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Different responses of soil respiration and its components to nitrogen and phosphorus addition in a subtropical secondary forest

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Abstract

Background: Nitrogen (N) and phosphorus (P) deposition have largely affected soil respiration (R_s) in forest ecosystems. However, few studies have explored how N and P individually or in combination to influence R_s and its components (autotrophic respiration, R_a ; heterotrophic respiration, R_h), especially in highly P-limited subtropical forests. To address this question, we conducted a field manipulation experiment with N and/or P addition in a 50-year-old subtropical secondary forest.

Results: We found that N addition on average reduced R_s , R_a , and R_h by 15.2%, 15%, and 11.7%, respectively during 2-year field study. P addition had an inconsistent effect on R_a , with R_a increasing by 50.5% in the first year but reducing by 26.6% in the second year. Moreover, P addition on average decreased R_h by 8.9%–30.9% and R_s by 6.7%–15.6% across 2 years. In contrast, N and P co-addition on average increased R_s , R_a , and R_h by 1.9%, 7.9%, and 2.1% during the experimental period. Though R_s and R_h were significantly correlated with soil temperature, their temperature sensitivities were not significantly changed by fertilization. R_a was predominantly regulated by soil nitrogen availability (NH_4^+ and NO_3^-), soil dissolved organic carbon (DOC), and enzyme activities, while the variation in R_h was mainly attributable to changes in soil microbial community composition and soil β -D-Cellubiosidase (CB) and β -Xylosidase (XYL) activities.

Conclusion: Our findings highlight the contrasting responses of R_s and its components to N or P addition against N and P co-addition, which should be differentially considered in biogeochemical models in order to improve prediction of forest carbon dynamics in the context of N and P enrichment in terrestrial ecosystems.

Keywords: Nitrogen deposition, Phosphorus enrichment, Heterotrophic respiration, Autotrophic respiration, Enzyme activities, Microbial community composition

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Introduction

Soil respiration (R_s) is considered as an important process of carbon dioxide (CO_2) exchange between the atmosphere and soils in terrestrial carbon (C) cycling. A small change in soil C emissions can lead to a great impact on atmospheric CO_2 concentration and thus global climate change (Xu and Shang 2016). Soil respiration includes two components, namely CO_2 release originated from roots and rhizosphere microbes (autotrophic respiration, R_a) that use C fixed by plant photosynthesis, and CO_2 emission derived from microbial decomposition of plant litter and soil organic matter (heterotrophic respiration, R_h) (Subke et al. 2006). Atmospheric nitrogen (N) deposition has been predicted to increase by 50%–100% by 2030 compared with 2000 and it is expected to exacerbate in many areas around the world, especially in the East and South Asia (Reay et al. 2008). The evidence is mounting that N enrichment can affect R_s by affecting soil nutrient availability, inducing soil acidification, and influencing plant growth and microbial activity (Tian and Niu 2015; Peng et al. 2017b; Wang et al. 2019b). Therefore, knowledge of responses of R_s and its components to the increase in N availability is critical to improve our ability to quantify ecosystem C balance, model terrestrial C cycling, and predict C-climate feedbacks (Robertson et al. 2013).

Considerable efforts have been devoted to explore the influence of N supply on R_s in forest ecosystems, which have high productivity and store vast amounts of C in soils (Pan et al. 2011; Sun et al. 2019). However, the results from field observations (Zhao et al. 2018), models (Devaraju et al. 2016), and meta-analyses (Zhou et al. 2014; Chen et al. 2016; Zhong et al. 2016; Feng and Zhu 2019) are inconsistent and controversial, exhibiting positive (Zhou et al. 2014; Chen et al. 2019; Wang et al. 2019a), negative (Wang et al. 2017a; Zhou et al. 2018; Song et al. 2020) and negligible effects on R_s (Zhong et al. 2016; Liu et al. 2017; Wei et al. 2020). Such discrepancies are largely ascribed to the different sensitivities of plant roots and soil micro-organisms to N enrichment, resulting in uncertainties and inconsistent results of R_a and R_h in response to N enrichment. For instance, a global meta-analysis has reported that N addition generally decreases R_h in diverse ecosystems (Zhang et al. 2018), which is likely due to decreased soil microbial biomass, suppression of extracellular enzyme activities, and changes in microbial composition that may increase microbial C use efficiency (Li et al. 2018; Wang et al. 2018; Zhang et al. 2018; Liu et al. 2019; Ma et al. 2020). In contrast, the response of R_a to N enrichment is disparate. Increasing N availability can alleviate N restriction on root growth and thus promoting R_a (Cheng et al. 2015; Li et al. 2017). However, excessive N may decrease R_a because the photosynthetic C allocated

