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Phosphorus addition alters the response of soil organic carbon decomposition to nitrogen deposition in a subtropical forest

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ABSTRACT

The continuous increase of nitrogen (N) deposition may exacerbate phosphorus (P) deficiency, which affects soil organic carbon (SOC) decomposition by changing microbial community characteristics in subtropical forests with highly weathered soils. However, there is currently little information about the role of P and the N × P interaction in SOC dynamics. Here, a field nutrient manipulation experiment was established in a subtropical plantation forest in China. Soils collected from simulated N deposition and P addition treatments for 5 years were incubated at 25 °C for 130 days. Soil microbial composition was measured using the phospholipid fatty acid method and the enzyme activities related to SOC hydrolysis were measured. The SOC concentration and δ^{13}C in bulk soil and three particle-size fracfractions were also determined. The cumulative CO_2 respired over 9 days, representing the utilization of carbon sources under field conditions, increased with N deposition levels under the without-P treatment, while no significant differences were found among the three N deposition levels in the with-P treatment. Meanwhile, P addition generally suppressed the SOC decomposition during 130 days incubation. Similarly, P addition decreased the potential organic carbon decomposition (C_0) and C_0/SOC ratio. In contrast, C_0 increased with N deposition in the without-P treatment, while was unaffected by N deposition under the with-P treatment, suggesting the response of SOC decomposition to N deposition was affected following P addition by alteration of SOC quality. Moreover, N deposition tended to deplete the δ^{13}C of the SOC and P addition enriched the δ^{13}C of the macro-particulate organic carbon. Addition of P increased total microbial, fungal and bacterial biomass values by 41.6%, 90.0% and 46.9%, respectively, whereas N deposition had no significant effect. Soil fungi/bacteria ratio significantly increased by N deposition and P addition, which partly explained the reduction of SOC decomposition after P addition. The cellobioside activity significantly decreased by 48.3% after P addition, while cellobioside and β-xylosidase activities increased with N deposition, suggesting that N deposition and P addition had opposite roles in the SOC stability. These results indicate that the positive effect of N deposition on SOC decomposition was suppressed when P was added by changing microbial community and enzyme activity and enhanced P availability may result in increased SOC accumulation under N deposition scenarios in subtropical forests.

1. Introduction

Soil plays a key role in carbon (C) storage in terrestrial ecosystems, but is also an important source of atmospheric CO_2 (Lal, 2004; Lehmann and Kleber, 2015). The pool of soil organic carbon (SOC) in terrestrial ecosystems is more than three times as large as that of atmospheric C (Wang et al., 2017). Therefore, any change of SOC decomposition rate could have a profound influence on SOC sequestration and atmospheric CO_2 concentration (Lal, 2004). Within the last century, there has been a three to fivefold increase in atmospheric nitrogen (N) due to human activities (Galloway et al., 2008). Increased reactive N input has caused extensive effects on quality and quantity of soil

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substrates, soil microbial biomass, composition and enzyme activity, which eventually alters the decomposition rate of SOC (Cusack et al., 2011; Liu et al., 2012; Camenzind et al., 2016). For instance, Wang et al. (2017) found that SOC decomposition and enzyme activity were inhibited following N deposition in a temperate forest. In contrast, Tu et al. (2013) revealed that N addition stimulated different components of soil respiration in a subtropical bamboo forest. Likewise, Janssens et al. (2010) concluded that N deposition typically impeded C cycling in forest soils through a meta-analysis, but this article commented that whether soil C cycling is also affected by N deposition in tropical and N-saturated forests remains an open question. Accordingly, despite considerable research effort, the effects of N deposition on SOC decomposition remain a matter for debate (Tu et al., 2013; Wang et al., 2017).

At the larger scale, the uncertainty of SOC decomposition to N deposition may be related to the degree of N limitation in different forest ecosystems (Bradford et al., 2008; Janssens et al., 2010). In general, N is the limiting element in temperate and the boreal forest ecosystems (Allison et al., 2008; Wang et al., 2017), and our cognition of the ecological effects of N deposition mostly came from N-limited temperate forests (Pregitzer et al., 2008), while tropical and subtropical forests are often rich in or saturated with available N, with rapid rates of N cycling and the lack of N limitation to net primary productivity (NPP) (Matson et al., 1999; Brookshire et al., 2012). The evidence that large dissolved organic N leached by simulated N deposition was reported in three typical subtropical forests in southern China (Fang et al., 2009b), which proved that the subtropical forest soils were under N-saturated conditions. Meanwhile, tropical and subtropical forests are often limited by phosphorus (P) (Camenzind et al., 2018), since much of the P is bound to aluminum and iron complexes during pedogenesis with highly weathered soils (Vitousek et al., 2010). Moreover, most ecosystems gradually became increasingly P deficient with the sustained N deposition, because there was a relative balance of N and P supply in many ecosystems (Elser et al., 2007). Increased N deposition could accelerate the soil P cycle and therefore change the characteristics of soil stoichiometry (Marklein and Houlton, 2012), thus P is considered to be the limiting element in tropical and subtropical forest ecosystems determining the NPP (Vitousek et al., 2010). For example, P addition could increase the belowground NPP and root turnover rates in low P availability Hawaiian montane forests (Ostertag, 2001).

Similarly, P is one of the important factors affecting the microbial community structure and enzyme activity that alters SOC dynamics in tropical or subtropical areas (Camenzind et al., 2016, 2018). It has been reported that microbial biomass and fungi to bacteria ratio increased by enhanced soil P input in a subtropical forest (Liu et al., 2012; Li et al., 2015). Producing hydrolases that depolymerize soil organic matter and convert organically bound nutrients into available forms to microbes is likely to play a key role influencing SOC dynamics (Keuskamp et al., 2015; Wang et al., 2018). Glycosidases, such as cellobioside (CB) and β-glucosidase (BG), are responsible for C degrading and their activities may change if nutrient addition alters microbial allocation to enzyme production or shifts the abundance of fungi or bacteria (Wang et al., 2018). In P-limited forest soil, microbes are likely to the production of extracellular CB and BG that mineralize organic matter, thereby increasing potential release of P (Camenzind et al., 2018). When inorganic P is adequate, soil microbes will decrease the investment of enzyme and therefore reduce the decomposition of organic matter (Zheng et al., 2015). In contrast, the addition of N was detected to decrease the soil microbial biomass (Zhang et al., 2018), but to increase the cellulase activities, accelerating cellulose decomposition in both subtropical and N-limited forest ecosystems (Janssens et al., 2010; Tian et al., 2017). If this pattern is true, additions of P and N may have different effects on SOC decomposition through their alterations on enzyme activities. Moreover, a recent meta-analysis study demonstrated that a variety of microbial processes were predominantly limited by P across tropical forests, and a additional N limitation was found in tropical montane forests (Camenzind et al., 2018). Ilstedt and Singh (2005) suggested P constrained the initial respiration rate, while the maximum level of microbial respiration was limited by N in the P-fixing tropical Acrisol, implying that P and N may work together affecting SOC dynamics in tropical or subtropical areas. However, these specific effects of P availability and its interaction with N on soil microbial community composition and enzyme activity in tropical/subtropical forests are not clear.

