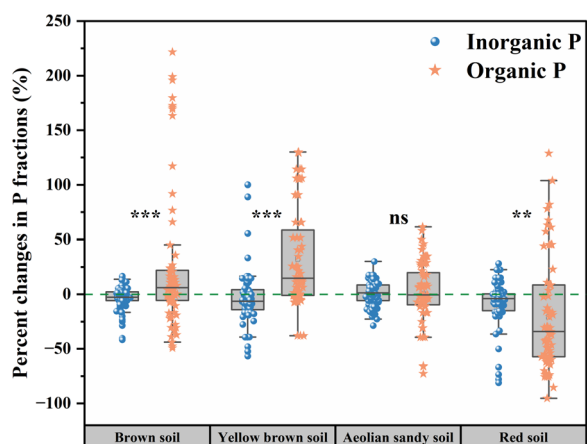


Fig. 2 Percent changes in inorganic and organic P fractions with N addition (inorganic P, $n = 108$; organic P, $n = 72$) in the four types of soils. Asterisks *, ** and *** indicate significant differences between inorganic P and organic P in each type of soils at the level of $p < 0.05$, $p < 0.01$ and $p < 0.001$, ns indicates no significant effect ($p > 0.05$)

soils, with the greatest elevation (1.14 times) in the yellow brown soil (Fig. 4A). The NaOH-Po concentration increased with N addition in the brown and aeolian sandy soils (5.68–34.19%), but decreased with N addition in the yellow brown and red soils in most of treatments (4.81–73.60%) (Fig. 4B). $\text{HCl}_{\text{conc.}}$ -Po concentration increased with N addition in the brown and yellow brown soils (51.81% – 2.06 times), while being reduced in the aeolian sandy and red soils (36.72–75.52%) (Fig. 4C). Total organic P concentration also varied significantly with N addition in all the soils, increased in the brown and yellow brown soils (11.77–23.58%), and reduced in the aeolian sandy and red soils in most treatments (11.02–62.87%) (Fig. 4D).

Effects of N addition on soil biochemical properties

N addition had different effects on the biochemical properties of the four soils, which also depended on the chemical form and rate of N addition. Glycine addition generally had greater effects on soil microbial and enzyme activities than NH_4NO_3 addition (Fig. 5). The soil APase significantly increased with glycine addition at any rate in the brown and red soils (by 17.17–47.11%), significantly changed with N addition only at the highest rate in the aeolian sandy soil, and did not respond to N addition in the yellow brown soil (Fig. 5A). Soil C_{mic} was not affected by NH_4NO_3 addition, but significantly reduced with glycine addition in the brown and yellow brown soils. NH_4NO_3 and glycine additions both significantly reduced C_{mic} (by 18.14–71.70%) in the aeolian sandy soil, and raised it (by 1.01–4.08 times) in the red soil (Fig. 5B). For soil P_{mic} , NH_4NO_3 addition significantly

reduced it in the brown and red soils in most cases, but raised it in the aeolian sandy soil, while did not affect it in the yellow brown soil. Glycine addition increased soil P_{mic} in the brown and red soils (by 19.29% – 1.33 times), and reduced it in the yellow brown and aeolian sandy soil in most cases (Fig. 5C).

N addition significantly increased soil inorganic N concentration in most treatments and soils, except that NO_3^- -N concentration did not change or even decreased with glycine addition in the yellow brown and red soils. N addition increased total N and reduced soil organic C concentrations in some cases (Table 2). Soil pH generally reduced with N addition (by 0.24–0.70 units), except that it was significantly increased with glycine addition in the red soil. Also, the N addition impacts on soil pH was weakest in the brown soil. N addition significantly reduced exchangeable Ca^{2+} and Mg^{2+} (by 6.01–15.22%) almost in all treatments of the brown soil, and in several cases of the red soil, but not in the other two soils (Table 2).

Relationships between variations in soil P fractions with biochemical properties

All inorganic P fractions were significantly and positively correlated with pH, exchangeable Ca^{2+} and Mg^{2+} ($r = 0.276$ – 0.972 , $p < 0.01$) (Supplementary Table S4), while all organic P fractions were significantly and positively correlated with APase, C_{mic} and P_{mic} ($r = 0.249$ – 0.898 , $p < 0.05$), with the exception that there were no significant correlations between $\text{HCl}_{\text{conc.}}$ -Po and P_{mic} (Supplementary Table S4).

The results of the RDA further showed the relationships between each P fraction and the environmental variables (soil acid-base properties and microbial properties), and the two axes together explained 97.51% of the variance under N addition (Fig. 6). Soil exchangeable Ca^{2+} was the main explanatory variable responsible for the variations in soil P fractions, with the highest explanation of the variance (93.3%, $p = 0.001$), followed by exchangeable Mg^{2+} (2.9%, $p = 0.001$) and organic C (0.8%, $p = 0.001$).

Pearson's correlation and RDA analyses were also conducted between the percent changes in soil P fractions and biochemical properties caused by N addition, for each soil type separately (Fig. 7 and Supplementary Fig. S2). The results showed the quite different correlations between percent changes in P fractions and biochemical properties among different soil types (Supplementary Fig. S2). The first two axes of RDA explained 51.06% to 89.69% of the total variance in the four soils. The percent changes in soil P fractions can be significantly explained by the percent changes in soil C_{mic} , APase, exchangeable Ca^{2+} and Mg^{2+} ($p < 0.05$) in the brown soil, by changes in

