

Responses of Soil C-, N-, and P-Hydrolyzing Enzyme Activities to N and P Addition in an Evergreen Broad-Leaved Forest in Southwest China

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Abstract

Background and Aims

Human activities-mediated input of nitrogen (N) and phosphorus (P) to ecosystem may significantly affect soil hydrolyzing enzyme activities (Hy-EAs). However, the mechanisms underlying the responses of soil Hy-EAs to change in N and P availability remains unclear.

Methods

Here, a two-year field N and P addition experiment was conducted in a subtropical evergreen broad-leaved forest to elucidate the effects of N addition, P addition, and NP co-additions on soil Hy-EAs and biochemistry properties.

Results

The invertase, cellulase, and acid phosphatase activities were increased in N treatment but reduced in P treatment. The urease activity was reduced in N treatment but did not alter in P treatment. NP treatment significantly increased the invertase and cellulase activities. Furthermore, the cellulase activity was positively correlated with soil organic carbon concentration. The acid phosphatase activity was negatively correlated with microbial biomass carbon (MBC), total P, and available P concentrations. Whereas the urease activity was not strongly dependent on total N concentrations, but positively correlated with soil pH and MBC. These Hy-EAs were significantly correlated with C-to-P and N-to-P ratios, while no significantly correlation with C-to-N ratio.

Conclusions

Overall, our results indicated that N and P addition significantly affected the soil C-, N-, and P-hydrolyzing enzyme activities. With ongoing imbalanced N and P input in our studied subtropical evergreen broad-leaved forest, N addition may exacerbate the limitation of soil C and P availability, while the exogenous P addition may improve the soil C and P availability.

Introduction

Nitrogen (N) and phosphorus (P) are the essential elements of the biology, and generally regarded as the two limiting factors for the net primary productivity of the forest ecosystem (Elser et al. 2007; Zhang et al. 2015). However, with the intensification of human activities (e.g., the large consumption of fossil fuels and fertilizers), the input rates of N and P in the global terrestrial ecosystem have been greatly increased and still maintained an upward trend (Vitousek et al. 1997; Galloway 2000, 2004; Du et al. 2016), but compared to the anthropogenic N input, the amount of P input is far less (Galloway et al. 2004;

Mahowald et al. 2008). The imbalance of N and P input may transform the forest ecosystem that was originally restricted by N into a state of N saturation, enhance the dependence of forest primary productivity on P (Burns et al. 2013; Zhu et al. 2016), and greatly affects the availability of carbon (C) and nutrients in the soil (Lu et al. 2013; Fang et al. 2019), especially in tropical/subtropical forests ecosystems which are generally limited by P (Cleveland et al. 2013; Du et al. 2016).

Soil hydrolytic enzyme activities (Hy-EAs) reflects the relationship between plant and microbial nutrient demand and soil nutrient supply (Sinsabaugh et al. 2008; Hill et al. 2012), and has been shown to be closely related to pH, microbial biomass, C, N, and P concentrations and their stoichiometric characteristics (Rodríguez-Loiñaz et al. 2008; Sinsabaugh et al. 2008; Burns et al. 2013). Changes in soil pH, microbial activity and N and P availability caused by human-induced N and P input not only have a direct impact on soil enzyme activities (Waring et al. 2014; Shi et al. 2018), but also may regulate enzyme activities indirectly by changing the microbial allocation to soil enzymes production and the abundance of specific enzyme-producing microorganisms (Sinsabaugh and Moorhead 1994; Allison et al. 2008; Yokoyama et al. 2017). However, the response of soil Hy-EAs to N and/or P inputs are complex and still uncertain.

In recent years, the effect of N addition or deposition on soil enzymes activities has been widely studied (Sinsabaugh et al. 2005; Song et al. 2009; Jing et al. 2017; Crim and Cumming 2020). However, the effects of N enrichment on soil C-, N- and P-hydrolyzing enzyme activities (C-, N-, and P-Hy EAs) generally showed difference in both direction and magnitude among different studies. For example, studies have shown that N addition had neutral effects on the soil C-, N- and P-Hy EAs in tropical/subtropical and temperate forest in eastern China (Jing et al. 2017), while had a significant promotion effect in a temperate deciduous broad-leaved forest in Northern China (Zhang et al. 2016), and had a significant inhibition effect in temperate broad-leaved forest and coniferous forest in North American (Fatemi et al. 2016). In addition, N addition had positive effects on soil C- and P-Hy EAs, while had no significant effect on N-Hy EAs, as indicated by recent meta-analyses (Jian et al. 2016; Xiao et al. 2018). These inconsistent results may not only due to the difference of vegetation and soil types, as well as the forms and levels of N addition, but also may be caused by the different effects of N enrichment on soil chemical properties and microbial biomass (DeForest et al. 2004; Shi et al. 2018).

Compared with the N addition, there are relatively few studies focusing on the effect of P addition on the soil Hy-EAs. Based on the resource allocation theory (Sinsabaugh and Moorhead 1994; Allison and Vitousek 2005), the higher levels of P availability will suppress the P-Hy EAs, but promote the Hy-EAs of involved in soil C and N cycling (Allison and Vitousek 2005; Marklein and Houlton 2012). This point has been proved in some P addition experiments (Olander and Vitousek 2000; Zheng et al. 2015). However, not all studies support the resource allocation theory, depending on research sites and enzymes assayed, positive (Mganga et al. 2015; Huang et al. 2018), neutral (Xiao et al. 2018), and negative (Turner and Joseph Wright 2014; Yokoyama et al. 2017) effects of P addition on C-, N-, and P-Hy EAs have been reported. These different results indicate that the effect of P addition on soil C-, N-, and P-Hy EAs are site-

specific. More research are needed to enhance our understanding of the underlying mechanism change in soil P availability affect hydrolytic enzymes activities.

Here, we conducted a two-year field N and P addition experiment in a natural evergreen secondary broad-leaved forest in Southwest China. In this study, we investigated the response of four soil Hy-EAs, i.e., invertase, cellulase, urease and acid phosphatase to N and/or P addition. We also simultaneously measured the soil pH, microbial biomass C, and nutrient (soil organic C, total N, total P and available P) concentrations. Our objective is to evaluate how the N and/or P addition affect soil C-, N-, and P-Hy EAs in subtropical evergreen broad-leaved forests, and to explore soil hydrolytic enzymes activities correlations with the soil MBC, pH, nutrient concentrations, and stoichiometric characteristics under the N and/or P addition. According to the production of soil enzymes follows the resource allocation theory (Allison and Vitousek 2005; Allison et al. 2007), enzyme producers tend to synthesize and secrete enzymes related to the decomposition of elements that limit their own growth. The N and/or P addition may increase the availability of N and/or P in the soil, but will increase soil microbial demands for C. Therefore, we hypothesized that: (1) N treatment would increase the soil invertase, cellulase and acid phosphatase activities, while decrease the soil urease activity. (2) P treatment would increase the soil invertase, cellulase and urease activities, while decrease soil acid phosphatase activity. (3) NP treatment would increase the soil invertase and cellulase activities, while decrease the soil urease and acid phosphatase activities.

Material And Methods

Study site

The study was conducted in the Bi Feng Gorge Science spot, Ya'an City, Sichuan Province, China (103°00'25"E, 30°04' 06"N, and 970 m a.s.l.). The region is located in a subtropical moist forest zone with a monsoon climate, mean annual precipitation is approximately 1658 mm (average from 1971 to 2017), which has distinct dry and wet seasons. About 60% of the rainfall from the wet season (June to August) and only 4.4% from dry season (December to February), the mean annual temperature is 16.4°C (Zhou et al. 2018). More details on study sites were described in Wei et al. (2020) .

Experimental design

The experiment was established with a complete randomized block design. Three replicate blocks were established in September 2017 and each block was separated by 20 meters. In every block, four plots (5 m× 5 m) were established and separated by a 2-m-wide buffer zone. With reference to the levels of N and P addition in the previous study (Jing et al. 2016; Zhou et al. 2017a), four treatments were established in this study: control (Ctr: no N or P addition), N addition (N, 100 kg N ha⁻¹ year⁻¹), P addition (P, 50 kg P ha⁻¹ year⁻¹), and N and P co-addition treatment (NP, 100 kg N ha⁻¹ year⁻¹+50 kg P ha⁻¹ year⁻¹). NH₄NO₃ and NaH₂PO₄ solutions were used as fertilizers, and were sprayed below the canopy monthly from

October 2017. During each application, the supplies were dissolved in 2 L of water and sprayed onto the forest floor using a hand-held sprayer. The control plots received 24 L of water without supply each year, this equivalent to an annual precipitation increase of 0.96 mm, which was negligible (Wei et al. 2020).

Field sampling

Surface soil (0-20cm) samples were collected using an auger (20 cm in height, 9 cm inside diameter) in April, July and October 2018, January, April, July and October 2019 as well as January 2020. Three cores were randomly collected from each plots, the stones and coarse roots in cores were removed, thoroughly mixed and sieved using a 2-mm mesh sieve, then divided into two subsamples. One subsample was stored at 4 °C and used to measure the soil hydrolase activity (measure within one week), the other was air-dried at ambient temperature for the chemical properties measurements.