The decomposition dynamics of SOC is closely related to the quality of soil substrates (Fang et al., 2015; Li et al., 2015). The ^{13}C natural abundance technique is a practical way to trace the soil substrates dynamics with diverse stability, since SOC is composed of various heterogeneous pools (Balesdent et al., 1988). Coarse-sized soil C fractions derived from recent plant residues with short-term decomposition typically represented by litter-derived ^{13}C-depleted signals, while fine-sized SOC fractions originating from old plant materials are represented by ^{13}C-enriched signals (Fang et al., 2009a). Several studies on soil ^{13}C natural abundance in relation to N deposition have been conducted. Neff et al. (2002) found that N addition increased the decomposition of soil light C fractions and stabilized the heavier and mineral-associated C fractions using ^{13}C analysis. Elevated P availability resulting from P addition could alter the quality of litter and shift microbial community composition to change the chemical structure and composition of soil substrates in P deficiency area (Chen et al., 2015; Li et al., 2015). Similar to N deposition, P addition potentially leads that microbes utilize labile organic matter primarily rather than decompose the organic matter with a complex structure to require P in P-limiting forest soils (Cleveland et al., 2002; Li et al., 2015), which may result in the increase of δ^{13}C through accelerating the decomposition of labile C with ^{13}C-depleted signals. It is largely unknown of the pattern that how the coupling effects of P addition and N deposition affect the ^{13}C abundance associated with C fractions in subtropical forest ecosystems.

Although previous researches have examined the effects of N deposition on SOC decomposition (Janssens et al., 2010; Wang et al., 2017), only a few studies have discussed the influence of P availability and N × P interaction on SOC decomposition coupled with organic carbon quality and microbial variables in subtropical forests (Liu et al., 2013). In the present study, we collected soils from a 5-year simulated N deposition and P addition experiment to test the following hypotheses: 1) P addition and N deposition would have opposite roles in SOC decomposition because of their different effects on microbial biomass and enzyme activity, and 2) P addition could alter soil quality (such as increase of δ^{13}C, especially in coarse-sized C fractions) by enhancing the decomposition of labile C.

2. Materials and methods

2.1. Study site

The study was conducted at the Qianyanzhou Ecological Research Station of the Chinese Academy of Sciences, Jiangxi province, China (26°42′N, 115°04′E). The site is characterized by a subtropical moist monsoon climate with a mean annual precipitation and air temperature of approximately 1600 mm and 17.9 °C, respectively. The soil is described as Typical Hapludult Ultisols (locally ‘red soil’) developed from Quaternary Red Clay and covers over 60% of 1.14 million km^2 of total land area in southeast China (Chen et al., 2015) and 30–200 m above sea level.

2.2. Experimental design and soil sampling

In December 2011, a field nutrient manipulation experiment was established to simulate N deposition and P addition in a 12-year-old Chinese fir plantation forest of subtropical China. The ecological responses to environmental changes are representative in a 12-year-old Chinese fir plantation, since it is a middle-aged forest ecosystem with stable biogeochemical cycle characteristics. The randomized block design was used with five replications and six treatments as follows: N0 (0 kg N hm^{-2} a^{-1}), N1 (50 kg N hm^{-2} a^{-1}), N2 (100 kg N hm^{-2} a^{-1}), N0P (0 kg N hm^{-2} a^{-1} + 50 kg P hm^{-2} a^{-1}), N1P (50 kg N hm^{-2} a^{-1} + 50 kg P hm^{-2} a^{-1}) and N2P (100 kg N hm^{-2} a^{-1} + 50 kg P hm^{-2} a^{-1}) (Chen et al., 2015). Five replications were established on five separate hills and the treatment with 20 m × 20 m randomly distributed plots within each replication. There was a 20 m buffer zone between two adjacent treatments. The N and P were added four times each year [in March (30%), June (30%), September (20%) and December (20%)] as $NH_4NO_3/CO(NH_2)_2$ and NaH_2PO_4, respectively.

In July 2016, we divided each treatment plot into four subplots and randomly collected nine soil cores (3 cm diameter and 10 cm depth) from each subplot. These soil cores (total 36 soil samples) were composited into one sample for each plot. Soils were immediately transported to the laboratory for pretreatment. After removing roots and organic debris, the soil samples were divided into two parts. One part of fresh soil was used to determine the SOC decomposition dynamics by incubation, microbial community and enzyme activities. The rest of the soil samples were air-dried for SOC and particle-size fraction analysis.

2.3. Sample analysis

2.3.1. SOC decomposition

The SOC decomposition was measured by incubating soil samples in the laboratory for 130 days. Briefly, 30 g soil samples were adjusted moisture to 50% field capacity with distilled water and placed in a 450 ml chamber at 25 °C. Soil moisture was constant during the incubation by spraying deionized water after CO_2 measurement to ensure the weight same as the beginning. Sealed chambers contained alkali CO_2 traps consisting of 10 ml 1 M NaOH in 25 ml beakers. The released CO_2 was measured at 3, 6, 9, 14, 19, 24, 34, 44, 54, 70, 95 and 130 days after incubation, and the CO_2 respired was determined by titration with standardized 0.25 M HCl after precipitation of CO_2 with the addition of 1 M $BaCl_2$ (Fang et al., 2015). The cumulative CO_2 respired and mean respiration rate at the beginning 9 days of the incubation were calculated, since these indices closely represented the utilization of C sources under field conditions.

To compare the SOC decomposition among the treatments, the key parameters of an SOC decomposition kinetic model were calculated using the formula $C_m = C_0 (1 - e^{-kt})$ (Fang et al., 2015), where C_m and C_0 represent the cumulative SOC decomposition during incubation and potential SOC decomposition (CO_2—C kg^{-1} soil), respectively; and k and t represent the decomposition constant and length of incubation (days), respectively.