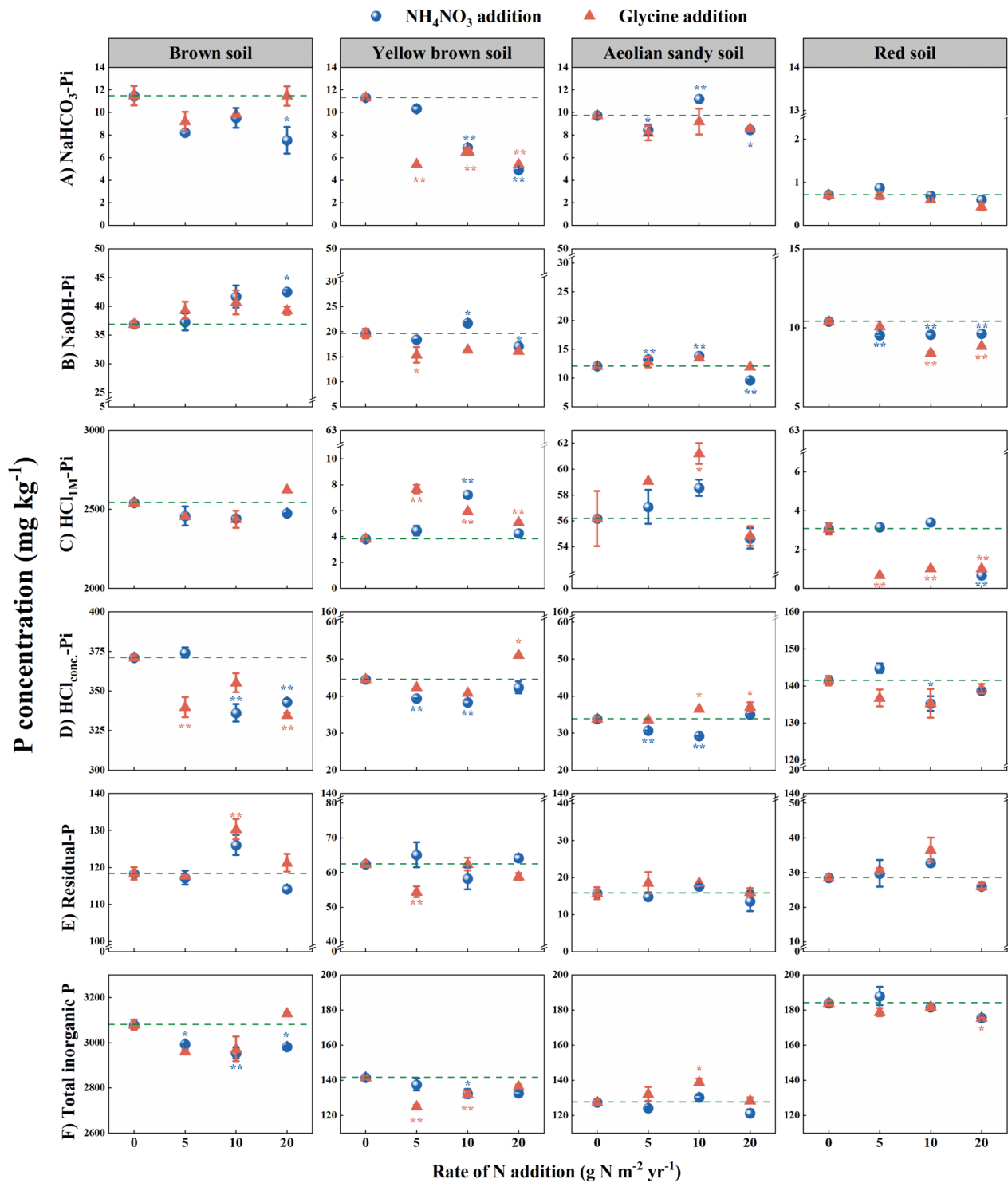


Fig. 3 Changes in inorganic P fractions with N addition in the four types of soils (Mean \pm SE, $n=3$). Asterisks * and ** indicate significant differences between N addition treatments and control in the four types of soils with Dunnett's t -test at the level of $p < 0.05$ and $p < 0.01$, respectively

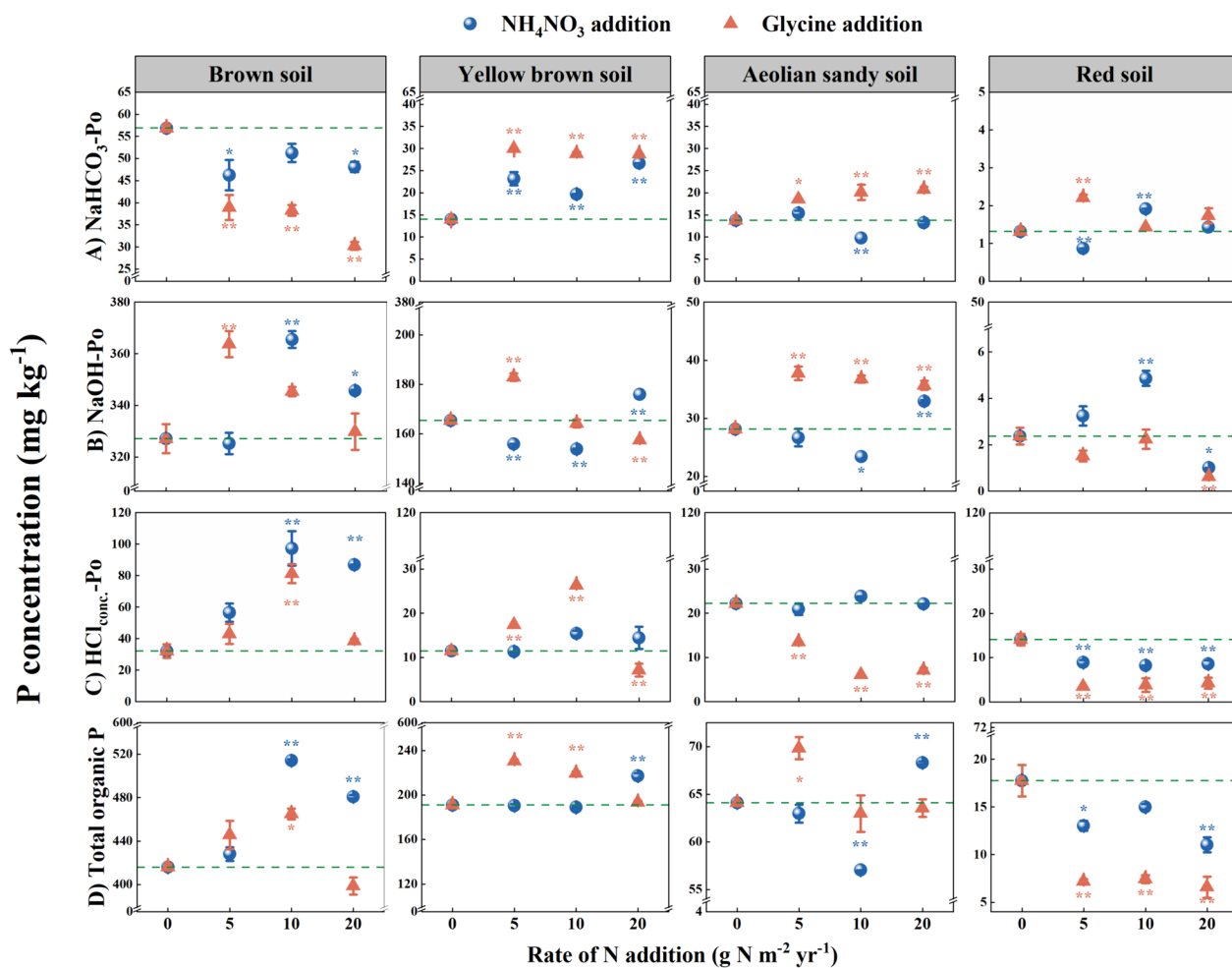


Fig. 4 Changes in organic P fractions with N addition in the four types of soils (Mean \pm SE, $n=3$). Asterisks * and ** indicate significant differences between N addition treatments and control in the four types of soils with Dunnett's t -test at the level of $p < 0.05$ and $p < 0.01$, respectively

C_{mic} , NH_4^+-N , $NO_3^- -N$, exchangeable Ca^{2+} ($p < 0.002$) and Mg^{2+} ($p = 0.013$) in the yellow brown soil, by changes in C_{mic} , P_{mic} , APase, $NO_3^- -N$, $NH_4^+ -N$ and pH ($p < 0.006$) in the aeolian sandy soil, and by the percent changes in C_{mic} , P_{mic} , APase, $NH_4^+ -N$, $NO_3^- -N$, pH and exchangeable Ca^{2+} ($p < 0.005$) in the red soil (Fig. 7).

Discussion

Greater impacts of N addition on organic P than on inorganic P fractions

Interestingly, the laboratory N addition had obviously greater impacts on soil organic P fractions than on inorganic P fractions in all the four soil types, even though inorganic P dominated soil P pool in most of soils (Fig. 2 and Supplementary Fig. S1). Under laboratory conditions, N addition influences soil P transformations by directly changing soil biochemical properties (Zhang et al. 2022). Therefore, these findings highlight the

contribution of N-induced changes in soil biochemical properties in mediating soil organic P transformation. In contrast, N deposition probably affect soil inorganic P transformations mainly through other indirect pathways (such as root activities).