Soil enzyme activities and chemical properties

The assays of the enzyme activities were based on the colorimetric determination of the products released by the enzymes (Guan 1986; Alef and Nannipieri 1995; Hopkins et al. 2008), with some modifications. The invertase (EC 3.2.1.26) activity was measured with 8% sucrose as substrate with an incubation period of 24 h at 37°C, the cellulase (EC 3.1.1.4) activity was measured with 1% sodium carboxymethylcellulose as the substrate with an incubation period of 72 h at 37°C. The activity of invertase and cellulase were expressed by determined the released reducing sugars. Urease (EC 3.5.1.5) activity was determined using 10% urea as the substrate under standard conditions (24h at 37°C), based on the number of enzymatic products to analyze the activity. Acid phosphatase (EC 3.1.3.2) activity was measured using 0.5% disodium phenyl phosphate as the substrate and incubated at 37°C for 2 h. Enzyme activities were expressed as the micro molar of specific products produced by soil enzymes in unit time unit dry soil mass ($\mu\text{mol}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$ / $\text{nmol}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$).

Soil pH was determined by a glass electrode in a soil/water (1:2.5) suspension, soil organic C (SOC) was determined using dichromate oxidation and titration with ferrous ammonium sulfate, the total N (TN) was determined by Kjeldahl digestion, and the total P was determined by molybdenum-antimony colorimetry (Allen et al. 1974). The soil available P (AP) was extracted using 0.72 mol L^{-1} sodium acetate anhydrous and 0.52 mol L^{-1} acetic acid and analyzed calorimetrically using a spectrophotometer (UV2550, Shimadzu, Japan). Soil microbial biomass carbon (MBC) and were measured using the chloroform fumigation extraction technique via the total C analyzer (Wu et al. 1990).

Statistical analyses

All variables were first tested for a normal distribution and homogeneity of variance using the Shapiro-Wilk test and Levene's test, respectively. The linear mixed effects models employing the restricted

maximum likelihood estimation method were used to determine the main effects of the N or/and P addition, sampling time and their interactions on the four soil Hy-EAs (invertase, cellulase, urease, and acid phosphatase), chemical properties (SOC, TN, TP and AP concentrations, and pH) and MBC. The N addition, P addition, and sampling time were designated as fixed effects, and the plots nested in blocks as random effects. After establishing the general linear model, the Bonferroni adjustment method was used to the confidence intervals and significance values to account for multiple comparisons. Linear regression analysis was applied to evaluate the relationship between soil C-, N-, and P-Hy EAs and soil MBC, pH, SOC, TN, TP (or AP) concentrations and their stoichiometric proportion. Prior to linear regression analysis, parameters were Ln-transformed to maintain the same order of magnitude. All statistical analyses were performed in SPSS 20.0 (SPSS Inc., USA).

Results

Soil hydrolytic enzymes

During the 2 years experiment, soil invertase and acid phosphatase activities did not show significant seasonal dynamic (Fig.1a, d). The activities of the soil cellulase and urease were seasonally dynamic, the maximum activities were observed in July and October, respectively; and the minimum occurred in January and April, respectively (Fig.1b, c). The linear mixed model showed that the soil invertase, cellulase, urease, and acid phosphatase activities were significantly affected by the main effect of N addition, P addition and sampling time ($P < 0.05$, Table 1). Notably, the interaction effect between N and/or P addition and sampling time significantly affected soil invertase, urease, and acid phosphatase activities ($P < 0.05$), while did not significantly affect soil cellulase activity ($P > 0.05$, Table 1).

Multiple comparisons showed that, compared with the control, the mean activities of soil invertase and cellulase were significantly increased by 27.5% and 25.2% in the N treatments, respectively; and NP treatment significantly increased the mean activities of invertase and cellulase by 30.9% and 20.3%, respectively ($P < 0.05$, Fig.1a, b). On the contrary, P treatment significantly decreased the mean activities of invertase and cellulase by 10.1% and 13.6%, respectively ($P < 0.05$, Fig.1a, b). There was no significant difference in the mean activity of urease under the P and NP treatments (Fig.1c), but significantly decreased by 11.2% in N treatment ($P < 0.05$, Fig.1c). Notably, we found that short-term (12 months) N treatment promoted urease activity, but it was reduced with continuous N addition (Fig.1c). The mean activity of soil acid phosphatase was not significantly affected by the NP treatment (Fig.1d), but significantly increased by 37.8% in N treatment and decreased by 18.4% in P treatment ($P < 0.05$, Fig.1d).

Soil chemical properties and MBC

Overall, the N and/or P addition had certain effects on soil chemical properties and MBC concentration during the experiment (Table 2, S1 and S2). Multiple comparisons showed that, compared with the control, soil mean total C:N ratio, TN, and TP mean concentrations did not responsive to N or P

treatments (Table 2). Soil SOC mean concentrations, mean total C:P and N:P ratios significantly increased by 4.7%, 9.7% and 11.9% in N treatment, respectively ($P < 0.05$, Table 2), but decreased by 3.3% ($P < 0.05$), 7.0% ($P < 0.05$), and 5.0% ($P > 0.05$) in P treatment, respectively (Table 2). Soil AP and MBC mean concentrations significantly decreased by 16.7% and 17.4% in N treatment, respectively ($P < 0.05$, Table 2), but increased by 21.5% ($P < 0.05$) and 4.0% ($P > 0.05$) in P treatment (Table 2). Soil mean pH significantly decreased by 2.3% and 1.9% in N and P treatment, respectively ($P < 0.05$, Table 2). The NP treatment significantly increased soil mean MBC concentration and pH by 23.6% and 1.6%, respectively ($P < 0.05$, Table 2), while did not significantly affect other soil chemical properties ($P > 0.05$, Table 2)

Relationships between soil chemical properties, MBC and hydrolytic enzymes activities

Soil invertase activity was positively correlated with total C:P ($P < 0.01$; Fig.2b) and total N:P ($P < 0.01$; Fig.2c) ratios, soil cellulase activity was positively correlated with soil SOC concentration ($P < 0.01$; Fig.2a), total C:P ratio ($P < 0.01$; Fig.2b). Notably, we did not detect a significant correlation between invertase activity and soil SOC concentration ($P = 0.65$, Fig.2a). Soil urease activity was negatively correlated with total C:P ($P < 0.01$; Fig.2d) and total N:P ($P < 0.01$; Fig.2e) ratios and positively correlated with soil pH ($P < 0.01$; Fig.2f) and MBC ($P < 0.01$; Fig.2g). Notably, there was no significant correlation between soil urease activity and TN concentration ($P = 0.14$, Fig.S2a). Soil acid phosphatase activity was negatively correlated with soil TP ($P < 0.01$; Fig.2h), AP ($P < 0.01$; Fig.2i), and MBC ($P < 0.01$; Fig.2l) concentrations, and positively correlated with total C:P ($P < 0.01$; Fig.2j) and total N:P ($P = 0.03$; Fig.2k) ratios.

Discussion

Effects of N addition on soil hydrolytic enzymes

In agreement with our first hypothesis and some previous studies (Wang et al. 2008; Heuck et al. 2018; Jia et al. 2020), N treatment significantly increased soil invertase and cellulase mean activities (Fig.1a, b), indicating that N addition had a positive effect on the process of soil invertase and cellulase participating in soil unstable C metabolism (Buchkowski et al. 2015; Sun et al. 2016). The increase of soil invertase and cellulase activities may be related to soil limited target nutrients, that is, N addition increases the N availability relative to C in the soil (Chen et al. 2017, 2018b). Using the excessive N resources, plant roots and microorganisms can synthesize C-hydrolyzing enzymes (such as invertase and cellulase) to accelerate soil labile C mineralization (Wang et al. 2015; Heuck et al. 2018). Notably, the increase of soil total C:P and N:P ratios after N addition maybe also promote soil invertase and cellulase activities, because we found significant correlation between soil invertase activity with total N:P ratio (Fig.2c), and between soil cellulase activity with C:P ratio (Fig.2d). These results support the premise that soil stoichiometric characteristics plays an important role in the regulation of enzymes activities (Fanin et al. 2015; Wang et al. 2020b). However, we found N treatment significantly increased SOC concentration in

our study (Table 2), indicating that the increase of soil invertase and cellulase activities did not reduce the accumulation of soil SOC. The increased of SOC concentration could be results from the N-induced suppression of soil microbial biomass (Table 2) (Chen et al. 2018a; Fang et al. 2019), soil respiration (Fang et al. 2019) and decomposition of litter (Whittinghill et al. 2012; Cenini et al. 2016). In our previous studies in the same region, we reported that N addition significantly suppressed soil heterotrophic respiration (Wei et al. 2020), and decomposition of litter and the degradation of lignin and cellulose (Zhou et al. 2018; Tie et al. 2020a). In addition, we found significant correlation between soil cellulase activities with SOC concentration (Fig.2a), this means that the increase of cellulase activity in soil may be related to the increase of substrate utilization (Pancholy and Rice 1973; Katsalirou et al. 2010). Therefore, we believe that although N addition increased SOC concentration, the soil microorganisms growth may also be limited by the C availability, and the increase of soil C-Hy EAs (such as invertase and cellulase) maybe promote the soil unstable C mineralization to improve the availability of C and thus to feed back the growth of microorganisms.