to belowground would decrease (Olsson et al. 2005; Adair et al. 2009). Moreover, excess N enrichment could give rise to ammonium toxicity and acid cation toxicity (Li et al. 2018), also resulting in the decrease of R_a . Nevertheless, most of those studies were conducted in N limited forests (Savage et al. 2013; Sun et al. 2014), tropical and subtropical forests where are highly phosphorus (P) limited instead of N limited are poorly understood and largely uncertain regarding the effects of N addition on R_s and its components (Li et al. 2016; Wei et al. 2020).

It is generally recognized that plant growth is limited by P in tropical and subtropical forests, because soils in these regions are particularly old, highly weathered and consequently poor in available P (Vitousek et al. 2010; Cleveland et al. 2011). Moreover, increasing N availability with N deposition may shift from N to P limitation in many forest ecosystems (Zeng and Wang 2015; Li et al. 2016; Wang et al. 2017b; Camenzind et al. 2018). Thus, P addition is likely to alleviate soil P limitation and accelerate root growth and R_a in forests (Naples and Fisk 2010). Similarly, increasing available P may stimulate R_h by enhancing microbial growth and activities (Ilstedt and Singh 2005) or inhibit R_h largely due to soil acidification and shifts in soil microbial community (Li et al. 2018; Feng and Zhu 2019). Multiple evidence suggests that the impacts of P enrichment on R_a and R_h are very complex in the context of N deposition (Liu et al. 2019). Nonetheless, the mechanisms underlying the divergent responses of R_a and R_h to P addition and its interaction with N addition are far from clear, which deserves further investigation, especially in the highly P limited forests.

R_a and R_h are determined by a complex interaction of controlling factors, including soil temperature and moisture, soil pH, soil nutrient availability, substrate supply, and soil microbial community (Zhang et al. 2014; Cheng et al. 2015; Li et al. 2018; Tang et al. 2020). Among them, soil temperature is a dominant factor that affects R_s and its components in forests (Wagai et al. 2013; Miao et al. 2020; Peng et al. 2020). Temperature sensitivity of R_s (Q_{10}), which is the relative change of soil CO_2 efflux for a 10 °C increase in soil temperature, is a critical parameter in assessing climate-C feedbacks in C cycle models (Davidson and Janssens 2006). A variety of factors can influence Q_{10} value, including soil moisture, substrate and nutrient availability, soil enzyme activity and microbial community composition (Curiel Yuste et al. 2004; Wagai et al. 2013; Dong et al. 2020). Both N and P enrichments could directly and indirectly change these factors, thereby influencing Q_{10} values of R_s and its components. However, inconsistent results regarding the response of Q_{10} to N addition have been found in previous studies, including increase (Deng et al. 2010),

decrease (Zhang et al. 2014), or no change (Jia et al. 2012). Although the previous studies have investigated the response of Q_{10} in R_s to N addition in temperate forests (Sun et al. 2014), few studies have addressed the effects of P addition and its association with N addition on Q_{10} values of R_s and its components in tropical and subtropical forests.

We conducted a 3-year field N and P addition experiment in a subtropical secondary forest, which is located in the transition belt from the warm temperate to subtropical forest of a mountain ecosystem. In this study, we measured soil R_s , R_a and R_h and explored their relationships with soil physicochemical properties, soil microbial biomass, cellulose degrading enzyme activities, microbial community composition under N and P enrichment. The objectives of this work were (1) to investigate the effects of N and P enrichment and their interaction on R_s and its components; (2) to explore the biotic and abiotic mechanisms underlying the responses of R_s , R_a and R_h to N and P enrichment; (3) to reveal the Q_{10} of R_a and R_h in response to N and P enrichment in this subtropical forest ecosystem.