Variations in microbial biomass and activity were responsible for the changes in organic P transformations, as indicated by their close correlations (Supplementary Fig. S2 and Table S4). N addition can exert both favorable and adverse effects on soil microbial biomass and acid phosphatase activities simultaneously through different pathways, and the net effects depend on which pathways predominate. Firstly, excess N addition can inhibit microbial growth, due to the toxic effects of excess NH_4^+ and NO_3^- ions on osmotic potentials and reduction in soil pH (Compton et al. 2004; Wang et al. 2018). These adverse effects occurred in the present study, since $NH_4^+ -N$ and $NO_3^- -N$ concentrations sharply increased and soil pH

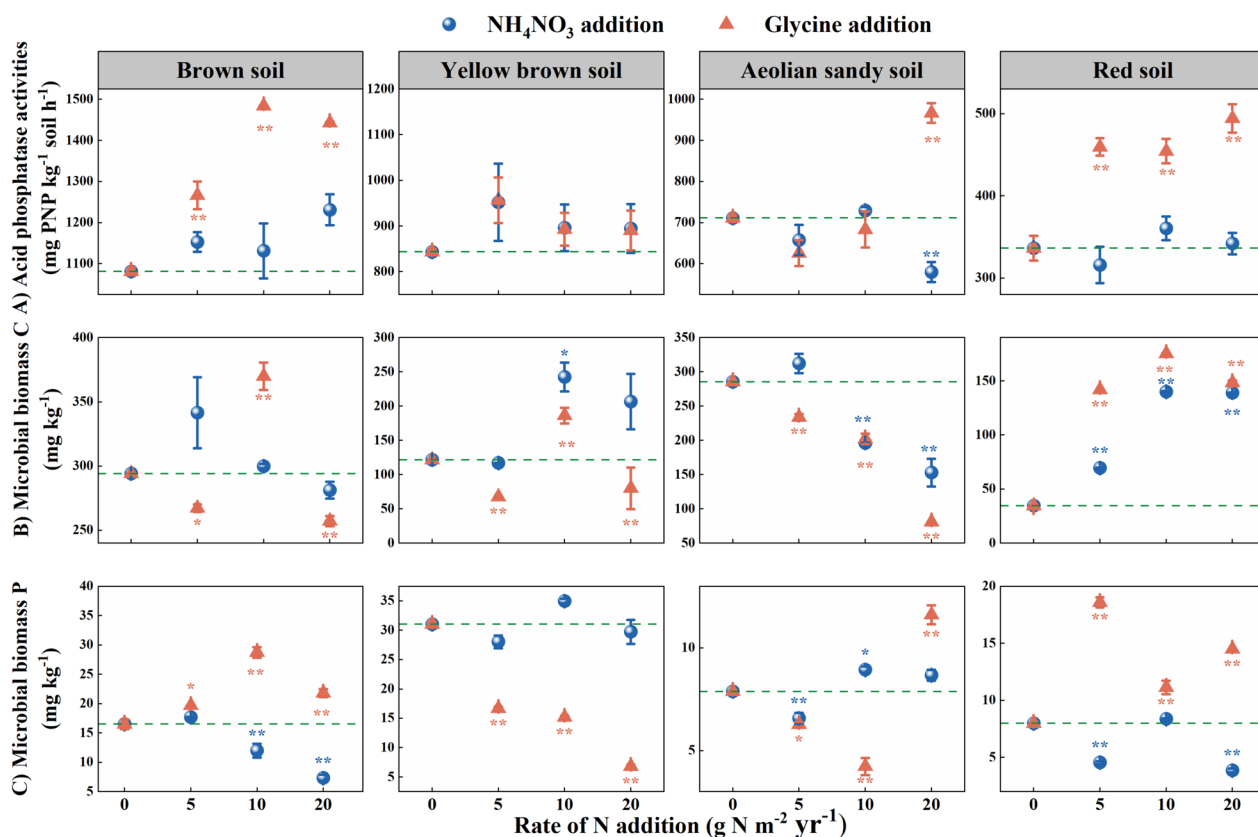


Fig. 5 Changes in soil acid phosphatase activities (A), microbial biomass C (B) and microbial biomass P (C) with N addition in the four types of soils (Mean \pm SE, $n=3$). Asterisks * and ** indicate significant differences between N addition treatments and control in the four types of soils with Dunnett's *t*-test at the level of $p < 0.05$ and $p < 0.01$, respectively

significantly reduced with N addition. N addition can also improve soil microbial biomass and promote synthesis of phosphatase activities in N limited soils (Hu et al. 2019). Although phosphatase activities and microbial biomass changed significantly with N addition in most cases, the net effects varied with soils and treatments.

In contrast to organic P fractions, inorganic P fractions responded to N addition to a less degree. Soil inorganic P fractions transform mainly through two reversible processes, i.e. adsorption/desorption of phosphate onto/from surfaces of Al and Fe oxides and soil minerals, and solubilization/precipitation of phosphate minerals (predominately calcium phosphate) (Hinsinger 2001; Shen et al. 2011). Soil pH is the principal factor controlling the both equilibriums (Helfenstein et al. 2020). This was supported by the significant correlations between pH and all inorganic P fractions in the present study (Supplementary Table S4). However, the reduction in soil pH with N addition in the present laboratory study was much lower than the corresponding field work, which could be the main reason for the slight responses of soil inorganic P fractions to

N addition. For example, soil pH reduced by less than 0.2 units in the brown and yellow brown soil in the present study, while it significantly reduced by 0.97 and 0.4 units in the corresponding field work (6 and 2 years of field NH_4NO_3 addition at $10 \text{ g N m}^{-2} \text{ yr}^{-1}$) (Hu et al. 2010; Zhang et al. 2021). Soil pH significantly reduced by 1.37 units with a 6-year of field N addition ($10 \text{ g N m}^{-2} \text{ yr}^{-1}$) in the Mongolian pine plantation on the aeolian sandy soil, which is much higher than the 0.5 units reduction in the present study (Zhang et al. 2022).

The much smaller reduction in soil pH in the present study than in the field work could be mainly because that the laboratory study excluded the impacts of root activities and leaching on acidification. Besides, in the soil incubation experiment without root uptake of phosphate, accumulation of phosphate ions in soil solution can hinder the mobilization of recalcitrant inorganic P (Grant and Heaney 1997; Bu et al. 2020). A field root exclusion experiment indicated that root P uptake and rhizosphere activities were the main drivers of mobilization of inorganic P fractions (Zhang et al. 2023). The slight responses of soil inorganic P fractions to N addition in