As expected, we found that N treatment significantly decreased the soil urease mean activity in this study (Fig.1c), which in line with some previous N addition experiments (Ajwa et al. 1999; Kang and Lee 2005; Feng et al. 2020). The inhibition effect of N addition on soil urease activity may be explained by the following possible mechanisms. On the one hand, we found that N addition reduced microbial the biomass and soil pH (Table 2), given that significantly positively correlations between soil urease activity with soil pH (Fig.2f) and MBC concentration (Fig.2g), N addition may lead to a decrease in urease activity via N-induced reduction of microbial biomass and the acidification (Table 2) (Burns et al. 2013; Jia et al. 2020). On the other hand, N addition promotes the soil C and P mineralization can continue to release availability N (Olander and Vitousek 2000; Zhu et al. 2014; Wang et al. 2020b), thus inhibit the soil urease activity. The negative correlation between soil urease activity and total C:P (Fig.2d) and N:P (Fig.2e) ratios supported this explanation. In addition, urease is involved in the hydrolysis of urea-type substrates, and its activity is crucial in soil N mineralization (Saiya-Cork et al. 2002; Enowashu et al. 2009; Hu et al. 2013). The continuous N supply to the soil satisfies the nutrient needs of microorganisms and plants, and the rate of N mineralization was reduced (Zhou et al. 2012; Song et al. 2014), then the lower urease activity was observed in our N treatment. However, although N treatment significantly reduced soil urease mean activity in our experiment (Fig.1c), positive effect and no respond also were observed during the first year (Fig.1c). Which mean that N addition may suppressed the soil urease activity by reducing its production, while the process required a certain time to make the urease activity at a lower level (Lloyd and Sheaffe 1973; Burns et al. 2013; Nannipieri et al. 2018). This maybe well explained that there was no significant negative correlation between soil urease activity and TN concentration (Fig.S1d). Being inconsistent with our findings, Sun et al. (2019) who reported that soil urease activity had no response to N addition in a nearby natural evergreen broad-leaved forest, which may be related to the difference of soil N concentration (Trasar-Cepeda et al. 2008; Shi et al. 2018).

Supporting our first hypothesis, we found that soil acid phosphatase activity increased in N treatment (Fig.1d), indicating that greater availability of N stimulated biotic demands of P (Johnson et al. 2005; Hou et al. 2015). Consistent with our results, studies on various ecosystems showed that N addition can

promote soil acid phosphatase activity (Yokoyama et al. 2017; Heuck et al. 2018; Jia et al. 2020; Tie et al. 2020b). In this study, N treatment significantly increased soil total C:P and N:P ratios (Table 2), indicating the increase of soil C and N concentrations led to the lack of P in the soil (Tian et al. 2010; Zechmeister-Boltenstern et al. 2011). We also found that soil acid phosphatase activity was positively correlated with total C:P (Fig.2j) and total N:P (Fig.2k) ratios, meaning that with the increase of soil C and N concentrations or the decrease of soil P concentrations, soil acid phosphatase activity will increase. Therefore, the increase of soil acid phosphatase activity may be the result of excessive N resources being used by microbial and plants for the synthesis of phosphatase to enhancing the release of inorganic P from organic P compounds (McGill and Cole 1981; Nasto et al. 2014; Zhang et al. 2018). Moreover, soil TP and AP concentrations decreased with the N addition (Table 2) in our research, suggesting that N addition increased soil P uptake by plants and microorganisms (Wang et al. 2007a; Schleuss et al. 2020), which providing additional evidence for microbial and plants production of phosphatase to obtain P in soil (Marklein and Houlton 2012). All of these results imply that soil microorganisms and plants can alleviate soil P deficiency caused by N addition to a certain extent by regulating acid phosphatase activity (Olander and Vitousek 2000; Treseder and Vitousek 2001; Marklein and Houlton 2012). However, the reason for the significantly negative association between the acid phosphatase activity and MBC concentrations was unclear in our study (Fig.1l). We infer the result may be related to the change of soil microbial community composition caused by N addition (Cusack et al. 2011; Jian et al. 2016). In our previous studies revealed that although N addition reduced soil total bacterial biomass, it increased the proportion of bacterial community (the main producer of soil hydrolase) in the 0-20 cm soil layer (Zhou et al. 2017b).

Effects of P addition soil hydrolytic enzymes

In agreement with our second hypothesis, we found that P treatment significantly decreased soil invertase and cellulase mean activities (Fig.1a, b). This result was consistent with the results found in other subtropical forests, where C-Hy EAs were strongly inhibited by P addition (Zheng et al. 2015; Fang et al. 2019; Wang et al. 2020). The decrease of soil invertase and cellulase activities after P addition may be attributed to the following three possible mechanisms: First, P addition may make microbes down regulate their C investments on phosphatase production (Turner and Wright 2014; Wang et al. 2020a). Second, P addition may increase in plant-derived C influx to soils by increased root biomass (Hector 2006; Brzostek et al. 2013; Zhu et al. 2013), and the enrichment in P could release degradable organic C from Fe/Al oxides into soil solution, thus elevating soil C availability (Mori et al. 2018), thereby reducing soil invertase and cellulase activities. This was also supported by our results that soil invertase and cellulase activities had significant positive correlation with soil total C:P ratio (Fig.2b). Third, P addition may reduce in C-hydrolyzing enzymes abundance in soil. On the one hand, P addition may reduce the gene abundance of enzymes that degrade labile C substrates in the soil and decreased the expression of C-hydrolyzing enzymes (Yao et al. 2018). On the other hand, the changes in microbial composition caused by P addition may also reduce soil C-hydrolyzing enzymes abundance. Previous studies have shown that soil MBC had no significant response to P addition in subtropical broad-leaved forests, but greatly

increased the relative abundance of symbiotic fungi (Liu et al. 2012), which may reduce the production of C-hydrolyzing enzymes in the soil, because fungi acquire C from host plants rather than producing C degrading enzymes (Gartner et al. 2012). In addition, in this study, we also found that the addition of P significantly reduced the SOC concentration (Table 2), which may be caused by the addition of P to increase the microbial activity in the soil, resulting in a higher rapid of soil organic matter transformation (Liu et al. 2012; Wang et al. 2020c). Therefore, it is not surprising that SOC concentration decreases even if C-Hy EAs is inhibited under P addition. This result may imply that the increase in soil P availability will have a negative impact on soil C sequestration compared with the positive effects of N addition (Reay et al. 2008; Stiles et al. 2017).

Contrary to our expectation, there was no significant effect of P treatment on soil urease mean activity in this study (Fig.1c), meaning that maybe there was no close relationship between soil N demand and urease activity after P addition (Olander and Vitousek 2000). Consistent with our results, P addition had no significant effect on soil urease and other N-hydrolyzing enzymes activities, as indicated by a recent meta-analyze (Xiao et al. 2018) and some experiments (Wang et al. 2020a; Zhang et al. 2020). We speculated that the neutral effect of P addition on soil urease activity observed in this study may be related to the N status of our forest ecosystem. In our study region, the annual average N deposition (wet) reached 95 kg ha^{-1} (Xu et al. 2013), the higher N deposition may provide sufficient available N for the forest ecosystem in this region (Zhou et al. 2017a). When there was enough N to meet the needs of plants and microorganisms, the production of microbial N-hydrolyzing enzymes will return to the constituent level (Chróst 1991), and soil N-Hy EAs may not change with P addition. In addition, since N is abundant and short-term P addition may not affect the soil N supply (Zhu et al. 2015), a short-term P addition might prevent us from detecting statistically significant changes in soil urease activity. From this, compared with the strict control of phosphatase activity by N addition, the regulation of N-hydrolyzing enzymes by P addition is much weaker in our experimental forest.

In our study, we found P treatment significantly decreased soil acid phosphatase mean activity (Fig.1d), which was consistent with our second hypothesis and the general pattern in most terrestrial ecosystems that P addition have inhibitory effect on soil phosphatase activities (Marklein and Houlton 2012; Xiao et al. 2018), especially in the tropical/subtropical forest ecosystems (Turner and Joseph Wright 2014; Zheng et al. 2015; Yokoyama et al. 2017). In our study, we also found that P treatment significantly reduced the soil total C:P ratio (Table 2). Compared with the higher soil total C:P ratio, the lower soil total C:P ratio after P addition indicated that increased availability of P in the soil, plant and soil microorganisms growth were not restricted by P (Cleveland and Townsend 2006; Mooshammer et al. 2012), the competition between plants and soil microorganisms for P therefore was weakened, thus reducing the microbial dependence on organic P mineralization as the main source of P (Rodríguez and Fraga 1999; Wang et al. 2007b). That is, when the supply of P is sufficient, microorganisms will reduce the investment in phosphatase and the rate of P mineralization will decrease (Marklein and Houlton 2012; Yokoyama et al. 2017). This was also supported by our results that soil AP and TP concentrations had a very significant negative correlation with soil acid phosphatase activity (Fig.2h, i).