2.3.2. SOC and particle-size fractions

Soil particle-size fractions were assessed with a wet-sieving method before the incubation (Fang et al., 2014). In brief, 20 g air-dried soil samples were placed in flasks with 100 ml 5 g L^{-1} $(NaPO_3)_6$ solution and shaken for 15 h at a rate of 90 rpm. After back-washing the sieve with 250 μm and 53 μm, the soil size of > 250 μm and 53–250 μm were defined as macro-particulate OC (macro-POC) and micro-particulate OC (micro-POC), respectively. Particulate organic carbon (POC) was the sum of macro-POC and micro-POC. The rest of the soil with a size of < 53 μm was mineral-associated OC (MAOC) fraction. All particle-size samples were dried at 60 °C and weighed.

The SOC concentration in bulk soil and soil particle-size fractions were determined with a CHN automatic analyzer (Euro Vector EA3000, Italy). Stable C isotope composition ($\delta^{13}C$) in soil samples was measured using an automated isotope ratio mass spectrometer (Delta V Advantage, Thermo Fisher, Germany).

2.3.3. Microbial community, enzyme activities

Phospholipid fatty acid (PLFA) analysis was used to characterize the soil microbial biomass and community before the incubation. The PLFA extraction protocol was executed as described by Dong et al. (2015). The fatty acid methyl esters were separated, quantified, and identified with a GC-MS system (Agilent GC6890/MS5973, Japan). The different groups of soil microorganisms were represented through quantifying the total amounts of the different PLFAs. The PLFAs of i15:0, a15:0, i16:0, i17:0 and a17:0 were markers for Gram-positive bacteria, while the 16:1ω7c, 16:1ω9c, cy17:0 and 18:1ω7 PLFAs served as markers for Gram-negative bacteria. Total bacterial biomass was quantified as the sum of 15:0, i15:0, a15:0, 16:0, i16:0, 16:1ω7c, 16:1ω9c, 17:0, i17:0, a17:0, cy17:0, 18:0, 18:1ω7 and 20:0 (Hill et al., 2000). The PLFAs of 8:1ω9c, 18:2ω6 and 18:2ω9c were defined as fungi, and the 10Me16:0, 10Me17:0 and 10Me18:0 PLFAs were markers for actinomycetes (Wang et al., 2017).

The enzyme activities of cellobioside (CB), β-glucosidase (BG), leucine aminopeptidase (LAP), β-N-acetylglucosaminidase (NAG), and acid phosphatase (AP) were measured with 96-well microplates following the method described by Saiya-Cork et al. (2002) before the incubation. Fluorescence was measured at an excitation wavelength of 365 and a 450 nm emission cutoff filter using a microplate fluorometer (BioTek SynergyH4, USA) after incubating assay plates at 20 °C for 4 h.

2.4. Statistical analysis

All data were tested for homogeneity of variance (Levene's test) before statistical analysis. The differences among the three N deposition levels and between the with-P and without-P treatments were analyzed by the one-way analysis of variance (ANOVA) and least significant difference methods. Two-way ANOVA in a randomized complete block design was used to examine the interaction effects of N deposition and P addition on soil properties. Principal component analysis (PCA) was performed to analyze all the soil properties based on a correlation similarity matrix. The standard 0.05 level was used throughout as the threshold for statistical significance. All statistical analyses were conducted using SPSS 16.0 (SPSS Inc., Chicago, US).

Redundancy analysis (RDA) was used to examine the relationship between soil microbial variables (PLFAs and enzyme activities) and SOC decomposition properties across the treatments, since microbes were the executor in the decomposition of SOC. Data were first analyzed by detrended correspondence analysis using Canoco Software 4.5 (Microcomputer Power, USA).

3. Results

3.1. Characteristics of SOC decomposition

The cumulative CO_2 respired over 9 days increased with N deposition levels under the without-P treatment and was 13.3% higher in the N2 treatment than in the N0 treatment, but no significant differences were found among the three N deposition levels in the with-P treatment (Fig. 1a). Meanwhile, the cumulative CO_2 respired over 9 days was 7.5% lower in the with-P treatment than in the without-P treatment (Fig. 1a). The mean respiration rate over 9 days had similar patterns among different treatments, which was significantly affected by P addition (Fig. 1b). The SOC decomposition exhibited by cumulative CO_2 respired measured over 9, 70 and 130 days (representing different stages of decomposition) was unaffected by N deposition and N × P interaction, but decreased with P addition (Fig. 1c).

Phosphorus addition suppressed the SOC decomposition, and the inhibition effects of P addition on SOC decomposition increased with the increasing N deposition levels (Fig. 2). The inhibition effect by P addition was higher in the N2 (−15.2%) and N1 (−4.0%) treatments than in the N0 treatment (−0.9%) at 9 days.

The potential SOC decomposition (C_0), decomposition constant (k) and decomposition days of half decomposable carbon ($t_{0.5}$) varied with N deposition and P addition (Table 1). The C_0 and $t_{0.5}$ significantly increased with N deposition and decreased with P addition. In the without-P treatment, the C_0 was higher by 38.2% in the N2 treatment