Table 2 Changes in biochemical properties in response to N addition in the four types of soils (Mean \pm SE, $n = 3$)

| Soil type | N addition | | Organic C (g kg ⁻¹) | Total N (g kg ⁻¹) | C:N ratio | NH ₄ ⁺ -N (mg kg ⁻¹) | NO ₃ ⁻ -N (mg kg ⁻¹) | pH | Exchangeable Ca ²⁺ (cmol kg ⁻¹) | Exchangeable Mg ²⁺ (cmol kg ⁻¹) |
|--------------------|---------------------------------|---|------------------------------------|----------------------------------|--------------------|---|---|-------------------|---|---|
| | Form | Rate (g N m ⁻² yr ⁻¹) | | | | | | | | |
| Brown soil | Control | 0 | 49.76 \pm 0.93 | 2.69 \pm 0.05 | 18.52 \pm 0.64 | 18.78 \pm 0.13 | 34.99 \pm 1.54 | 5.45 \pm 0.01 | 11.77 \pm 0.05 | 2.70 \pm 0.06 |
| | | 5 | 50.34 \pm 0.13 | 2.80 \pm 0.00 | 17.99 \pm 0.05 | 27.19 \pm 0.31** | 88.38 \pm 3.46** | 5.41 \pm 0.01 | 11.07 \pm 0.18 | 2.39 \pm 0.04* |
| | NH ₄ NO ₃ | 10 | 49.56 \pm 0.28 | 3.13 \pm 0.01** | 15.82 \pm 0.16** | 38.02 \pm 1.00** | 66.05 \pm 5.11** | 5.38 \pm 0.03 | 10.56 \pm 0.07** | 2.32 \pm 0.05* |
| | | 20 | 46.92 \pm 0.28* | 3.10 \pm 0.02** | 15.15 \pm 0.08** | 48.00 \pm 0.67** | 17.10 \pm 0.62* | 5.36 \pm 0.06 | 10.64 \pm 0.30** | 2.41 \pm 0.10* |
| | Glycine | 5 | 50.23 \pm 0.11 | 2.38 \pm 0.07 | 21.15 \pm 0.67* | 24.65 \pm 0.50* | 46.46 \pm 0.28** | 5.43 \pm 0.00 | 10.80 \pm 0.11** | 2.39 \pm 0.09* |
| | | 10 | 52.13 \pm 0.28 | 2.81 \pm 0.02 | 18.58 \pm 0.11 | 28.59 \pm 0.03** | 44.72 \pm 1.72** | 5.44 \pm 0.05 | 10.29 \pm 0.03** | 2.30 \pm 0.02** |
| Yellow brown soil | Control | 0 | 48.60 \pm 2.27 | 2.77 \pm 0.12 | 17.58 \pm 0.69 | 63.10 \pm 2.60** | 51.86 \pm 0.27** | 5.25 \pm 0.02** | 10.30 \pm 0.03** | 2.29 \pm 0.01** |
| | | 5 | 25.54 \pm 0.27 | 1.27 \pm 0.02 | 20.08 \pm 0.46 | 14.96 \pm 1.11 | 39.90 \pm 0.01 | 4.33 \pm 0.01 | 1.42 \pm 0.19 | 0.29 \pm 0.06 |
| | NH ₄ NO ₃ | 10 | 23.86 \pm 1.04 | 1.42 \pm 0.02** | 16.75 \pm 0.48** | 34.96 \pm 4.43** | 132.8 \pm 2.81** | 4.20 \pm 0.01* | 1.27 \pm 0.11 | 0.24 \pm 0.01 |
| | | 20 | 23.27 \pm 1.14 | 1.56 \pm 0.04** | 14.95 \pm 0.67** | 49.17 \pm 0.48** | 49.29 \pm 3.33* | 4.23 \pm 0.01 | 1.56 \pm 0.10 | 0.26 \pm 0.03 |
| | Glycine | 5 | 25.08 \pm 0.24 | 1.27 \pm 0.03 | 19.76 \pm 0.23 | 63.07 \pm 0.49** | 34.25 \pm 1.15 | 4.21 \pm 0.05* | 1.70 \pm 0.04 | 0.28 \pm 0.06 |
| | | 10 | 23.59 \pm 1.10 | 1.12 \pm 0.01 | 20.97 \pm 0.85 | 13.27 \pm 1.25 | 44.42 \pm 0.90 | 4.05 \pm 0.00** | 1.23 \pm 0.00 | 0.31 \pm 0.14 |
| Aeolian sandy soil | Control | 0 | 25.55 \pm 1.65 | 1.09 \pm 0.10 | 23.70 \pm 1.31* | 27.61 \pm 0.38** | 39.58 \pm 0.01 | 4.29 \pm 0.01 | 2.51 \pm 0.22** | 0.39 \pm 0.09 |
| | | 5 | 23.00 \pm 1.01 | 1.19 \pm 0.08 | 19.47 \pm 0.53 | 66.59 \pm 3.48** | 47.33 \pm 3.96 | 4.22 \pm 0.01* | 1.46 \pm 0.04 | 0.45 \pm 0.15 |
| | NH ₄ NO ₃ | 10 | 16.20 \pm 0.52 | 0.90 \pm 0.00 | 17.93 \pm 0.59 | 14.18 \pm 0.28 | 62.06 \pm 2.52 | 5.39 \pm 0.01 | 3.24 \pm 0.13 | 1.40 \pm 0.02 |
| | | 20 | 12.45 \pm 0.83** | 0.78 \pm 0.02* | 16.08 \pm 1.55 | 14.87 \pm 0.63 | 82.45 \pm 0.56** | 5.35 \pm 0.00** | 3.14 \pm 0.04 | 1.37 \pm 0.03 |
| | Glycine | 5 | 11.23 \pm 0.03** | 0.74 \pm 0.05** | 15.26 \pm 0.95 | 35.70 \pm 0.79** | 59.79 \pm 5.40 | 5.21 \pm 0.00** | 3.06 \pm 0.06 | 1.29 \pm 0.01 |
| | | 10 | 8.08 \pm 0.22** | 0.65 \pm 0.00** | 12.40 \pm 0.33* | 29.79 \pm 1.01** | 77.09 \pm 0.57* | 5.26 \pm 0.01** | 3.36 \pm 0.32 | 1.51 \pm 0.05 |
| Red soil | Control | 0 | 13.10 \pm 0.54** | 0.79 \pm 0.03* | 16.58 \pm 0.04 | 4.90 \pm 0.36** | 95.94 \pm 1.09** | 4.94 \pm 0.01** | 3.15 \pm 0.18 | 1.36 \pm 0.08 |
| | | 5 | 15.78 \pm 0.09 | 0.96 \pm 0.03 | 16.53 \pm 0.57 | 12.76 \pm 0.42 | 111.33 \pm 6.55** | 4.92 \pm 0.04** | 2.93 \pm 0.04 | 1.29 \pm 0.02 |
| | NH ₄ NO ₃ | 10 | 12.88 \pm 0.09** | 1.03 \pm 0.02* | 12.50 \pm 0.35** | 14.47 \pm 0.56 | 141.10 \pm 9.29** | 4.83 \pm 0.01** | 2.73 \pm 0.09* | 1.13 \pm 0.02** |
| | | 20 | 2.92 \pm 0.17 | 0.25 \pm 0.01 | 11.87 \pm 0.68 | 4.43 \pm 0.69 | 3.09 \pm 0.01 | 4.89 \pm 0.01 | 0.45 \pm 0.03 | 0.32 \pm 0.01 |
| | Glycine | 5 | 2.46 \pm 0.09 | 0.19 \pm 0.05 | 12.99 \pm 0.47 | 16.63 \pm 0.66** | 54.93 \pm 1.92** | 4.46 \pm 0.00** | 0.22 \pm 0.02** | 0.10 \pm 0.01** |
| | | 10 | 3.35 \pm 0.14 | 0.14 \pm 0.06 | 23.90 \pm 1.01** | 34.08 \pm 1.68** | 58.45 \pm 1.54** | 4.34 \pm 0.01** | 0.30 \pm 0.04* | 0.26 \pm 0.01 |
| Red soil | Glycine | 5 | 3.23 \pm 0.11 | 0.32 \pm 0.00 | 10.23 \pm 0.36 | 57.91 \pm 0.67** | 64.21 \pm 0.71** | 4.19 \pm 0.00** | 0.77 \pm 0.05** | 0.34 \pm 0.03 |
| | | 10 | 3.52 \pm 0.01* | 0.18 \pm 0.02 | 19.70 \pm 0.06** | 27.50 \pm 1.23** | 0.68 \pm 0.01** | 4.87 \pm 0.01 | 0.36 \pm 0.04 | 0.22 \pm 0.02 |
| | Control | 5 | 3.16 \pm 0.01 | 0.24 \pm 0.04 | 13.28 \pm 0.02 | 54.30 \pm 6.57** | 2.27 \pm 0.07** | 5.15 \pm 0.02** | 0.54 \pm 0.05 | 0.17 \pm 0.05* |
| | | 20 | 3.68 \pm 0.17** | 0.28 \pm 0.02 | 13.17 \pm 0.24 | 86.69 \pm 2.95** | 1.27 \pm 0.00** | 5.31 \pm 0.02** | 0.44 \pm 0.01 | 0.29 \pm 0.03 |