Effects of NP co-addition soil hydrolytic enzymes

In our study, NP treatment significantly increased soil invertase and cellulase mean activities (Fig.1 a-b), which is consistent with our third hypothesis. We also found that NP treatment significantly increased soil pH and MBC (Table 2). Therefore, we speculated that the increase in soil invertase and cellulases activities caused by NP treatment might be the result of the increased demand for availability C by soil biological growth (Cleveland et al. 2002; Li et al. 2015). However, unexpectedly, we found NP treatment did not significantly affect the SOC concentration (Table 2), in our study, possible explanation could be the positive effects of N addition on SOC sequestration counteracted by the negative effects of P addition on SOC sequestration, indicating that the SOC mineralization was limited by soil P availability, and P addition could mitigate the effects of N on soil C sequestration in this system (Bradford et al. 2008; Street et al. 2018). In contrast to our third hypothesis that NP treatment would inhibit soil urease and acid phosphatase activities, we found that NP treatment did not significantly change soil urease and acid phosphatase mean activities in our study (Fig.1c, d). The results showed that that under the combination N and P addition, the addition of P might counteract the inhibition of N availability on soil urease activity and the promotion of acid phosphatase activity in subtropical forest soil. We also found that soil N and P concentrations and their stoichiometric ratios were not significantly affected by NP treatment (Table 2), which may explain the minor response of soil urease and phosphatase activities to NP treatment in this study. Consistent with our results, recent meta-analyses also indicated that NP co-addition significantly increased soil C-Hy EAs but the effect on soil N- and P-Hy EAs were not significantly (Xiao et al. 2018).

Different from the effect of alone N or P addition, the effect of NP co-addition on soil hydrolytic enzymes activities may also be the result of the interaction effect of N and P, because there is a widespread close coupling relationship between N and P in the ecosystem (Peñuelas et al. 2013a, b; Gao et al. 2014). Indeed, our results also showed that the interaction of N and P addition had a significant effect on soil invertase, urease, and acid phosphatase activities (Table 1), the interactions found in this study may be attributed to the opposing responses of alone N and P addition on soil C-, P-, and N-Hy EAs, and further research is needed.

Conclusions

In summary, in our study, N addition increased soil invertase, cellulase, and acid phosphatase activities, but decreased soil urease activity. In contrast, P addition decreased soil invertase, cellulase, and acid phosphatase activities, while not affect soil urease activity. The responses of N and P addition on soil hydrolase activity may be related to the change of soil nutrient availability and microbial biomass. Overall, in our studied subtropical evergreen broad-leaved forest, ongoing N deposition may increase soil C sequestration, but will result in the limitation of soil C and P availability. Although the exogenous P input may reduce the soil C sequestration, while alleviates the limitation of soil C and P availability caused by the long-term of N deposition.

Declarations

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

1. Ajwa HA, Dell CJ, Rice CW (1999) Changes in enzyme activities and microbial biomass of tallgrass prairie soil as related to burning and nitrogen fertilization. *Soil Biol Biochem* 31:769–777. [https://doi.org/10.1016/S0038-0717\(98\)00177-1](https://doi.org/10.1016/S0038-0717(98)00177-1)
2. Allen SE, Grimshaw AH, Parkinson JA QC (1974) *Chemical Analysis of Ecological Materials*
3. Allison SD, Czimczik CI, Treseder KK (2008) Microbial activity and soil respiration under nitrogen addition in Alaskan boreal forest. *Glob Chang Biol* 14:1156–1168. <https://doi.org/10.1111/j.1365-2486.2008.01549.x>
4. Allison SD, Vitousek PM (2005) Responses of extracellular enzymes to simple and complex nutrient inputs. *Soil Biol Biochem* 37:937–944. <https://doi.org/10.1016/j.soilbio.2004.09.014>
5. Allison VJ, Condon LM, Peltzer DA, Richardson SJ, Turner BL (2007) Changes in enzyme activities and soil microbial community composition along carbon and nutrient gradients at the Franz Josef chronosequence, New Zealand. *Soil Biol Biochem* 39:1770–1781. <https://doi.org/10.1016/j.soilbio.2007.02.006>
6. Bradford MA, Fierer N, Reynolds JF (2008) Soil carbon stocks in experimental mesocosms are dependent on the rate of labile carbon, nitrogen and phosphorus inputs to soils. *Funct Ecol* 22:964–974. <https://doi.org/10.1111/j.1365-2435.2008.01404.x>
7. Brzostek ER, Greco A, Drake JE, Finzi AC (2013) Root carbon inputs to the rhizosphere stimulate extracellular enzyme activity and increase nitrogen availability in temperate forest soils. *Biogeochemistry* 115:65–76. <https://doi.org/10.1007/s10533-012-9818-9>
8. Buchkowski RW, Schmitz OJ, Bradford MA (2015) Microbial stoichiometry overrides biomass as a regulator of soil carbon and nitrogen cycling. *Ecology* 96:1139–1149. <https://doi.org/10.1890/14-1327.1>
9. Burns RG, DeForest JL, Marxsen J, Sinsabaugh RL, Stromberger ME, Wallenstein MD, Weintraub MN, Zoppini A (2013) Soil enzymes in a changing environment: Current knowledge and future directions. *Soil Biol Biochem* 58:216–234. <https://doi.org/10.1016/j.soilbio.2012.11.009>

10. Cenini VL, Fornara DA, McMullan G, Ternan N, Carolan R, Crawley MJ, Clément JC, Lavorel S (2016) Linkages between extracellular enzyme activities and the carbon and nitrogen content of grassland soils. *Soil Biol Biochem* 96:198–206. <https://doi.org/10.1016/j.soilbio.2016.02.015>
11. Chen J, Luo Y, García-Palacios P, Cao J, Dacal M, Zhou X, Li J, Xia J, Niu S, Yang H, Shelton S, Guo W, van Groenigen KJ (2018a) Differential responses of carbon-degrading enzyme activities to warming: Implications for soil respiration
12. Chen J, Luo Y, Li J, Zhou X, Cao J, Wang RW, Wang Y, Shelton S, Jin Z, Walker LM, Feng Z, Niu S, Feng W, Jian S, Zhou L (2017) Costimulation of soil glycosidase activity and soil respiration by nitrogen addition. *Glob Chang Biol* 23:1328–1337. <https://doi.org/10.1111/gcb.13402>
13. Chen J, Luo Y, Van Groenigen KJ, Hungate BA, Cao J, Zhou X, Wang R wu (2018b) A keystone microbial enzyme for nitrogen control of soil carbon storage. *Sci Adv* 4:2–8. <https://doi.org/10.1126/sciadv.aag1689>
14. Chróst RJ (1991) Environmental Control of the Synthesis and Activity of Aquatic Microbial Ectoenzymes
15. Cleveland CC, Houlton BZ, Smith WK, Marklein AR, Reed SC, Parton W, Del Grosso SJ, Running SW (2013) Patterns of new versus recycled primary production in the terrestrial biosphere. *Proc Natl Acad Sci U S A* 110:12733–12737. <https://doi.org/10.1073/pnas.1302768110>
16. Cleveland CC, Townsend AR (2006) Nutrient additions to a tropical rain forest drive substantial soil carbon dioxide losses to the atmosphere. *Proc Natl Acad Sci U S A* 103:10316–10321. <https://doi.org/10.1073/pnas.0600989103>
17. Cleveland CC, Townsend AR, Schmidt SK (2002) Phosphorus limitation of microbial processes in moist tropical forests: Evidence from short-term laboratory incubations and field studies. *Ecosystems* 5:680–691. <https://doi.org/10.1007/s10021-002-0202-9>
18. Crim PM, Cumming JR (2020) Extracellular Soil Enzyme Activities in High-Elevation Mixed Red Spruce Forests in Central Appalachia,U.S.A. *Forests* 11:1–13. <https://doi.org/10.3390/F11040468>
19. Cusack DF, Silver WL, Torn MS, Burton SD, Firestone MK (2011) Changes in microbial community characteristics and soil organic matter with nitrogen additions in two tropical forests. *Ecology* 92:621–632. <https://doi.org/10.1890/10-0459.1>
20. DeForest JL, Zak DR, Pregitzer KS, Burton AJ (2004) Atmospheric Nitrate Deposition, Microbial Community Composition, and Enzyme Activity in Northern Hardwood Forests. *Soil Sci Soc Am J* 68:132–138. <https://doi.org/10.2136/sssaj2004.1320>
21. Du E, De Vries W, Han W, Liu X, Yan Z, Jiang Y (2016) Imbalanced phosphorus and nitrogen deposition in China's forests. *Atmos Chem Phys* 16:8571–8579. <https://doi.org/10.5194/acp-16-8571-2016>
22. Eivazi F, Tabatabai MA (1990) Factors affecting glucosidase and galactosidase activities in soils. *Soil Biol Biochem* 22:891–897. [https://doi.org/10.1016/0038-0717\(90\)90126-K](https://doi.org/10.1016/0038-0717(90)90126-K)
23. Elser JJ, Bracken MES, Cleland EE, Gruner DS, Harpole WS, Hillebrand H, Ngai JT, Seabloom EW, Shurin JB, Smith JE (2007) Global analysis of nitrogen and phosphorus limitation of primary