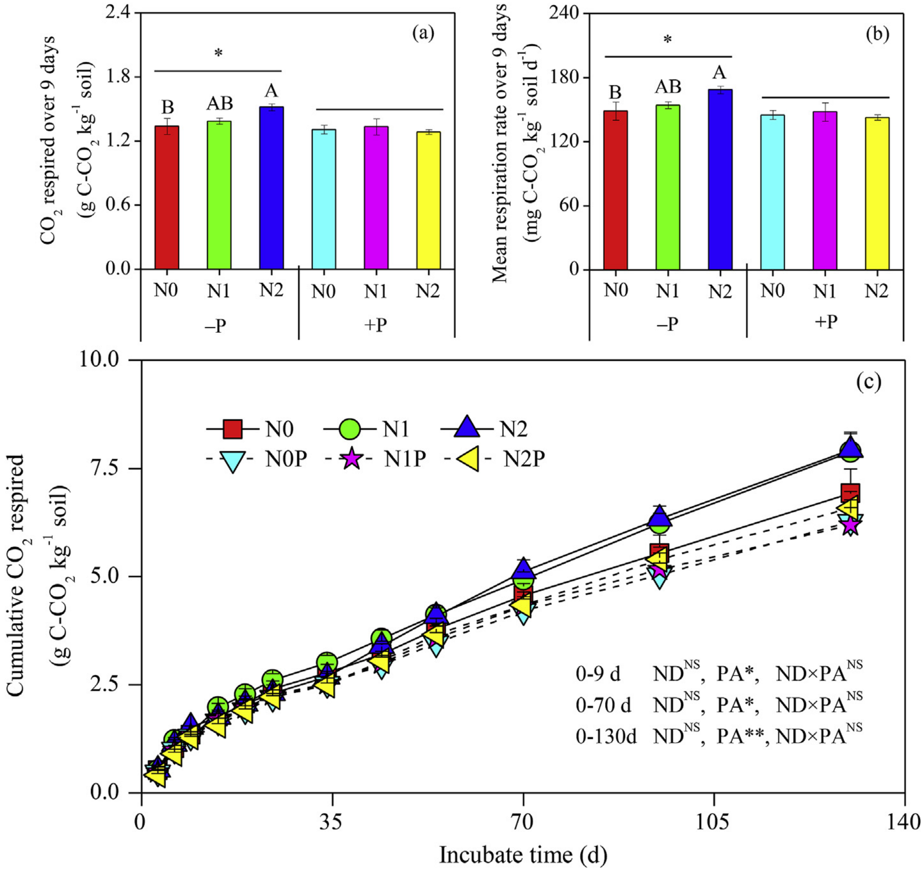

Fig. 1. Responses of cumulative CO_2 respired (a and c) and mean respiration rate (b) to N deposition and P addition in a subtropical forest. Error bars indicate standard error. ND: nitrogen deposition; PA: phosphorus addition; The capital letters indicate the differences among the three levels of N deposition ($p < 0.05$) in the with-P or without-P treatment. The * on the line indicates the difference between the with-P and without-P treatments. $^{*}p < 0.05$; $^{**}p < 0.01$; ^{NS} not significant.

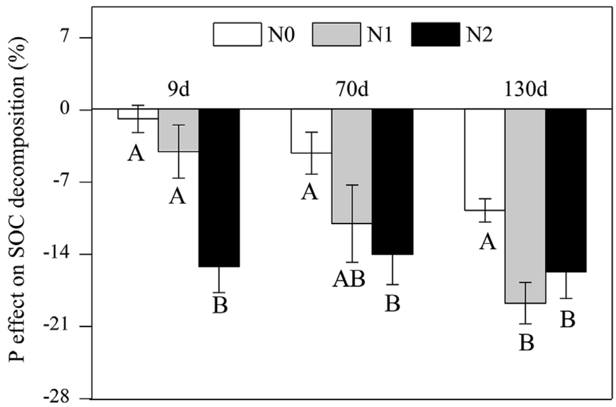

Fig. 2. Response of the P addition effect on SOC decomposition to three N deposition levels. The capital letters indicate the differences among the three levels of N deposition ($p < 0.05$).

than in the N0 treatment. The C_0/SOC ratio reached roughly 9.8% and 12.2% in the with-P and without-P treatments, respectively, and a significant difference was detected between the with-P and without-P treatments. However, N deposition and P addition did not affect k and R^2 and no N × P interaction was found for the parameters of SOC decomposition kinetic model (Table 1).

3.2. SOC concentration and δ ^{13}C value in bulk soil and various particle-size fractions

SOC and micro-POC were significantly affected following N deposition, while the macro-POC was influenced by P addition (Fig. 3). SOC and micro-POC increased with N deposition levels, but did not change with P addition (Fig. 3a and c). Addition of P significantly decreased macro-POC concentration, while macro-POC increased with the levels of N deposition under with-P treatment (Fig. 3b). The macro-POC was 42.0% higher in the N2P treatment than in the N0P treatment (Fig. 3b). The effects of N deposition and P addition were not observed for MAOC (Fig. 3d). POC/SOC decreased with P addition but increased with increasing N deposition levels (Fig. 4a). POC/SOC was significantly lower by 19.4% in the N0P treatment than in the N2P treatment. Similarly, N deposition increased the value of POC/MAOC under the with-P treatment (Fig. 4b).

Deposition of N significantly decreased the $\delta^{13}C_{SOC}$ value (Fig. 3e). The $\delta^{13}C_{macro\text{-}POC}$ increased with P addition but showed significant decrease with N deposition under the with-P treatment (Fig. 3f). The value of $\delta^{13}C_{micro\text{-}POC}$ significantly decreased with N deposition in the with-P treatment (Fig. 3g). The $\delta^{13}C_{micro\text{-}POC}$ was lower in the N1P and N2P treatments than in the N0P treatment by 0.97% and 0.86%, respectively. The N deposition and P addition had no significant effects on $\delta^{13}C_{MAOC}$ (Fig. 3h).

3.3. Soil microbial community composition

The P addition significantly enhanced total microbial, fungal and bacterial biomasses. There were insignificant effects of N deposition on biomasses of total microbes, fungi and bacteria, while these indicators increased with the levels of N deposition in the with-P addition treatment (Fig. 5a–c). The total microbial biomass was higher in the N1P and N2P treatments than that in the N0P treatment by 64.5% and 50.9%, respectively. In contrast, the biomasses of Gram-positive, Gram-negative and actinomycetes were not significantly different among the N deposition and P addition treatments (Fig. 5d–f). In addition, N × P interaction was not found for all the soil microbial indicators (Fig. 5). The fungi/bacteria ratio generally increased with P addition and the levels of N deposition, while the ratio of Gram-positive to Gram-negative was unaltered by N deposition and P addition (Fig. 6a and b).

Table 1
Parameters of soil organic carbon (SOC) decomposition kinetic model under N deposition and P addition situations in a subtropical forest.

N deposition	P addition	C_0 (CO_2–C kg^{-1} soil)	k (day^{-1})	R^2	$t_{0.5}$ (day)	C_0/SOC (%)
N0	−P	8.22 ± 0.84b	0.013 ± 0.002	0.96 ± 0.01	54.7 ± 4.92b	11.30 ± 1.45
N1	−P	9.76 ± 0.66 ab	0.012 ± 0.002	0.96 ± 0.01	61.43 ± 4.55 ab	11.38 ± 0.85
N2	−P	11.36 ± 0.88a	0.010 ± 0.001	0.97 ± 0.01	78.45 ± 5.92a	13.60 ± 0.99
N0	+P	7.24 ± 0.51	0.014 ± 0.002	0.96 ± 0.01	51.82 ± 3.83	11.14 ± 0.78
N1	+P	7.23 ± 0.91	0.016 ± 0.002	0.96 ± 0.01	50.02 ± 7.81	8.36 ± 1.35
N2	+P	8.32 ± 0.93	0.012 ± 0.002	0.97 ± 0.01	60.07 ± 7.01	9.92 ± 0.74
Variance analysis of *F*-statistics[#]						
ND		3.49*	1.28	2.73	4.38*	1.51
PA		10.87**	1.79	0.01	5.27*	6.42*
ND × PA		0.91	0.34	0.21	0.91	1.47

Note: Mean ± 1 standard error. ND: nitrogen deposition; PA: phosphorus addition; C_0: the potential SOC decomposition; k: the decomposition constant; $t_{0.5}$: the decomposition days of half decomposable carbon. The small letters indicate the differences among the three levels of N deposition ($p < 0.05$) in the with-P or without-P treatment. [#] Significance of F values: $^*p < 0.05$; $^{**}p < 0.01$.