Materials and methods

Site description

This study was carried out at the Jigong Mountain Biosphere Reserve in the south of Henan Province, China (31°51'58" N, 114°5'12" E). The region is characterized by a transitional climate, which is from warm temperate to subtropical climate. The annual mean air temperature is 15.2 °C. The mean lowest temperature in January is approximately 1.9 °C, and the mean highest temperature in July is 27.5 °C. This region has a mean annual precipitation of 1118.7 mm per year. The main soil type is classified as yellow-brown soil with soil thickness ranging from 30 to 60 cm (Yan et al. 2014). The studied forest is considered as a 50-year-old secondary forest. The most common tree species at the canopy layer are *Quercus acutissima* and *Q. variabilis*. Soil properties at the beginning of the experiment were described as the study in Li et al. (2018).

Experimental design

This experiment was performed in July 2013 with a complete randomized block design. Four treatments with four replications were set up. Each plot was 10 m × 10 m and separated by a 10 m-wide buffer strip. The treatments were implemented as follows: control (CK), N addition (N, 10 g·m⁻²·yr⁻¹ NH₄NO₃), P addition (P, 10 g·m⁻²·yr⁻¹ NaH₂PO₄), and N and P co-addition (NP, 10 g·m⁻²·yr⁻¹ NH₄NO₃ plus 10 g·m⁻²·yr⁻¹ NaH₂PO₄). The fertilizer was dissolved in 50 L of water and sprayed using a portable sprayer. The same amount of water was employed in the control plots, equivalent to an annual

precipitation increase of 0.5 mm each year. N and P supplies were conducted monthly from May to October during the growing season in each year.

Soil respiration and its autotrophic and heterotrophic components

For soil total respiration (R_s) measurements, three polyvinyl chloride (PVC) collars (20 cm in diameter and 8 cm in height) were inserted into 5 cm depth in each plot. The trenching method was used to separate autotrophic respiration (R_a) and heterotrophic respiration (R_h) from R_s . Specifically, one subplot (1 m × 1 m) was set and trenched to 60 cm depth in each plot in July 2013. The polyethylene boards were installed inside the trenches to avoid root regrowth into the subplots. Living plants were cut to keep the trenched subplots free of roots with the minimum disturbance to the soil. Three PVC collars were also established in each trenched subplot. Because the respiration in the trenched subplots was originated only from microbes but not from roots, it refers to R_h . R_a was calculated by subtracting R_h from R_s . R_s and its components were measured once a month from May to October during the study period. The Li-8100 automatic soil CO₂ flux system (LI-COR Biosciences, Lincoln, Nebraska, USA) was used to measure soil respiration rates between 9:00 and 11:00 of the local time. Soil temperature and moisture at 5 cm depth were measured synchronously with monitoring of soil CO₂ fluxes. In order to reduce the effect of dead root decomposition in the trenched plots, R_s and its components were initially measured in May 2014, nearly 1 year after the trenching implementation.

Soil measurements

Soil chemical measurements and fine root biomass

We collected soil samples in October 2014 and 2015. At each sampling plot, five 0–15 cm soil samples (3.75 cm in diameter) were collected randomly after removing the litters. These five soil cores were combined to one composite sample and passed through a 2-mm mesh sieve immediately. Soil dissolved organic carbon (DOC), ammonium nitrogen (NH₄⁺-N), nitrate nitrogen (NO₃⁻-N), and microbial biomass carbon (MBC) contents were measured with fresh soils from one part of soil samples. The other part of soil samples was air-dried, crushed, and then sieved for soil available phosphorus (AP), extractable cations (Al³⁺, Ca²⁺, Mg²⁺, and Na⁺) and pH measurements. For soil DOC measurement, fresh soil sample was extracted with 0.5 M K₂SO₄ and the extracts in filtered through a 0.45 μm cellulose acetate filter (Millipore) were analyzed with a TOC analyzer (multi N/C 3100, Analytik Jena, Germany). Soil inorganic N concentrations (NH₄⁺-N, NO₃⁻-N) were determined after extraction using 2 M KCl solution on a FIAstar

5000 Analyzer (Foss Tecator, Denmark). Soil available phosphorus was measured with a spectrophotometer (UV2550, Shimadzu, Japan). The extractable cations were measured according to the method in Mao et al. (2017). Soil pH was measured with a ratio of 1:2.5 (soil: water solution). Soil MBC was determined by the method of chloroform fumigation extraction (Liu et al. 2009). Fine root biomass was determined in November 2014 and 2015 with an auger (10 cm diameter, 15 cm deep). The roots were collected from soil cores through a 1-mm mesh sieve, dried for 48 h at 65 °C to a constant weight and measured to estimate fine root biomass.