Asterisks * and ** indicate significant differences between N addition treatments and control with Dunnett's r-test at the level of $p < 0.05$ and $p < 0.01$, respectively

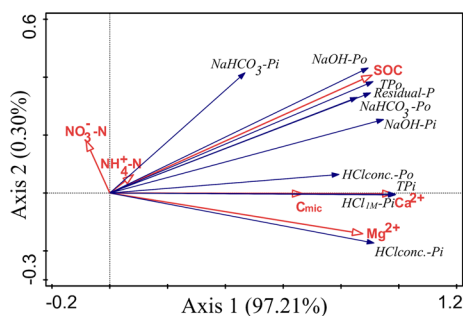


Fig. 6 Ordination diagram from redundancy analysis of the relationships between P fractions and soil biochemical properties. SOC: soil organic C; C_{mic} : microbial biomass C; P_{mic} : microbial biomass P; APase: acid phosphatase activity; Ca^{2+} : exchangeable Ca^{2+} ; Mg^{2+} : exchangeable Mg^{2+}

the laboratory study further confirmed the major role of plant roots in mobilizing soil inorganic P.

Greater impacts of glycine addition on soil P fractions than NH_4NO_3 addition

Being partially consistent with our third hypothesis, glycine addition had significantly greater impacts on organic and inorganic P fractions in some of the soils. The greater impacts of glycine addition can be ascribed to its greater impacts on soil microbial biomass and activities, as well as soil pH than NH_4NO_3 addition (Fig. 5). Many studies found that plants and microorganisms can directly absorb low-molecular-weight amino acids, and the uptake rate of glycine can be comparable to NH_4^+ (Näsholm et al. 1998; Harrison et al. 2007). Moreover, compared to inorganic N, organic N can be more rapidly assimilated by microorganisms, and thus can improve microbial activities to a greater degree (Pan et al. 2023).

Greater improvement of glycine addition on soil organic P fractions only occurred in the aeolian sandy and red soils, but not in the brown and yellow brown soils (Fig. 1C). This is probably because soil organic C was much lower in the aeolian sandy and red soils than the other two soil types. Microbial utilization of mineral and amino N sources was found to be dependent on soil C availability (Farrell et al. 2014; Yang et al. 2021). Soil microorganisms prefer to absorb glycine rather than NH_4^+ when soil C availability is deficient, because they can directly acquire C from glycine at the same time of N absorption (McFarland et al. 2010; Pan et al. 2023). Nevertheless, soil microorganisms prefer NH_4^+ over organic N when there is a high availability of C (Farrell et al. 2014).

Differences between glycine and NH_4NO_3 additions in affecting soil pH could be another important reason responsible for their different impacts on soil P fractions

in some soils. Soil pH increased with glycine addition, due to the decarboxylation and oxidative deamination of glycine (Yan et al. 1996; Hartley et al. 2010). Nevertheless, variation in soil pH with glycine addition depended on initial soil pH and acid buffering capacity (Xu et al. 2006). If initial soil pH is less than the pKa of the weak acid groups on added organic N, there will be an increase in soil pH due to association of H^+ from the soil with some of the organic anions, and vice versa (Xu et al. 2006). Also, the nitrification would be depressed in soils with low pH (Yan et al. 1996; Qiu et al. 2022). Therefore, glycine addition increased soil pH only in the red soil with low soil pH and acid buffering capacity in the present study. Although amino N is important component of atmospheric deposited N, most of N deposition simulating experiments were conducted with only inorganic N addition. So, our findings suggested that soil acidification and the adverse impacts of N deposition on soil microbial biomass activities were overestimated previously, particularly in soils with low C availability and acid buffering capacity.

Contrasting impacts of N addition on soil P fractions in different forest soils

Direction and magnitude of the N addition impacts on soil P fractions were quite different in the four soil types (Figs. 3 and 4). However, contrary to our hypotheses, the differences in N addition impacts on soil P fractions among the four soil types cannot be predicted by one specific soil variable, but generated by the interactions of several key soil variables.

For organic P fractions, the extent of soil microbial N limitation and soil acid buffering capacity could be the predominant factors determining their response to N addition. As discussed above, soil microbial biomass and activities controlled organic P mineralization, and which can be improved by N addition in N-poor soils due to the alleviation of N limitation, which can also be depressed by direct toxic effects of excess ions in N-rich soils and by N-induced acidification in soils with low acid buffering capacity (Zhou et al. 2017, 2018). The net effects in a specific soil depended on which processes dominate.

Specifically, in the brown soil with very high acid buffering capacity, N addition did not acidify the soil, and significantly increased microbial biomass and phosphatase activities, and thereby accelerated the mineralization of labile P and accumulation of recalcitrant organic P fractions (Tables 1, 2 and Figs. 4 and 5). Increases in acid phosphatase activities with N addition was also observed in field studies on temperate forests on brown soils (Yang and Zhu 2015; Zheng et al. 2023). Compared to the brown soil, in the red soil microbial biomass and activities were also improved by N addition, but oppositely,