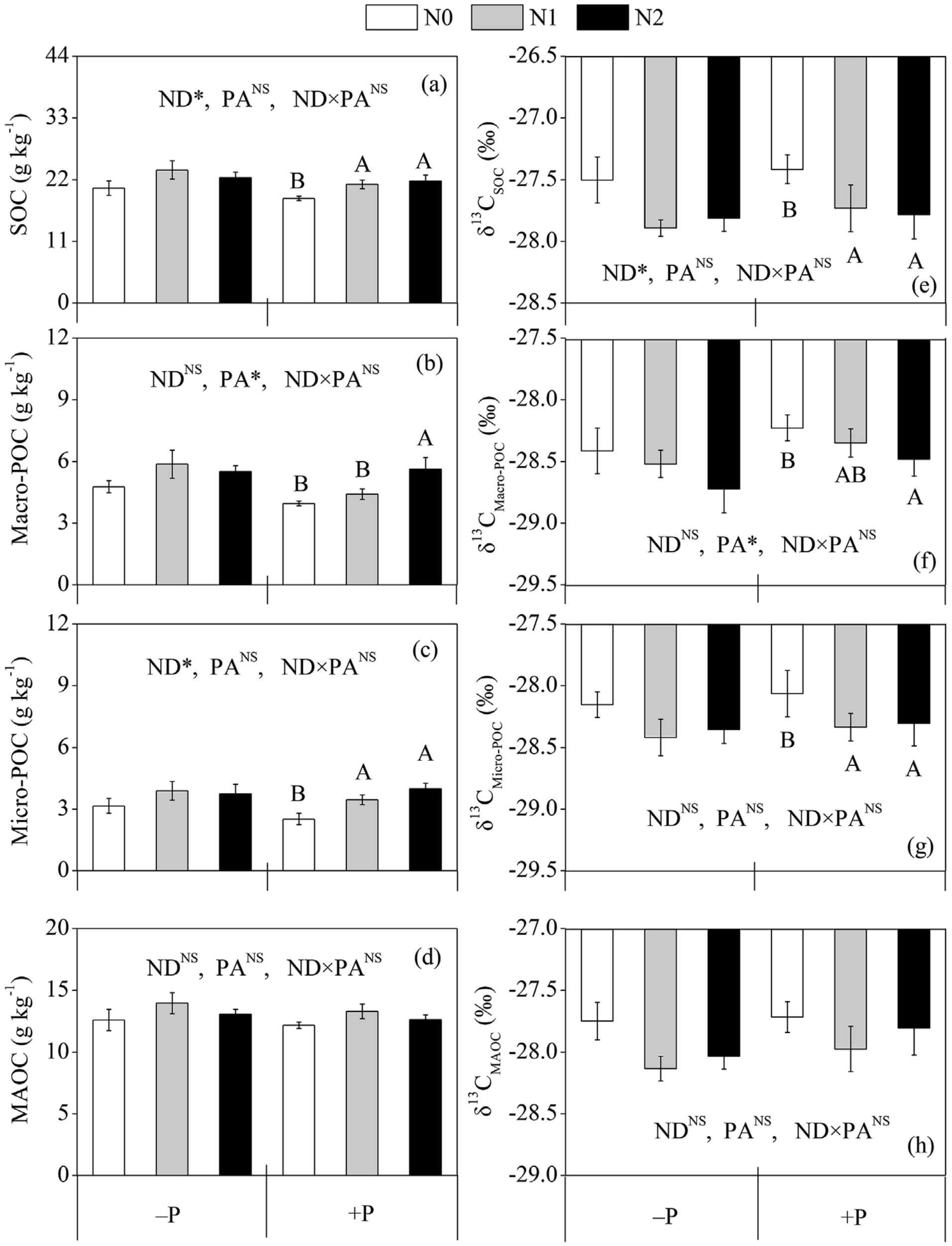

Fig. 3. Effects of N deposition and P additions on SOC (a), particle-size fractions (b–d) and $\delta^{13}C$ in bulk soil (e) and soil particle samples (f–h) in a subtropical forest. Error bars indicate standard error. ND: nitrogen deposition; PA: phosphorus addition; macro-POC: macro-particulate organic C; micro-POC: micro-particulate organic C; MAOC: mineral-associated organic C; The capital letters indicate the differences among the three levels of N deposition ($p < 0.05$) in the with-P or without-P treatment. $^*p < 0.05$; ^{NS} not significant.

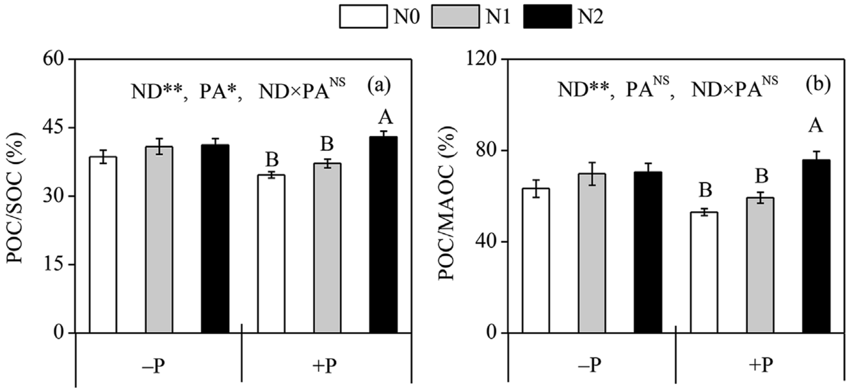

Fig. 4. Effects of N deposition and P addition on the ratios of POC/SOC (a) and POC/MAOC (b) in a subtropical forest. Error bars indicate standard error. ND: nitrogen deposition; PA: phosphorus addition; POC: particulate organic carbon, the sum of macro-POC and micro-POC. MAOC: mineral-associated organic C; The capital letters indicate the differences among the three levels of N deposition ($p < 0.05$) in the with-P or without-P treatment. $^{*}p < 0.05$; $^{**}p < 0.01$; ^{NS} not significant.