- producers in freshwater, marine and terrestrial ecosystems. *Ecol Lett* 10:1135–1142.
<https://doi.org/10.1111/j.1461-0248.2007.01113.x>
24. Enowashu E, Poll C, Lamersdorf N, Kandeler E (2009) Microbial biomass and enzyme activities under reduced nitrogen deposition in a spruce forest soil. *Appl Soil Ecol* 43:11–21.
<https://doi.org/10.1016/j.apsoil.2009.05.003>
 25. Fang H, Geng J, Cheng S, Xu M, Lu, Yu G, Cao Z (2019) Effects of Nitrogen and Phosphorus Enrichment on Carbon Sequestration in Forest. *Acta Pedol Sin* 56:1–11
 26. Fang X, Zhang X, Chen F, Zong Y, Bu W, Wan S, Luo Y, Wang H (2019) Phosphorus addition alters the response of soil organic carbon decomposition to nitrogen deposition in a subtropical forest. *Soil Biol Biochem* 133:119–128. <https://doi.org/10.1016/j.soilbio.2019.03.005>
 27. Fanin N, Hättenschwiler S, Schimann H, Fromin N (2015) Interactive effects of C, N and P fertilization on soil microbial community structure and function in an Amazonian rain forest. *Funct Ecol* 29:140–150. <https://doi.org/10.1111/1365-2435.12329>
 28. Fatemi FR, Fernandez IJ, Simon KS, Bryan D (2016) Nitrogen and phosphorus regulation of soil enzyme activities in acid forest soils. *Soil Biol Biochem* 98:171–179.
<https://doi.org/10.1016/j.soilbio.2016.02.017>
 29. Feng H, Yu M, Xue L (2020) Effects of nitrogen and phosphorus additions on soil enzyme activities in *Acacia auriculiformis* stands under different planting densities. *Acta Ecol Sin* 40:4894–4902.
<https://doi.org/10.5846/stxb201910162163>
 30. Galloway JN (2000) Nitrogen mobilization in Asia. *Nutr Cycl Agroecosystems* 57:1–12.
<https://doi.org/10.1023/A:1009832221034>
 31. 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, Vörösmarty CJ (2004) Nitrogen cycles: Past, present, and future. *Biogeochemistry* 70:153–226. <https://doi.org/10.1007/s10533-004-0370-0>
 32. Gao Y, He N, Yu G, Chen W, Wang Q (2014) Long-term effects of different land use types on C, N, and P stoichiometry and storage in subtropical ecosystems: A case study in China. *Ecol Eng* 67:171–181.
<https://doi.org/10.1016/j.ecoleng.2014.03.013>
 33. Gartner TB, Treseder KK, Malcolm GM, Sinsabaugh RL (2012) Extracellular enzyme activity in the mycorrhizospheres of a boreal fire chronosequence. *Pedobiologia* 55:121–127.
<https://doi.org/10.1016/j.pedobi.2011.12.003>
 34. Guan Y (1986) *Soil Enzymes and its Methodology*. Agricultural Press, Beijing
 35. Hector A (2006)overyielding and stable species coexistence. *New Phytol* 172:1–3.
<https://doi.org/10.1111/j.1469-8137.2006.01865.x>
 36. Heuck C, Smolka G, Whalen ED, Frey S, Gundersen P, Moldan F, Fernandez IJ, Spohn M (2018) Effects of long-term nitrogen addition on phosphorus cycling in organic soil horizons of temperate forests. *Biogeochemistry* 141:167–181. <https://doi.org/10.1007/s10533-018-0511-5>

37. Hill BH, Elonen CM, Seifert LR, May AA, Tarquinio E (2012) Microbial enzyme stoichiometry and nutrient limitation in US streams and rivers. *Ecol Indic* 18:540–551.
<https://doi.org/10.1016/j.ecolind.2012.01.007>
38. Hopkins DW, Sparrow AD, Shillam LL, English LC, Dennis PG, Novis P, Elberling B, Gregorich EG, Greenfield LG (2008) Enzymatic activities and microbial communities in an Antarctic dry valley soil: Responses to C and N supplementation. *Soil Biol Biochem* 40:2130–2136.
<https://doi.org/10.1016/j.soilbio.2008.03.022>
39. Hou E, Chen C, Wen D, Liu X (2015) Phosphatase activity in relation to key litter and soil properties in mature subtropical forests in China. *Sci Total Environ* 515–516:83–91.
<https://doi.org/10.1016/j.scitotenv.2015.02.044>
40. Hu YL, Jung K, Zeng DH, Chang SX (2013) Nitrogen- and sulfur-deposition-altered soil microbial community functions and enzyme activities in a boreal mixedwood forest in western Canada. *Can J For Res* 43:777–784. <https://doi.org/10.1139/cjfr-2013-0049>
41. Huang J, Chen W, Qi K, Yang B, Bao W, Pang X (2018) Distinct effects of N and P addition on soil enzyme activities and C distribution in aggregates in a subalpine spruce plantation. *Biogeochemistry* 141:199–212. <https://doi.org/10.1007/s10533-018-0513-3>
42. Jia X, Zhong Y, Liu J, Zhu G, Shangguan Z, Yan W (2020) Effects of nitrogen enrichment on soil microbial characteristics: From biomass to enzyme activities. *Geoderma* 366:.
<https://doi.org/10.1016/j.geoderma.2020.114256>
43. Jian S, Li J, Chen J, Wang G, Mayes MA, Dzantor KE, Hui D, Luo Y (2016) Soil extracellular enzyme activities, soil carbon and nitrogen storage under nitrogen fertilization: A meta-analysis. *Soil Biol Biochem* 101:32–43. <https://doi.org/10.1016/j.soilbio.2016.07.003>
44. Jing X, Chen X, Tang M, Ding Z, Jiang L, Li P, Ma S, Tian D, Xu L, Zhu J, Ji C, Shen H, Zheng C, Fang J, Zhu B (2017) Science of the Total Environment Nitrogen deposition has minor effect on soil extracellular enzyme activities in six Chinese forests. *Sci Total Environ* 607–608:806–815.
<https://doi.org/10.1016/j.scitotenv.2017.07.060>
45. Jing X, Yang X, Ren F, Zhou H, Zhu B, He JS (2016) Neutral effect of nitrogen addition and negative effect of phosphorus addition on topsoil extracellular enzymatic activities in an alpine grassland ecosystem. *Appl Soil Ecol* 107:205–213. <https://doi.org/10.1016/j.apsoil.2016.06.004>
46. Johnson D, Leake JR, Read DJ (2005) Liming and nitrogen fertilization affects phosphatase activities, microbial biomass and mycorrhizal colonisation in upland grassland. *Plant Soil* 271:157–164. <https://doi.org/10.1007/s11104-004-2267-z>
47. Kang H, Lee D (2005) Inhibition of extracellular enzyme activities in a forest soil by additions of inorganic nitrogen. *Commun Soil Sci Plant Anal* 36:2129–2135.
<https://doi.org/10.1080/00103620500194650>
48. Kassem Alef and Paolo Nannipieri (1995) *Methods in Applied Soil Microbiology and Biochemistry*. Academic Press, London

49. Kathleen K. Treseder PMV (2001) Effects of soil nutrient availability on investment in acquisition of N AND P in Hawaiian rain forests. *Ecology* 82:946–954
50. Katsalirou E, Deng S, Nofziger DL, Gerakis A (2010) Long-term management effects on organic C and N pools and activities of C-transforming enzymes in prairie soils. *Eur J Soil Biol* 46:335–341. <https://doi.org/10.1016/j.ejsobi.2010.06.004>
51. Li J, Li Z, Wang F, Zou B, Chen Y, Zhao J, Mo Q, Li Y, Li X, Xia H (2015) Effects of nitrogen and phosphorus addition on soil microbial community in a secondary tropical forest of China. *Biol Fertil Soils* 51:207–215. <https://doi.org/10.1007/s00374-014-0964-1>
52. Liu L, Gundersen P, Zhang T, Mo J (2012) Effects of phosphorus addition on soil microbial biomass and community composition in three forest types in tropical China. *Soil Biol Biochem* 44:31–38. <https://doi.org/10.1016/j.soilbio.2011.08.017>
53. Lloyd AB, Sheaffe MJ (1973) Urease activity in soils. *Plant Soil* 39:71–80. <https://doi.org/10.1007/BF00018046>
54. Lu X, Gilliam FS, Yu G, Li L, Mao Q, Chen H, Mo J (2013) Long-term nitrogen addition decreases carbon leaching in a nitrogen-rich forest ecosystem. *Biogeosciences* 10:3931–3941. <https://doi.org/10.5194/bg-10-3931-2013>
55. Mahowald N, Jickells TD, Baker AR, Artaxo P, Benitez-Nelson CR, Bergametti G, Bond TC, Chen Y, Cohen DD, Herut B, Kubilay N, Losno R, Luo C, Maenhaut W, McGee KA, Okin GS, Siefert RL, Tsukuda S (2008) Global distribution of atmospheric phosphorus sources, concentrations and deposition rates, and anthropogenic impacts. *Global Biogeochem Cycles* 22:1–19. <https://doi.org/10.1029/2008GB003240>
56. Marklein AR, Houlton BZ (2012) Nitrogen inputs accelerate phosphorus cycling rates across a wide variety of terrestrial ecosystems. *New Phytol* 193:696–704. <https://doi.org/10.1111/j.1469-8137.2011.03967.x>
57. McGill WB, Cole CV (1981) Comparative aspects of cycling of organic C, N, S and P through soil organic matter. *Geoderma* 26:267–286. [https://doi.org/10.1016/0016-7061\(81\)90024-0](https://doi.org/10.1016/0016-7061(81)90024-0)
58. Mganga KZ, Razavi BS, Kuzyakov Y (2015) Microbial and enzymes response to nutrient additions in soils of Mt. Kilimanjaro region depending on land use. *Eur J Soil Biol* 69:33–40. <https://doi.org/10.1016/j.ejsobi.2015.05.001>
59. Mooshammer M, Wanek W, Schneckner J, Wild B, Leitner S, Hofhansl F, Blochl A, Hammerle I, Frank AH, Fuchslueger L, Keiblinger KM, Zechmeister-Boltenstern S, Richter A (2012) Stoichiometric controls of nitrogen and phosphorus cycling in decomposing beech leaf litter. *Ecology* 93:770–782. <https://doi.org/10.1890/11-0721.1>
60. Mori T, Lu X, Aoyagi R, Mo J (2018) Reconsidering the phosphorus limitation of soil microbial activity in tropical forests. *Funct Ecol* 32:1145–1154. <https://doi.org/10.1111/1365-2435.13043>
61. Nannipieri P, Trasar-Cepeda C, Dick RP (2018) Soil enzyme activity: a brief history and biochemistry as a basis for appropriate interpretations and meta-analysis. *Biol Fertil Soils* 54:11–19. <https://doi.org/10.1007/s00374-017-1245-6>