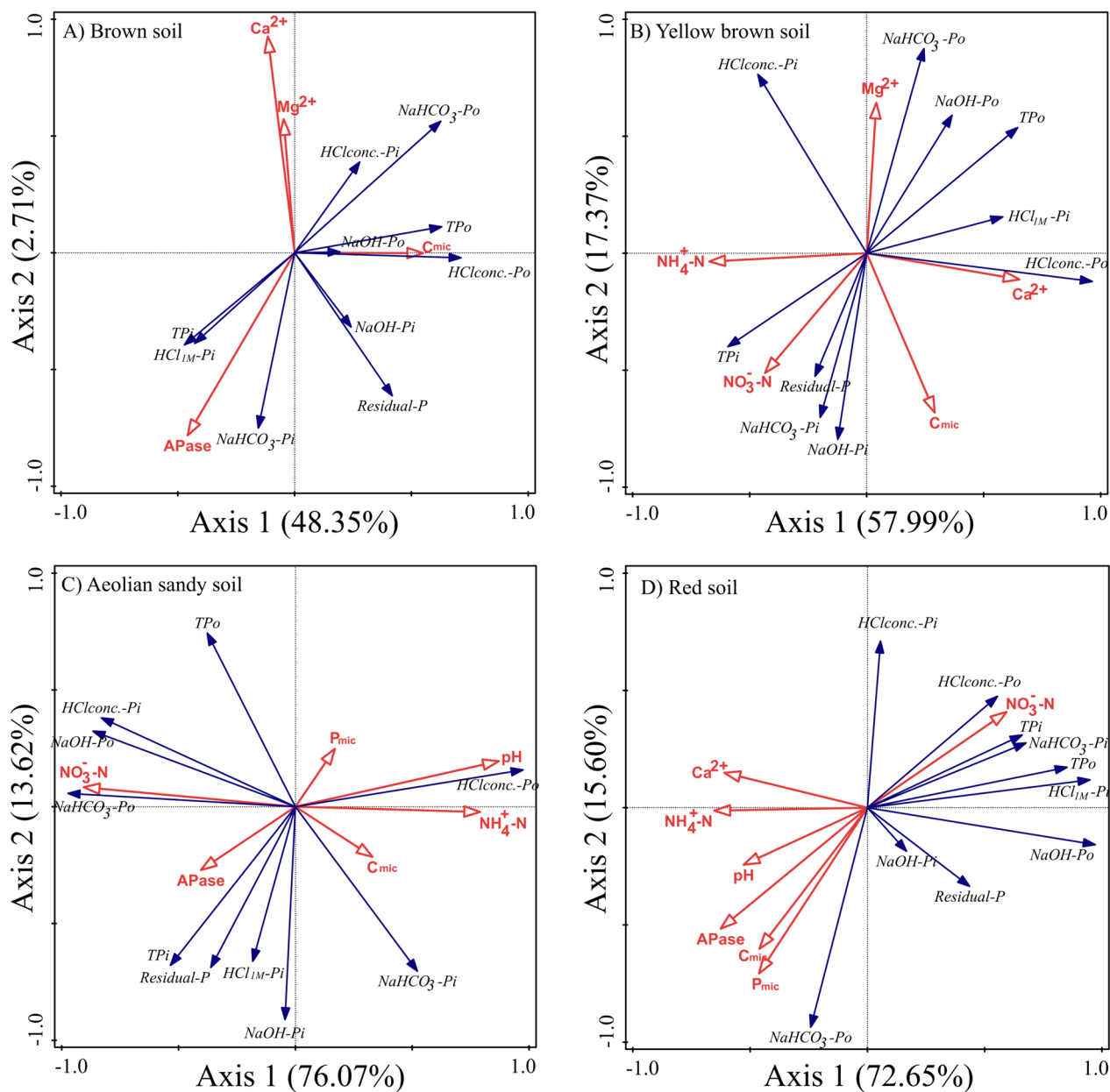


Fig. 7 Ordination diagram from redundancy analysis of the relationships between the percent changes in P fractions and changes in soil biochemical properties in the four types of soils. C_{mic} : microbial biomass C; P_{mic} : microbial biomass P; APase: acid phosphatase activity; Ca^{2+} : exchangeable Ca^{2+} ; Mg^{2+} : exchangeable Mg^{2+}

they led to the accumulation of labile organic P and mineralization of recalcitrant organic P, probably because of the low P status (Fig. 7D). Similar responses of organic P fractions to N addition were observed in field studies on forest red soils (Fan et al. 2018). Soil labile organic P concentration depends on the equilibrium between organic P mineralization and microbial P immobilization (Häussling and Marschner 1989; Deforest and Scott 2010; Arenberg and Arai 2019). In P-deficient soils, improved

microbial biomass greatly stimulated microbial P immobilization, but slightly elevated organic P mineralization, thus leading to net P immobilization (Jiang et al. 2014).

In the yellow brown and aeolian sandy soils with medium level of fertility and acid buffering capacity, N-induced acidification played the dominant role in affecting microorganisms. Consequently, N addition depressed mineralization of organic P and resulted in the accumulation of most organic P fractions. Increased

moderately labile organic P and reduced pH with N addition were observed in a mixed deciduous forest on yellow brown soil (Zhang et al. 2020).

For soil inorganic P fractions, their responses to N addition in the present study not only depended on the changes in soil pH, but also on the variations in related metal cations (i.e. Ca^{2+} , Mg^{2+}), and release of phosphate by mineralization of organic P. Therefore, soil acid buffering capacity, together with cation exchange capacity and microbial activities controlled responses of soil inorganic P transformations to N addition. The predominant processes varied in different soils and thus generated different responses to N addition.

Specifically, in the brown soil with very high buffering capacity, soil pH did not change, while exchangeable Ca^{2+} and Mg^{2+} significantly decreased with N addition, due to the competition of NH_4^+ and H^+ for ion exchange sites. The decreased Ca^{2+} and Mg^{2+} concentrations drove the dissolution of calcium phosphate (Fig. 3 and Table 2). Reduced inorganic P fractions were also observed in a field N addition experiment in *Larix gmelinii* plantations on the brown soil (Zheng et al. 2023). In the yellow brown soil, the significant reduction in labile inorganic P concentration with N addition in most treatments can be ascribed to the depressed organic P mineralization, since there was a significant correlation between $\text{NaHCO}_3\text{-Pi}$ and $\text{NaHCO}_3\text{-Po}$. So, reduction in soil pH and changes in soluble phosphate concentration caused by organic P mineralization concomitantly controlled inorganic P fractions. In the aeolian sandy soil, variations in soil pH played the predominant role in controlling inorganic P fractions, since exchangeable metal cations and labile inorganic P concentrations did not change significantly. Similarly, positive correlation between residual-P concentration and soil pH was observed in a previous field N addition experiment in the Mongolian pine plantation on aeolian sandy soil (Zhang et al. 2022). In the red soil, both soil pH and base cations changed significantly with N addition, which was responsible for the reduction in some inorganic P fractions. Being similar to our results, N addition decreased moderately labile P and occluded P concentrations, as well as pH and exchangeable cations in a *Castanopsis carlesii* forest on red soil (Fan et al. 2019).

Conclusions

In summary, our laboratory N addition experiment revealed the important role of edaphic characteristics in regulating the impacts of N addition on soil P transformations. Extent of soil microbial N limitation, acid buffering capacity, cation exchange capacity are the key soil properties determining the responses of soil P transformations to N addition. Besides, addition

of amino and mineral N sources had similar impacts on soil P fractions, but the former had greater positive impacts on soil microbial biomass and activities and thus organic P transformations in soils with low organic C concentration. Amino and mineral N additions also had different effects on soil pH in some soils, and thus exert different impacts on inorganic P transformations. Besides, N addition had obviously greater impacts on organic P than on inorganic P fractions in all soils, highlighting the significance of N induced changes in biochemical processes in driving organic P transformations. These findings greatly advance our understanding of the role and mechanisms of edaphic characteristics and chemical form of N addition in regulating N addition impacts on soil P transformations.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s13717-024-00523-7>.

Additional file 1.