3.4. Soil hydrolytic enzyme activities

The AP activity was significantly influenced by P addition, but did not change with N deposition (Table 2). AP activity was lower by 45.2% in the with-P treatment than in the without-P treatment. The BG activity increased with N deposition and a significant difference was found between the N0P and N2P treatments. The N deposition and P addition had significant effects on CB and NAG activities. The CB and NAG activities increased with N deposition, whereas they decreased with P addition. The LAP activity did not change with N deposition and P addition (Table 2).

3.5. Relationships among soil properties and OC decomposition

The PCA showed that there was a significant separation between the with-P and without-P treatments (Fig. S1). The PC1 and PC2 explained 38.5% and 19.2% of the variances of the data, respectively. High correlation coefficients for the PC1 were macro-POC, micro-POC, MAOC, SOC, POC/MAOC, POC/SOC, $\delta^{13}C_{SOC}$, $\delta^{13}C_{MAOC}$, AP, BG, CB, NAG, cumulative CO_2 respired, C_0 and $t_{0.5}$, while the high correlation coefficients for the PC2 were $\delta^{13}C_{macro\text{-}POC}$, $\delta^{13}C_{micro\text{-}POC}$, Gram-positive, Gram-negative, biomass of total microbes, fungi and bacteria, actinomycetes and the fungi/bacteria ratio.

Eigenvalues of RDA indicated that axes 1 and 2 explained 34.6% and 11.5% of the overall variances among the six treatments, respectively (Fig. S2). Species–environment correlation for both axes was 0.71 for all treatments, indicating that SOC decomposition properties strongly correlated with microbial parameters. The RDA showed that the mean respiration rate over 9 days, representing utilization rate of C sources under field conditions, positively correlated with CB activity and G+/G-ratio as well as negatively correlated with fungi/bacteria ratio, bacterial biomass, total microbial biomass and fungal biomass.

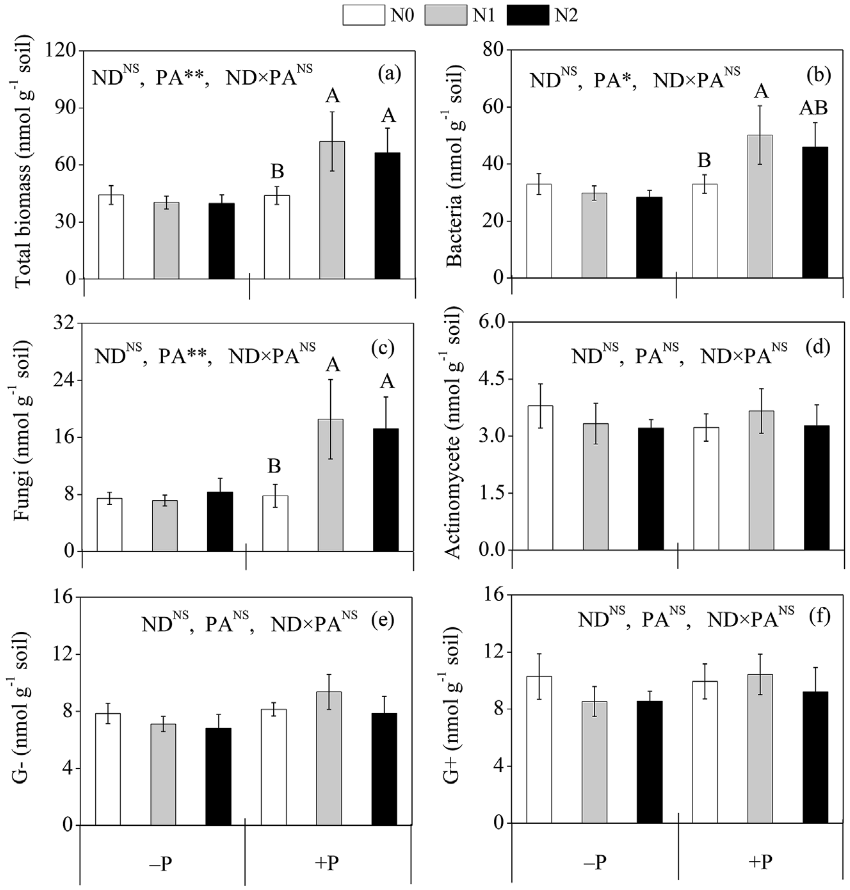

Fig. 5. Effects of N deposition and P addition on concentration of PLFAs (a–f) in a subtropical forest. Error bars indicate standard error. ND: nitrogen deposition; PA: phosphorus addition; G+: Gram-positive; G-: Gram-negative. The capital letters indicate the differences among the three levels of N deposition ($p < 0.05$) in the with-P or without-P treatment. $^{*}p < 0.05$; $^{**}p < 0.01$; ^{NS} not significant.

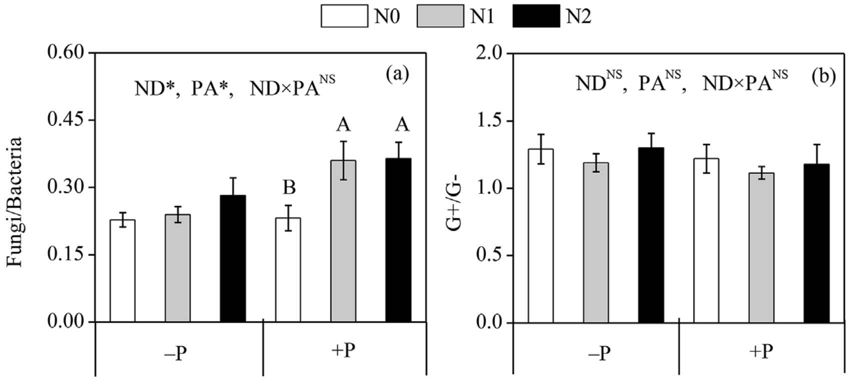

Fig. 6. Effects of N deposition and P addition on the ratios of fungi/bacteria (a) and G+/G- (b) in a subtropical forest. Error bars indicate standard error. ND: nitrogen deposition; PA: phosphorus addition; G+: Gram-positive; G-: Gram-negative. The capital letters indicate the differences among the three levels of N deposition ($p < 0.05$) in the with-P or without-P treatment. $^{*}p < 0.05$; ^{NS} not significant.

Table 2
Effects of N deposition and P addition on the hydrolytic enzyme activities (nmol h^{-1} g^{-1}) in a subtropical forest.