62. Nasto MK, Alvarez-Clare S, Lekberg Y, Sullivan BW, Townsend AR, Cleveland CC (2014) Interactions among nitrogen fixation and soil phosphorus acquisition strategies in lowland tropical rain forests. *Ecol Lett* 17:1282–1289. <https://doi.org/10.1111/ele.12335>
63. Olander LP, Vitousek PM (2000) Regulation of soil phosphatase and chitinase activity by N and P availability. *Biogeochemistry* 49:175–190. <https://doi.org/10.1023/A:1006316117817>
64. Pancholy Sunil K, Elroy LRice (1973) Carbohydrases in Soil as Affected by Successional Stages of Revegetation. *Soil Sci Soc Am J* 37:227–229
65. Peñuelas J, Poulter B, Sardans J, Ciais P, Van Der Velde M, Bopp L, Boucher O, Godderis Y, Hinsinger P, Llusia J, Nardin E, Vicca S, Obersteiner M, Janssens IA (2013a) Human-induced nitrogen-phosphorus imbalances alter natural and managed ecosystems across the globe. *Nat Commun* 4: <https://doi.org/10.1038/ncomms3934>
66. Peñuelas J, Sardans J, Estiarte M, Ogaya R, Carnicer J, Coll M, Barbeta A, Rivas-Ubach A, Llusia J, Garbulsky M, Filella I, Jump AS (2013b) Evidence of current impact of climate change on life: A walk from genes to the biosphere. *Glob Chang Biol* 19:2303–2338. <https://doi.org/10.1111/gcb.12143>
67. Reay DS, Dentener F, Smith P, Grace J, Feely RA (2008) Global nitrogen deposition and carbon sinks. *Nat Geosci* 1:430–437. <https://doi.org/10.1038/ngeo230>
68. Rodríguez-Loinaz G, Onaindia M, Amezaga I, Mijangos I, Garbisu C (2008) Relationship between vegetation diversity and soil functional diversity in native mixed-oak forests. *Soil Biol Biochem* 40:49–60. <https://doi.org/10.1016/j.soilbio.2007.04.015>
69. Rodríguez H, Fraga R (1999) Phosphate solubilizing bacteria and their role in plant growth promotion. *Biotechnol Adv* 17:319–339. [https://doi.org/10.1016/S0734-9750\(99\)00014-2](https://doi.org/10.1016/S0734-9750(99)00014-2)
70. Saiya-Cork KR, Sinsabaugh RL, Zak DR (2002) The effects of long term nitrogen deposition on extracellular enzyme activity in an *Acer saccharum* forest soil. *Soil Biol Biochem* 34:1309–1315. [https://doi.org/10.1016/S0038-0717\(02\)00074-3](https://doi.org/10.1016/S0038-0717(02)00074-3)
71. Schleuss PM, Widdig M, Heintz-Buschart A, Kirkman K, Spohn M (2020) Interactions of nitrogen and phosphorus cycling promote P acquisition and explain synergistic plant-growth responses PER. *Ecology* 101:1–14. <https://doi.org/10.1002/ecy.3003>
72. Shi B, Zhang J, Wang C, Ma J, Sun W (2018) Responses of hydrolytic enzyme activities in saline-alkaline soil to mixed inorganic and organic nitrogen addition. *Sci Rep* 8:1–12. <https://doi.org/10.1038/s41598-018-22813-9>
73. Sinsabaugh RL, Gallo ME, Lauber C, Waldrop MP, Zak DR (2005) Extracellular enzyme activities and soil organic matter dynamics for northern hardwood forests receiving simulated nitrogen deposition. *Biogeochemistry* 75:201–215. <https://doi.org/10.1007/s10533-004-7112-1>
74. Sinsabaugh RL, Lauber CL, Weintraub MN, Ahmed B, Allison SD, Crenshaw C, Contosta AR, Cusack D, Frey S, Gallo ME, Gartner TB, Hobbie SE, Holland K, Keeler BL, Powers JS, Stursova M, Takacs-Vesbach C, Waldrop MP et al (2008) Stoichiometry of soil enzyme activity at global scale. *Ecol Lett* 11:1252–1264. <https://doi.org/10.1111/j.1461-0248.2008.01245.x>

75. Sinsabaugh RL, Moorhead DL (1994) Resource allocation to extracellular enzyme production: A model for nitrogen and phosphorus control of litter decomposition. *Soil Biol Biochem* 26:1305–1311. [https://doi.org/10.1016/0038-0717\(94\)90211-9](https://doi.org/10.1016/0038-0717(94)90211-9)
76. Song XG, Hu TX, Xian JR, Xiao CL (2009) Soil enzyme activities and its response to simulated nitrogen deposition in an evergreen broad-leaved forest, southern Sichuan. *Shengtai Xuebao/ Acta Ecol Sin* 29:1234–1240
77. Song Y, Song C, Tao B, Wang J, Zhu X, Wang X (2014) Short-term responses of soil enzyme activities and carbon mineralization to added nitrogen and litter in a freshwater marsh of Northeast China. *Eur J Soil Biol* 61:72–79. <https://doi.org/10.1016/j.ejsobi.2014.02.001>
78. Stiles WAV, Rowe EC, Dennis P (2017) Long-term nitrogen and phosphorus enrichment alters vegetation species composition and reduces carbon storage in upland soil. *Sci Total Environ* 593–594:688–694. <https://doi.org/10.1016/j.scitotenv.2017.03.136>
79. Street LE, Mielke N, Woodin SJ (2018) Phosphorus Availability Determines the Response of Tundra Ecosystem Carbon Stocks to Nitrogen Enrichment. *Ecosystems* 21:1155–1167. <https://doi.org/10.1007/s10021-017-0209-x>
80. Sun T, Dong L, Wang Z, Lü X, Mao Z (2016) Effects of long-term nitrogen deposition on fine root decomposition and its extracellular enzyme activities in temperate forests. *Soil Biol Biochem* 93:50–59. <https://doi.org/10.1016/j.soilbio.2015.10.023>
81. Sun Y, Peng T, Li S, Zhou P, Wang T, Qian X, Liu X, Chen Y, Ma H, Li Z, Tu L (2019) Effects of simulated nitrogen deposition on soil carbon and nitrogen fractions and enzyme activity in moist evergreen broad-leaved secondary forest. *J Soil Water Conserv* 33:235–243, 250
82. Tian H, Chen G, Zhang C, Melillo JM, Hall CAS (2010) Pattern and variation of C:N:P ratios in China's soils: A synthesis of observational data. *Biogeochemistry* 98:139–151. <https://doi.org/10.1007/s10533-009-9382-0>
83. Tie L, Fu R, Peñuelas J, Sardans J, Zhang S, Zhou S, Hu J, Huang C (2020a) The additions of nitrogen and sulfur synergistically decrease the release of carbon and nitrogen from litter in a subtropical forest. *Forests* 11:1–16. <https://doi.org/10.3390/f11121280>
84. Tie L, Zhang S, Peñuelas J, Sardans J, Zhou S, Hu J, Huang C (2020b) Responses of soil C, N, and P stoichiometric ratios to N and S additions in a subtropical evergreen broad-leaved forest. *Geoderma* 379:114633. <https://doi.org/10.1016/j.geoderma.2020.114633>
85. Trasar-Cepeda C, Leirós MC, Gil-Sotres F (2008) Hydrolytic enzyme activities in agricultural and forest soils. Some implications for their use as indicators of soil quality. *Soil Biol Biochem* 40:2146–2155. <https://doi.org/10.1016/j.soilbio.2008.03.015>
86. Turner BL, Joseph Wright S (2014) The response of microbial biomass and hydrolytic enzymes to a decade of nitrogen, phosphorus, and potassium addition in a lowland tropical rain forest. *Biogeochemistry* 117:115–130. <https://doi.org/10.1007/s10533-013-9848-y>
87. Vitousek PM, Aber JD, Howarth RW, Likens GE, Matson PA, Schindler DW, Schlesinger WH, Tilman DG (1997) Human alteration of the global nitrogen cycle: Sources and consequences. *Ecol Appl* 7:737–