Microbial community composition

Soil microbial community composition was measured by phospholipid fatty acids (PLFAs) analysis following the method from Bossio et al. (1998). The fatty acids were analyzed with the Agilent 6890 (Agilent Technologies, Palo Alto, CA, USA) and the MIDI Sherlock Microbial Identification System (MIDI Inc., Newark, DE, USA). Phospholipid fatty acids concentrations were determined based on the internal standard (19:0 nonadecanoic methyl ester). The PLFAs of i14:0, i15:0, a15:0, i16:0, 10Me 16:0, i17:0, a17:0, 16:1 ω 7c, 17:1 ω 8, 18:1 ω 9, 18:1 ω 7c, cy17:0 and cy19:0 were used as bacteria, and 18:2 ω 6, 9 was used as fungi. The 10Me 16:0, 10Me 17:0, and 10Me 18:0 were used as actinobacteria (Frostegård and Bååth 1996).

Soil enzyme activity

We measured four cellulose degrading enzymes activities, including α -Glucosidase (AG), β -Glucosidase (BG), β -D-Cellulobiosidase (CB), and β -Xylosidase (XYL) activity following the method proposed by Jing et al. (2016) and Li et al. (2018). In brief, approximately 1 g of homogenized fresh soil was slurried in 250 mL of 50 mM, pH 5 acetate buffer in blender for 2 min. Soil slurries of 800 μ L was pipetted into 96-well microplate, and 200 μ L of 200 μ M substrate (4-methylumbelliferyl, MUB) was added to each assay. Six analytical replicates were used for each soil sample. The microplates were incubated at 25 °C for 3 h in the dark. The fluorescence was determined with a SynergyMX microplate reader (Biotek, VT, USA) at 365 nm for excitation and 450 nm for emission. The enzyme activity was expressed as nmol·g dry weight⁻¹·h⁻¹.

Statistical analysis

The relationships between soil respiration rates and soil temperature were fitted with the following equation, $R = ae^{bT}$. In this equation, R is soil respiration rate ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), T is soil temperature at 5 cm depth (°C). With reference to previous studies (Zou et al. 2018; Liu et al. 2019; Wei et al. 2020), the temperature sensitivity (Q_{10}) of soil respiration rates was calculated as $Q_{10} = e^{10b}$. Repeated-measured ANOVA tests were used to examine the

treatment effect on the rates of R_s , R_h and R_a in 2014 and 2015. Two-way ANOVA was performed to investigate the effects of N addition, P addition, and their interaction on the mean R_s and its components, soil enzymes activity, microbial community composition, as well as soil characteristics during the growing season in 2014 and 2015. One-way ANOVA with Tukey's HSD was used to test the differences among treatments in the fine root biomass, soil properties, MBC, and DOC, as well as the average values of R_s , R_h , and R_a . R_a and R_h in relation to abiotic and biotic controlling factors were further examined using linear regression models. A random forest algorithm was finally applied to assess the relative importance of abiotic and biotic drivers on R_a and R_h (Wang et al. 2019b). This method does not need any distribution assumptions and can deal with non-normal data and nonlinear relationships in the dataset. The importance values of each controlling factors were assessed by running the random forest 100 times (van Elsas et al. 2012). The normality and homogeneity tests of the data were performed before analyses and were transformed if the assumptions were not met. Statistical analyses were conducted with SPSS 19.0 (SPSS Inc., Chicago, IL, USA) for windows. The significance throughout the analyses was set at $P < 0.05$.