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Author contributions

All authors contributed to the study's conception and design. Material preparation, data collection and analysis, manuscript draft were performed by PZ. Soil sample analysis and data processing were carried out with the assistance of J-YH and XZ. The manuscript was reviewed and edited by QZ, BM, Q-YS and W-GW. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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References

- Aitken R, Moody P (1994) The effect of valence and ionic strength on the measurement of pH buffer capacity. *Aust J Soil Res* 32:975–984
- Arenberg MR, Arai Y (2019) Uncertainties in soil physicochemical factors controlling phosphorus mineralization and immobilization processes. In: Sparks DL (ed) *Advances in agronomy*. Academic Press, Cambridge, pp 153–200
- Boot CM, Hall EK, Denef K, Baron JS (2016) Long-term reactive nitrogen loading alters soil carbon and microbial community properties in a subalpine forest ecosystem. *Soil Biol Biochem* 92:211–220
- Bremner JM (1996) Nitrogen-Total. In: Sparks DL et al (eds) *Methods of soil analysis: Part 3 chemical methods*. Soil Science Society of America and American Society of Agronomy, Madison, pp 1085–1121
- Brookes PC, Powlson DS, Jenkinson DS (1982) Measurement of microbial biomass phosphorus in soil. *Soil Biol Biochem* 14:319–329
- Bu WS, Gu HJ, Zhang CC, Zhang Y, Singh AN, Fang XM, Fan J, Wang HM, Chen FS (2020) Mixed broadleaved tree species increases soil phosphorus availability but decreases the coniferous tree nutrient concentration in subtropical China. *Forests* 11:461
- Compton JE, Watrud LS, Arlene Porteous L, DeGroot S (2004) Response of soil microbial biomass and community composition to chronic nitrogen additions at Harvard forest. *For Ecol Manage* 196:143–158
- Cornell SE (2011) Atmospheric nitrogen deposition: revisiting the question of the importance of the organic component. *Environ Pollut* 159:2214–2222
- Deforest JL, Scott LG (2010) Available organic soil phosphorus has an important influence on microbial community composition. *Soil Sci Soc Am J* 74:2059–2066
- Deng Q, Hui DF, Dennis S, Reddy KC (2017) Responses of terrestrial ecosystem phosphorus cycling to nitrogen addition: a meta-analysis. *Glob Ecol Biogeogr* 26:713–728
- Doane TA, Horwath WR (2003) Spectrophotometric determination of nitrate with a single reagent. *Anal Lett* 36:2713–2722
- EPA (1971) *Methods of chemical analysis of water and wastes*. Environmental Protection Agency, Cincinnati
- Fan YX, Lin F, Yang LM, Zhong XJ, Wang MH, Zhou JC, Chen YM, Yang YS (2018) Decreased soil organic P fraction associated with ectomycorrhizal fungal activity to meet increased P demand under N application in a subtropical forest ecosystem. *Biol Fertil Soils* 54:149–161
- Fan YX, Zhong XJ, Lin F, Liu CC, Yang LM, Wang MH, Chen GS, Chen YM, Yang YS (2019) Responses of soil phosphorus fractions after nitrogen addition in a subtropical forest ecosystem: insights from decreased Fe and Al oxides and increased plant roots. *Geoderma* 337:246–255
- Farrell M, Prendergast-Miller M, Jones DL, Hill PW, Condon LM (2014) Soil microbial organic nitrogen uptake is regulated by carbon availability. *Soil Biol Biochem* 77:261–267
- Galloway JN, Dentener FJ, Capone DG, Boyer EW, Howarth RW, Seitzinger SP, Asner GP, Cleveland CC, Green PA, Holland EA, Karl DM, Michaels AF, Porter JH, Townsend AR, Vöosmarty CJ (2004) Nitrogen cycles: past, present, and future. *Biogeochemistry* 70:153–226
- Grant RF, Heaney DJ (1997) Inorganic phosphorus transformation and transport in soils: mathematical modeling in ecosys. *Soil Sci Soc Am J* 61:752–764
- Harrison KA, Bol R, Bardgett RD (2007) Preferences for different nitrogen forms by coexisting plant species and soil microbes. *Ecology* 88:989–999
- Hartley IP, Hopkins DW, Sommerkorn M, Wookey PA (2010) The response of organic matter mineralisation to nutrient and substrate additions in subarctic soils. *Soil Biol Biochem* 42:92–100
- Häussling M, Marschner H (1989) Organic and inorganic soil phosphates and acid phosphatase activity in the rhizosphere of 80-year-old Norway spruce [*Picea abies* (L.) Karst.] trees. *Biol Fertil Soils* 8:128–133
- Helpenstein J, Pistocchi C, Oberson A, Tamburini F, Goll DS, Frossard E (2020) Estimates of mean residence times of phosphorus in commonly considered inorganic soil phosphorus pools. *Biogeosciences* 17:441–454
- Hinsinger P (2001) Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: a review. *Plant Soil* 237:173–195
- Hu YL, Zeng DH, Liu YX, Zhang YL, Chen ZH, Wang ZQ (2010) Responses of soil chemical and biological properties to nitrogen addition in a Dahurian larch plantation in northeast China. *Plant Soil* 333:81–92
- Hu L, Ade LJ, Wu XW, Zi HB, Luo XP, Wang CT (2019) Changes in soil C:N:P stoichiometry and microbial structure along soil depth in two forest soils. *Forests* 10:113
- Huang SN, Wang F, Elliott EM, Zhu FF, Zhu WX, Koba K, Yu ZJ, Hobbie EA, Michalski G, Kang RH, Wang AZ, Zhu JJ, Fu SL, Fang YT (2020) Multiyear measurements on $\Delta^{17}\text{O}$ of stream nitrate indicate high nitrate production in a temperate forest. *Environ Sci Technol* 54:4231–4239
- Inagaki Y, Miura S (2002) Soil $\text{NO}_3\text{-N}$ production and immobilization affected by $\text{NH}_4\text{-N}$, glycine, and $\text{NO}_3\text{-N}$ addition in different forest types in Shikoku, southern Japan. *Soil Sci Plant Nutr* 48:679–684
- Jiang Y, Bu F, Zhang C, Chen LX (2014) Research advances on soil organic phosphorus mineralization. *J Nanjing For Univ* 38:160–166
- Li Q, Lv JH, Peng CH, Xiang WH, Xiao WF, Song XZ (2021) Nitrogen addition accelerates phosphorus cycling and changes phosphorus use strategy in a subtropical Moso bamboo forest. *Environ Res Lett* 16:024023
- Lu RK (2000) *Analytical methods of soil and agricultural chemistry*. China Agricultural Science and Technology Publishing House, Beijing
- Lu XK, Mo JM, Gilliam FS, Fang H, Zhu FF, Fang YT, Zhang W, Huang J (2012) Nitrogen addition shapes soil phosphorus availability in two reforested tropical forests in southern China. *Biotropica* 44:302–311
- Lu XK, Mao Q, Mo J, Gilliam FS, Zhou G, Luo Y, Zhang W, Huang J (2015) Divergent responses of soil buffering capacity to long-term N deposition in three typical tropical forests with different land-use history. *Environ Sci Technol* 49:4072–4080
- Lu XF, Hou EQ, Guo JY, Gilliam FS, Li JL, Tang SB, Kuang YW (2021) Nitrogen addition stimulates soil aggregation and enhances carbon storage in terrestrial ecosystems of China: a meta-analysis. *Glob Change Biol* 27:2780–2792
- Lv YN, Wang CY, Zhao GY, Tian XJ, Pu GZ, Wang FY, Ma X, Tian XJ (2013) Effects of nitrogen addition on litter decomposition, soil microbial biomass, and enzyme activities between leguminous and non-leguminous forests. *Ecol Res* 28:793–800
- McFarland JW, Russ RW, Kielland K, Pregitzer K, Hendrick R (2010) Glycine mineralization in situ closely correlates with soil carbon availability across six north American forest ecosystems. *Biogeochemistry* 99:175–191
- Murphy J, Riley JP (1962) A modified single solution method for the determination of phosphate in natural waters. *Anal Chim Acta* 27:31–36
- Näsholm T, Ekblad A, Nordin A, Giesler R, Högberg M, Högberg P (1998) Boreal forest plants take up organic nitrogen. *Nature* 392:914–916
- Nelson DW, Sommers LE (1996) Total carbon, organic carbon, and organic matter. In: Sparks DL et al (eds) *Methods of soil analysis: Part 3 chemical methods*. Soil Science Society of America and American Society of Agronomy, Madison, pp 961–1010
- Olsen SR, Sommers LE (1982) Phosphorus. In: Page AL (ed) *Methods of soil analysis: Part 2 chemical and microbiological properties*. American Society of Agronomy, Soil Science Society of America, Madison, pp 403–430
- Pan WK, Zhou JJ, Tang S, Wu LH, Ma QX, Marsden KA, Chadwick DR, Jones DL (2023) Utilisation and transformation of organic and inorganic nitrogen by soil microorganisms and its regulation by excessive carbon and nitrogen availability. *Biol Fertil Soils* 59:379–389
- Qiu QY, Mgelwa AS, Jin SF, Hu YL (2022) Nitrogen-induced changes in soil environmental factors are more important than nitrification and denitrification gene abundance in regulating N_2O emissions in subtropical forest soils. *Front Plant Sci* 13:950367
- Robertson GP, Sollins P, Ellis BG, Lajtha K (1999) Exchangeable ions, pH, and cation exchange capacity. In: Robertson GP, Coleman DC, Bledsoe CS, Sollins P (eds) *Standard Soil Methods for Long-Term Ecological Research*. Oxford University Press, Oxford, pp 106–114
- Shen J, Yuan L, Zhang J, Li H, Bai Z, Chen X, Zhang W, Zhang F (2011) Phosphorus dynamics: from soil to plant. *Plant Physiol* 156:997–1005
- Tabatabai MA (1994) Soil enzymes. In: Weaver RW et al (eds) *Methods of soil analysis: Part 2 microbiological and biochemical properties*. Soil Science Society of America, Madison, pp 775–833
- Tian DS, Niu SL (2015) A global analysis of soil acidification caused by nitrogen addition. *Environ Res Lett* 10:024019
- Tian D, Jiang L, Ma SH, Fang WJ, Schmid B, Xu LC, Zhu JX, Li P, Losapio G, Jing X, Zheng CY, Shen HH, Xu XN, Zhu B, Fang JY (2017) Effects of nitrogen