750. [https://doi.org/10.1890/1051-0761\(1997\)007\[0737:HAOTGN\]2.0.CO;2](https://doi.org/10.1890/1051-0761(1997)007[0737:HAOTGN]2.0.CO;2)
88. Wang C, Mori T, Mao Q, Zhou K, Wang Z, Zhang Y, Mo H, Lu X, Mo J (2020a) Long-term phosphorus addition downregulates microbial investments on enzyme productions in a mature tropical forest. *J Soils Sediments* 20:921–930. <https://doi.org/10.1007/s11368-019-02450-z>
89. Wang QK, Wang SL, Liu YX (2008) Responses to N and P fertilization in a young *Eucalyptus dunnii* plantation: Microbial properties, enzyme activities and dissolved organic matter. *Appl Soil Ecol* 40:484–490. <https://doi.org/10.1016/j.apsoil.2008.07.003>
90. Wang R, Cao Y, Wang H, Dijkstra FA, Jiang J, Zhao R, Ma W, Li T, Dorodnikov M, Wang Z, Sardans J, Peñuelas J (2020b) Exogenous P compounds differentially interacted with N availability to regulate enzymatic activities in a meadow steppe. *Eur J Soil Sci* 71:667–680. <https://doi.org/10.1111/ejss.12906>
91. Wang S, Mori T, Mo J, Zhang W (2020c) The responses of carbon- and nitrogen-acquiring enzymes to nitrogen and phosphorus additions in two plantations in southern China. *J For Res* 31:1319–1324. <https://doi.org/10.1007/s11676-019-00905-0>
92. Wang Y, Houlton BZ, Field CB (2007a) A model of biogeochemical cycles of carbon, nitrogen, and phosphorus including symbiotic nitrogen fixation and phosphatase production A model of biogeochemical cycles of carbon, nitrogen, and phosphorus including symbiotic nitrogen fixation and phosph. *Global Biogeochem Cycles*. <https://doi.org/10.1029/2006GB002797>
93. Wang YP, Houlton BZ, Field CB (2007b) A model of biogeochemical cycles of carbon, nitrogen, and phosphorus including symbiotic nitrogen fixation and phosphatase production. *Global Biogeochem Cycles* 21:1–15. <https://doi.org/10.1029/2006GB002797>
94. Wang YS, Cheng SL, Yu GR, Fang HJ, Mo JM, Xu MJ, Gao WL (2015) Response of carbon utilization and enzymatic activities to nitrogen deposition in three forests of subtropical china. *Can J For Res* 45:394–401. <https://doi.org/10.1139/cjfr-2014-0445>
95. Waring BG, Weintraub SR, Sinsabaugh RL (2014) Ecoenzymatic stoichiometry of microbial nutrient acquisition in tropical soils. *Biogeochemistry* 117:101–113. <https://doi.org/10.1007/s10533-013-9849-x>
96. Wei S, Tie L, Liao J, Liu X, Du M, Lan S, Li X, Li C, Zhan H, Huang C (2020) Nitrogen and phosphorus co-addition stimulates soil respiration in a subtropical evergreen broad-leaved forest. *Plant Soil* 450:171–182. <https://doi.org/10.1007/s11104-020-04498-0>
97. Whittinghill KA, Currie WS, Zak DR, Burton AJ, Pregitzer KS (2012) Anthropogenic N Deposition Increases Soil C Storage by Decreasing the Extent of Litter Decay: Analysis of Field Observations with an Ecosystem Model. *Ecosystems* 15:450–461. <https://doi.org/10.1007/s10021-012-9521-7>
98. Wu J, Joergensen RG, Pommerening B, Chaussod R, Brookes PC (1990) Measurement of soil microbial biomass C by fumigation-extraction-an automated procedure. *Soil Biol Biochem* 22:1167–1169. [https://doi.org/10.1016/0038-0717\(90\)90046-3](https://doi.org/10.1016/0038-0717(90)90046-3)
99. Xiao W, Chen X, Jing X, Zhu B (2018) A meta-analysis of soil extracellular enzyme activities in response to global change. *Soil Biol Biochem* 123:21–32.

- <https://doi.org/10.1016/j.soilbio.2018.05.001>
100. Xu Z, Tu L, Hu T, Schädler M (2013) Implications of greater than average increases in nitrogen deposition on the western edge of the Szechwan Basin, China. *Environ Pollut* 177:201–202. <https://doi.org/10.1016/j.envpol.2012.12.031>
 101. Yao Q, Li Z, Song Y, Wright SJ, Guo X, Tringe SG, Tfaily MM, Paša-Tolić L, Hazen TC, Turner BL, Mayes MA, Pan C (2018) Community proteogenomics reveals the systemic impact of phosphorus availability on microbial functions in tropical soil. *Nat Ecol Evol* 2:499–509. <https://doi.org/10.1038/s41559-017-0463-5>
 102. Yokoyama D, Imai N, Kitayama K (2017) Effects of nitrogen and phosphorus fertilization on the activities of four different classes of fine-root and soil phosphatases in Bornean tropical rain forests. *Plant Soil* 416:463–476. <https://doi.org/10.1007/s11104-017-3225-x>
 103. Zechmeister-Boltenstern S, Michel K, Pfeffer M (2011) Soil microbial community structure in European forests in relation to forest type and atmospheric nitrogen deposition. *Plant Soil* 343:37–50. <https://doi.org/10.1007/s11104-010-0528-6>
 104. Zhang C, Zhang X, Kuzyakov Y, Wang H, Fu X, Yang Y, Chen F, Dungait JAJ, Green SM, Fang X (2020) Responses of C-, N- and P-acquiring hydrolases to P and N fertilizers in a subtropical Chinese fir plantation depend on soil depth. *Appl Soil Ecol* 150:103465. <https://doi.org/10.1016/j.apsoil.2019.103465>
 105. Zhang W, Zhao J, Pan F, Li D, Chen H, Wang K (2015) Changes in nitrogen and phosphorus limitation during secondary succession in a karst region in southwest. *Plant Soil* 391:77–91. <https://doi.org/10.1007/s11104-015-2406-8>
 106. Zhang X, Yang Y, Zhang C, Niu S, Yang H, Yu G, Wang H, Blagodatskaya E, Kuzyakov Y, Tian D, Tang Y, Liu S, Sun X (2018) Contrasting responses of phosphatase kinetic parameters to nitrogen and phosphorus additions in forest soils. *Funct Ecol* 32:106–116. <https://doi.org/10.1111/1365-2435.12936>
 107. Zhang Yi, Wang C, YX, Xu Ke (2016) Effect of simulated atmospheric nitrogen deposition on soil microbial community structure in a temperate forest. *Chinese J Ecol* 35:2676–2683. <https://doi.org/10.13292/j.1000-4890.201610.012>
 108. Zheng Mianhai H, Juan C, Hao, Wang Hui MJ (2015) Responses of soil acid phosphatase and beta-glucosidase to nitrogen and phosphorus addition in two subtropical forests in southern China. *Eur J Soil Biol* 68:77–84. <https://doi.org/10.1016/j.ejsobi.2015.03.010>
 109. Zhou S, Xiang Y, Tie L, Han B, Huang C (2018) Simulated nitrogen deposition significantly reduces soil respiration in an evergreen broadleaf forest in western China. *PLoS One* 13:1–15. <https://doi.org/10.1371/journal.pone.0204661>
 110. Zhou S, Huang C de, Han B, han, Xiao Y, xiang, Tang J dong, Xiang Y bin, Luo C (2017a) Simulated nitrogen deposition significantly suppresses the decomposition of forest litter in a natural evergreen broad-leaved forest in the Rainy Area of Western China. *Plant Soil* 420:135–145. <https://doi.org/10.1007/s11104-017-3383-x>

111. Zhou SX, Xiang Y, Bin, Xiao YX, Huang C, De, Tang JD, Luo C, Han BH, Liang KG (2017b) Response of culturable soil microorganisms to simulated nitrogen deposition in a natural evergreen broadleaf forest in the Rainy Area of Western China. *Acta Ecol Sin* 37:1191–1198. <https://doi.org/10.5846/stxb201605040842>
112. Zhou X, Zhang Y, Downing A (2012) Non-linear response of microbial activity across a gradient of nitrogen addition to a soil from the Gurbantunggut Desert, northwestern China. *Soil Biol Biochem* 47:67–77. <https://doi.org/10.1016/j.soilbio.2011.05.012>
113. Zhu B, Gutknecht JLM, Herman DJ, Keck DC, Firestone MK, Cheng W (2014) Rhizosphere priming effects on soil carbon and nitrogen mineralization. *Soil Biol Biochem* 76:183–192. <https://doi.org/10.1016/j.soilbio.2014.04.033>
114. Zhu F, Lu X, Liu L, Mo J (2015) Phosphate addition enhanced soil inorganic nutrients to a large extent in three tropical forests. *Sci Rep* 5:19–21. <https://doi.org/10.1038/srep07923>
115. Zhu F, Yoh M, Gilliam FS, Lu X, Mo J (2013) Nutrient limitation in three lowland tropical forests in southern China receiving high nitrogen deposition: Insights from fine root responses to nutrient additions. *PLoS One* 8:1–8. <https://doi.org/10.1371/journal.pone.0082661>
116. Zhu J, Wang Q, He N, Smith MD, Elser JJ, Du J, Yuan G, Yu G, Yu Q (2016) Imbalanced atmospheric nitrogen and phosphorus depositions in China: Implications for nutrient limitation. *J Geophys Res Biogeosciences* 121:1605–1616. <https://doi.org/10.1002/2016JG003393>