N deposition	P addition	AP	BG	CB	LAP	NAG
N0	−P	686.3 ± 34.8	32.3 ± 6.3	6.5 ± 1.2b	4.3 ± 0.7	24.0 ± 5.2
N1	−P	629.7 ± 50.9	43.7 ± 7.1	15.1 ± 3.1a	5.3 ± 0.5	25.6 ± 4.2
N2	−P	645.3 ± 74.3	39.0 ± 8.3	13.8 ± 2.4 ab	5.3 ± 1.0	28.5 ± 1.6
N0	+P	322.8 ± 32.4	19.7 ± 6.8b	2.6 ± 0.3b	4.0 ± 0.5	11.9 ± 2.8b
N1	+P	366.3 ± 19.0	37.3 ± 7.4 ab	8.2 ± 1.1a	4.4 ± 0.8	17.6 ± 2.3b
N2	+P	384.7 ± 44.9	44.4 ± 8.7a	7.5 ± 2.4a	5.1 ± 0.3	29.3 ± 2.5a
Variance analysis of *F*-statistics#						
ND	0.07	2.74*	7.40**	1.53	5.71**	
PA	61.82***	0.55	12.41**	0.75	5.74*	
ND × PA	0.81	0.75	0.32	0.19	2.02	

Note: Mean ± 1 standard error. ND: nitrogen deposition; PA: phosphorus addition; AP: acid phosphatase; BG: β-glucosidase; CB: cellobioside; LAP: leucine aminopeptidase; NAG: β-N-acetylglucosaminidase; The small letters indicate the differences among the three levels of N deposition ($p < 0.05$) in the with-P or without-P treatment. # Significance of *F* values: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

And the C_0 positively correlated with NAG, AP and BG activities and negatively correlated with fungi/bacteria ratio, total microbial biomass and bacterial biomass (Fig. S2).

4. Discussion

4.1. *The overall effects of N deposition and P addition on SOC decomposition*

The SOC decomposition was significantly inhibited following P addition, which was inconsistent with the general idea that N deposition decreases the heterotrophic respiration of soil organisms, and thus reduces the decomposition of SOC that often observed in temperate forests (Pregitzer et al., 2008; Wang et al., 2017). The decrease of SOC decomposition following P addition rather than N deposition in this case might be explained by the theory of nutrient limitation on microbial C utilization (Craine et al., 2007). Temperate forests are often N-limited under natural conditions (Pregitzer et al., 2008), while P is considered to be the main element restricting microbial biomass in subtropical and tropical areas (Liu et al., 2012; Camenzind et al., 2018). Addition of P can provide available P source, thereby decreasing the microbial attack on SOC for the purpose of acquiring P, thus reducing SOC decomposition (Li et al., 2015). In addition, the demands for P and N balances of microbes can also explain the decreased SOC decomposition when the P is provided in a N-sufficient environment according to N and P stoichiometry (Elser et al., 2007; Vitousek et al., 2010). Moreover, the decrease of SOC decomposition under the with-P treatment could partly be explained by the changes in the structure of the soil microbial community. Because fungi typically utilize the more SOC having a complex structure as their carbon source (Six et al., 2006), the increasing value of fungi/bacteria ratio after P addition suggested that SOC was more stable under the with-P treatment than the without-P treatment. Furthermore, the greatly declining activity of CB in the with-P treatment may also explain the higher inhibition of SOC decomposition, because low CB activity represented less SOC decomposition under the with-P treatment. These finding suggested that P availability needed to be considered when evaluating the effects of N deposition on SOC dynamics in P-restricted subtropical forests.

Interestingly, the cumulative CO_2 respired over 9 days increased with N deposition under the without-P treatment, while was unaffected by N deposition under the with-P treatment in the present study (Fig. 1). Soil respiration depends on the quality and quantity of substrate and the activities of extracellular enzyme and microbes (Ryan and Law, 2005; Keuskamp et al., 2015). A previous study also found that the microbial respiration in a root-free soil was stimulated after two years of N addition in a subtropical forest (Tu et al., 2013). The increase of soil respiration under N deposition may be explained by its positive effects on SOC and C_0, because the SOC concentration significantly increased with N deposition levels and C_0, representing the size of labile C pool, was higher in the N2 treatment than in the N0 treatment. Moreover, CB and BG are the hydrolytic enzymes involved in the degradation of soil carbon compounds, such as cellulose. The opposite roles of N deposition and P addition on the activities of CB and BG were also responsible for the stimulatory effect of N deposition and restraining effect of P addition on soil respiration, since enhanced glycosidases activities mean more organic matter degradation (Keuskamp et al., 2015; Tian et al., 2017; Wang et al., 2018). Therefore, our result suggested that N deposition could increase the soil respiration in ambient P condition and P addition might counteract the stimulatory effect of N deposition on soil respiration in subtropical forests, despite more case studies should be conducted to validate this evidence further.

In addition, the subtropical area is one of the regions with high N deposition in China. The precipitation dissolved inorganic (DIN) and organic N (DON) input reached 34 kg N $ha^{-1}yr^{-1}$ and 18 kg N $ha^{-1}yr^{-1}$ in subtropical forests, respectively (Fang et al., 2007).

Although whether soil N leaching occurred was not directly determined, we speculated that N deposition would lead to the N loss in the predominant form of NO_3^- in our study area according to the previous research (Fang et al., 2007). Moreover, loss of N as DON was not ignored by abiotic incorporation of excess NO_3^- into soil organic matter (Fang et al., 2009b).

4.2. The responses of substrates quality related to SOC decomposition after N deposition and P addition

Similarly, our results showed the opposite effects of N deposition and P addition on SOC stability through the synthesis of SOC decomposition kinetic model. Here, C_0 represents the size of the soil active organic carbon pool, which is used to evaluate the substrate availability for heterotrophic populations in soils (Fang et al., 2015). In this study, the C_0 and C_0/SOC ratio increased under N deposition but decreased following P addition, implying that P addition had the ability to increase SOC stability. This speculation was supported by the finding that P addition decreased the macro-POC concentration (Fig. 3). Soil POC is thought of as an unprotected C pool and shall be impacted by anthropogenic disturbances (Cheng et al., 2010; Fang et al., 2014). The decreased POC after P addition suggested that the decomposition of soil labile carbon increased, since the microbes are likely to use active organic matter when the limitation by a nutrient is alleviated (Cheng et al., 2010). In addition, changes in the POC/MAOC and POC/SOC are also outcomes caused by the effects of N deposition and P addition on SOC decomposition. The decrease of POC/SOC under P addition treatment could be an result that the more POC decomposed via the increased microbial biomass. Liu et al. (2012) found P addition decreased the concentrations of total SOC and light C fractions in an old-growth tropical forest. Large POC/MAOC and POC/SOC values represent less stable SOC (Cheng et al., 2010; Fang et al., 2014). Therefore, P addition will enhance the SOC stability through altering SOC composition.