- deposition on soil microbial communities in temperate and subtropical forests in China. *Sci Total Environ* 607–608:1367–1375
- Tian D, Du EZ, Jiang L, Ma SH, Zeng WJ, Zou AL, Feng CY, Xu LC, Xing AJ, Wang W, Zheng CY, Ji CJ, Shen HH, Fang JY (2018) Responses of forest ecosystems to increasing N deposition in China: a critical review. *Environ Pollut* 243:75–86
- Tiessen H, Moir JO (1993) Characterization of available P by sequential extraction. In: Carter MR, Gregorich EG (eds) *Soil Sampling and Methods of Analysis*. CRC Press, pp 75–86
- Vance ED, Brookes PC, Jenkinson DS (1987) An extraction method for measuring soil microbial biomass C. *Soil Biol Biochem* 19:703–707
- Wang C, Liu DW, Bai E (2018) Decreasing soil microbial diversity is associated with decreasing microbial biomass under nitrogen addition. *Soil Biol Biochem* 120:126–133
- Weatherburn MW (1967) Phenol-hypochlorite reaction for determination of ammonia. *Anal Chem* 39:971–974
- Xu JM, Tang C, Chen ZL (2006) The role of plant residues in pH change of acid soils differing in initial pH. *Soil Biol Biochem* 38:709–719
- Xu W, Zhao YH, Liu XJ, Dore AJ, Zhang L, Liu L, Cheng MM (2018) Atmospheric nitrogen deposition in the Yangtze river basin: spatial pattern and source attribution. *Environ Pollut* 232:546–555
- Yan F, Schubert S, Mengel K (1996) Soil pH increase due to biological decarboxylation of organic anions. *Soil Biol Biochem* 28:617–624
- Yang K, Zhu JJ (2015) The effects of N and P additions on soil microbial properties in paired stands of temperate secondary forests and adjacent larch plantations in northeast China. *Soil Biol Biochem* 90:80–86
- Yang K, Zhu JJ, Gu JC, Yu LZ, Wang ZQ (2015) Changes in soil phosphorus fractions after 9 years of continuous nitrogen addition in a *Larix gmelinii* plantation. *Ann For Sci* 72:435–442
- Yang LJ, Yu CX, Zhang LL, Bai W, Li WT, Li DP, Cui YL, Song YC, Gong P, Xue Y, Wu ZJ (2021) Substrate availability affects the partitioning of C and N in glycine between plants and soil microorganisms. *Arch Agron Soil Sci* 67:109–121
- Zhang Y, Song L, Liu XJ, Li WQ, Lu SH, Zheng LX, Bai ZC, Cai GY, Zhang FS (2012) Atmospheric organic nitrogen deposition in China. *Atmos Environ* 46:195–204
- Zhang HZ, Shi LL, Fu SL (2020) Effects of nitrogen deposition and increased precipitation on soil phosphorus dynamics in a temperate forest. *Geoderma* 380:114650
- Zhang JJ, Li Y, Wang JS, Chen WN, Tian DS, Niu SL (2021) Different responses of soil respiration and its components to nitrogen and phosphorus addition in a subtropical secondary forest. *For Ecosyst* 8:37
- Zhang X, Zhao Q, Sun QY, Mao B, Zeng DH (2022) Understory vegetation interacts with nitrogen addition to affect soil phosphorus transformations in a nutrient-poor *Pinus sylvestris* var. *mongolica* plantation. *For Ecol Manage* 507:120026
- Zhang X, Zhao Q, Wei LM, Sun QY, Zeng DH (2023) Tree roots exert greater impacts on phosphorus fractions than aboveground litter in mineral soils under a *Pinus sylvestris* var. *mongolica* plantation. *For Ecol Manage* 545:121242
- Zhao Q, Zeng DH (2019) Nitrogen addition effects on tree growth and soil properties mediated by soil phosphorus availability and tree species identity. *For Ecol Manage* 449:117478
- Zheng LL, Zhao Q, Lin GG, Hong X, Zeng DH (2023) Nitrogen addition impacts on soil phosphorus transformations depending upon its influences on soil organic carbon and microbial biomass in temperate larch forests across northern China. *Catena* 230:107252
- Zhou ZH, Wang CK, Zheng MH, Jiang LF, Luo YQ (2017) Patterns and mechanisms of responses by soil microbial communities to nitrogen addition. *Soil Biol Biochem* 115:433–441
- Zhou KJ, Lu XK, Mori T, Mao QG, Wang C, Zheng MH, Mo H, Hou EQ, Mo JM (2018) Effects of long-term nitrogen deposition on phosphorus leaching dynamics in a mature tropical forest. *Biogeochemistry* 138:215–224

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