Figures

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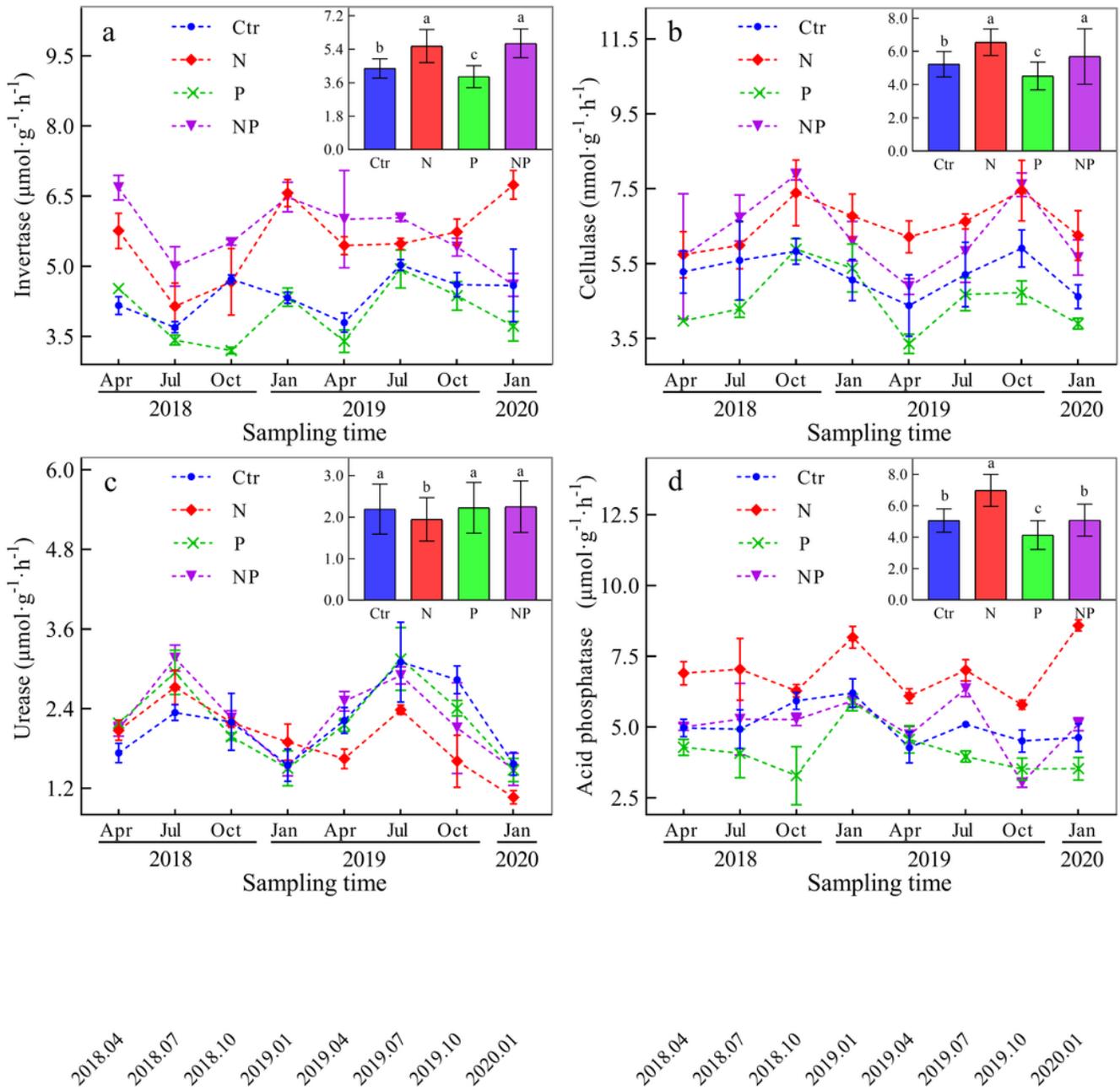


Figure 1

The temporal dynamics and mean value of soil invertase (a), cellulase (b), urease (c) and acid phosphatase (d) activities under N or/and P addition in the subtropical natural evergreen broad-leaved forest. Error bars represent the standard deviations of means ($n=3$). Different lowercase letters denote significant differences (multiple comparisons with Bonferroni adjustment, $P < 0.05$) among different

treatments during two years of fertilization. Ctr: control, without N or P addition; N: N addition treatment; P: P addition treatment; NP: N and P co-addition treatment.

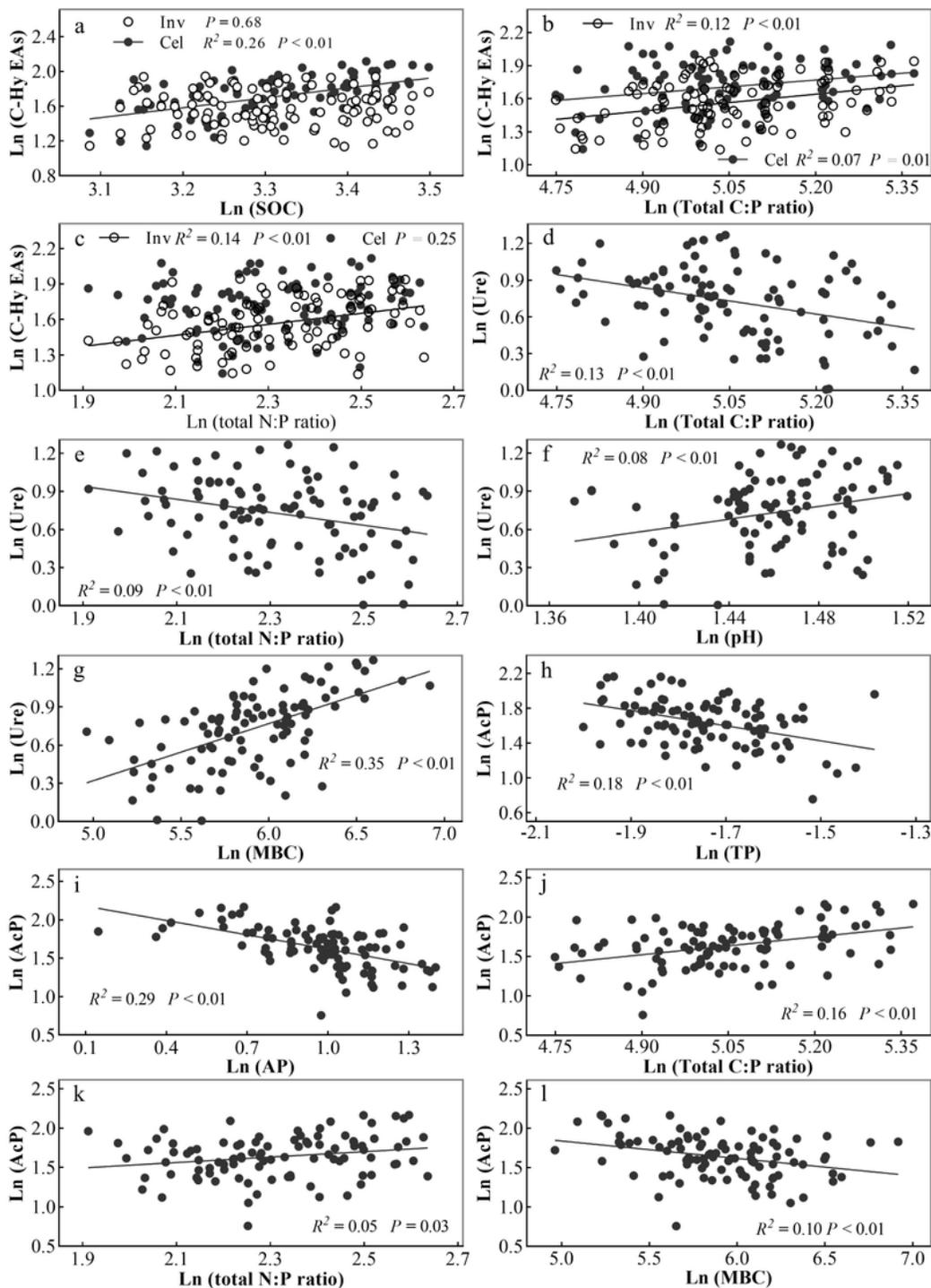


Figure 2

Relationships between the soil invertase (Inv), cellulase (Cel); urease (Ure) and acid phosphatase (AcP) activities with soil organic carbon (SOC), total phosphorus (TP), available phosphorus (AP), and microbial

biomass carbon (MBC) concentrations, pH, total C:P and N:P ratios. The parameters were Ln-transformed to maintain the same order of magnitude.

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