Moreover, the study of ^{13}C natural abundance provided substantial information for insight into the N deposition and P addition effects on SOC (Neff et al., 2002). Our study suggested that soil substrate quality could be altered responding to the effects of nutrient additions on soil organic matter decomposition. In this study, addition of P enriched the $\delta^{13}C$ values of the macro-POC, which could induce by stimulating the decomposition of readily available C from newly-formed plant residues, such as POC, after P addition (Fig. 3). It was reported that the decomposition of microbial residual products and bulk soil often respires relatively ^{13}C-depleted CO_2 (Cadisch et al., 1996; Wynn et al., 2006). Thus, the ^{13}C-depleted CO_2 loss from macro-POC decomposition was the main reason for ^{13}C-enrichment in macro-POC fractions following P addition. In contrast, N deposition significantly decreased $\delta^{13}C_{SOC}$, $\delta^{13}C_{macro\text{-}POC}$ and $\delta^{13}C_{micro\text{-}POC}$ values in the with-P treatment, which illustrated relatively more fresh plant residues input in the combined N deposition and P addition treatments. Furthermore, the $\delta^{13}C$ values of macro-POC and micro-POC were smaller than those of MAOC, which demonstrated that MAOC had a lower loss of ^{13}C-depleted CO_2 because this particle-size fraction was associated with soil silt and clay and thereby decreased the decomposition by microbes (Balesdent et al., 1988; Fang et al., 2009a).

4.3. The responses of microbial variables related to SOC decomposition after N deposition and P addition

We observed an overall increase in the biomasses of total microbes, fungi and bacteria after P addition, and these indicators were unaffected by N deposition levels. Some previous studies have also reported increase of microbial biomass after P addition in subtropical or tropical forests, which was in relation to soils characterized by low P availability (Liu et al., 2012, 2013). For instance, a recent study showed that the microbial biomass was unaffected by N addition, whereas P addition increased the total biomass after fertilization for 3 years in a secondary tropical forest (Li et al., 2015). The underlying mechanisms may be related to the fact that the soil in subtropical or tropical forests is P-restricted. Fertilization of reactive P relieves the constraint of microbial proliferation and then induces soil microbial biomass increase, because it is generally believed that microbial utilization of soil C in subtropical/tropical forests with old soils is predominantly P limited (Cleveland et al., 2002). Moreover, the C restriction of soil microbes could be another reason for the increase of microbial biomass after P addition (Torsvik and Øvreås, 2002; Li et al., 2015). Our previous research found that girth growth rate at breast height of Chinese fir significantly increased after 2 years of P addition in the same study plots as the current study (Chen et al., 2015) and thus we speculate that the increased plant growth rate would improve plant C input into soils and stimulate microbial activity, although we did not have the data for fine root biomass and root secretion after P addition. In addition, the soil total microbial, fungal and bacterial biomasses were not influenced by N deposition, but these indicators increased with the levels of N deposition under the with-P treatment, implying an interaction role of N deposition and P addition on microbial biomass in P-restricted forest, since N and P may exist in a balance that determines the microbial growth.

Soil microbial community composition is often altered by nutrient addition (Allison et al., 2008; Cusack et al., 2011). The N deposition and P addition significantly increased the soil fungi/bacteria ratio, indicating that soil fungi were more sensitive to N deposition and P addition than bacteria, which was consistent with some former studies in tropical forest systems (Liu et al., 2013; Li et al., 2015). Liu et al. (2012) indicated the fungal relative abundances and the fungi/bacteria ratio significantly increased following P addition in an old-growth tropical forest. Similarly, a study using the gene copies method revealed soil fungi abundance was more sensitive than soil bacterial abundance to soil fertilization and the abundance of soil microbes was restrained by P in the tropical area (He et al., 2008). High value of the fungi/bacteria ratio was considered as an ecosystem with high stability with lower environmental impacts following N deposition and P addition (Dong et al., 2015).

Furthermore, the AP, CB and NAG activities significantly decreased after P addition, whereas BG, CB and NAG activities increased with N deposition, suggesting the opposite roles of N deposition and P addition in the cycles of C and N due to the regulation of CB and NAG on C and N hydrolysis respectively (Wang et al., 2018). It has been observed that glycosidase activities increased with N addition in a range of ecosystems (Chen et al., 2017; Tian et al., 2017; Wang et al., 2018). Liang et al. (2014) considered that higher concentration of organic matter may lead to increased microbial activity and higher enzyme activity levels. The enhanced CB activity can be partly explained by the modeling results that N deposition was intended to increase the SOC concentration and the size of labile carbon pool (C_0), resulting in the enhanced CB for cellulose degradation (Dong et al., 2015). In contrast, the lower CB activity under P addition meant the reduced investment in C-acquiring enzymes of soil microbes because of the less labile substrate. Moreover, consistent with previous studies (Keuskamp et al., 2015; Wang et al., 2018), N deposition stimulated the N-acquiring enzymes (e.g. NAG) activity, because NAG activities contribute more to C and energy uptake than to N uptake reported by Keuskamp et al. (2015) that the NAG activity still increased under N addition with enough available mineral N when glucose was added to soil. In addition, when a resource is limiting, microbes may benefit from producing enzymes to obtain it and vice versa. Thus, the AP activity was lower in the with-P treatment than in the without- P treatment.

5. Conclusions

Our data reveal the effects of N deposition and P addition on SOC decomposition in a subtropical forest. The cumulative CO_2 respired increased with N deposition in ambient P condition while was unaffected by N deposition in with-P treatment, implying that P addition could counteract the stimulatory effect of N deposition on SOC decomposition. Phosphorus addition decreased while N deposition increased the C_0 and CB activity, supporting this speculation. Moreover, addition of P generally suppressed SOC decomposition, which was due to the increases in total microbial biomass and fungi/bacteria ratio after P addition. Our results considered P addition could alter the effect of N deposition on SOC decomposition related to the changes in microbial variables and substrate quality and higher P availability might further result in the increased C accumulation under the context of enhanced N deposition in subtropical forests. However, this study was conducted after 5 years of simulated N deposition and P addition treatments, which may differ from the results of a long-term experiment. A long-term monitoring study focusing on the distinction and interaction of ecological effects induced by N deposition and P addition should be conducted in future.

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Competing interests

The authors have declared that no competing interest exists.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2019.03.005.

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