Results

Soil chemical properties and fine root biomass

Soil temperature and moisture showed clear seasonal variations and were not responsive to N and/or P addition (Fig. S1). Soil dissolved organic carbon (DOC) was not changed by any treatment in 2014 ($P > 0.05$), but it was significantly increased by 7.2% under P addition and by 10.0% under NP addition in 2015 compared with the control (Table 1, Table S1). Soil microbial biomass carbon (MBC) was not significantly affected by N, P or NP addition in either year ($P > 0.05$). Soil NH_4^+ -N was increased by 35.0% under N addition in 2014 and decreased by 13.0% under P addition in 2015 ($P < 0.05$). Nitrogen addition increased soil NO_3^- -N by 10%–18% across 2 years, while P addition decreased it by 8.9% in 2014 (all $P < 0.05$). Nitrogen addition did not significantly change soil AP, which was on average increased by 3.8 to 4.4 times, 3.5 to 4.5 times across 2 years under P and NP addition, respectively (all $P < 0.05$). Soil pH was not altered by any treatment in 2014, however, it was significantly decreased by 0.4, 0.2 and 0.3 unit under N, P and NP addition, respectively in 2015 (Table 1). Compared to the control conditions, soil Al^{3+} was increased under N, P, and NP treatments, while Ca^{2+} was decreased significantly under N and NP treatments (all $P < 0.05$). N, P, and NP treatments did not change the concentrations of Mg^{2+} or Na^+ ($P > 0.05$). Fine root biomass was reduced by 17.3%, 8.4%, and 14.3% under N, P, and NP addition, respectively across 2 years (all $P < 0.05$, Table 1).

Table 1 Soil available carbon, nitrogen, and phosphorus, soil cations, as well as fine root biomass under different treatments

	Year	CK	N	P	NP
Available C&N&P					
DOC (mg·kg ⁻¹)	2014	299.2 ± 8.3 a	281.6 ± 6.1 a	310.4 ± 19.1 a	314.1 ± 28.3 a
	2015	294.8 ± 2.8 b	288.9 ± 5.5 b	316.0 ± 3.1 a	324.3 ± 5.3 a
MBC (mg·kg ⁻¹)	2014	571.7 ± 3.8 a	557.1 ± 3.5a	589.3 ± 2.9 a	588.2 ± 19.6 a
	2015	563.4 ± 15 ab	545 ± 10.3 b	593.2 ± 4.8 a	587.0 ± 1.7 a
NH ₄ ⁺ (mg·kg ⁻¹)	2014	8.1 ± 0.2 b	10.9 ± 0.6 a	6.8 ± 0.1 b	7.4 ± 0.5 b
	2015	7.7 ± 0.1 a	8.7 ± 0.6 a	6.7 ± 0.1 b	8.1 ± 0.03 a
NO ₃ ⁻ (mg·kg ⁻¹)	2014	38.4 ± 0.9 b	42.4 ± 1.1 a	35.0 ± 0.3 c	37.1 ± 0.9 bc
	2015	40.6 ± 0.1 b	48.1 ± 2.3 a	36.9 ± 0.3 b	41.6 ± 0.7 b
AP (mg·kg ⁻¹)	2014	27.5 ± 8.4 b	24.8 ± 4.3 b	149.3 ± 11.9 a	150.6 ± 21.4 a
	2015	29.3 ± 3.6 b	22.3 ± 4.1 b	139.4 ± 12.8 a	131.9 ± 9.8 a
Soil cations					
Soil pH	2014	4.7 ± 0.04 a	4.5 ± 0.07 a	4.6 ± 0.03 a	4.5 ± 0.1 a
	2015	4.6 ± 0.08 a	4.2 ± 0.04 b	4.4 ± 0.1 b	4.3 ± 0.01 b
Al ³⁺ (mmol·kg ⁻¹)	2014	38.0 ± 1.0 c	50.2 ± 1.0 a	42.4 ± 0.8 b	44.8 ± 0.9 b
	2015	38.4 ± 3.2 c	60.4 ± 0.4 a	48.7 ± 0.8 b	61.0 ± 1.4 a
Ca ²⁺ (mmol·kg ⁻¹)	2014	33.6 ± 3.1 a	25.1 ± 1.8 b	28.8 ± 0.3 ab	25.0 ± 0.7 b
	2015	30.3 ± 2.9 a	20.1 ± 0.5 c	26.0 ± 0.2 ab	22.6 ± 0.5 bc
Mg ²⁺ (mmol·kg ⁻¹)	2014	4.7 ± 0.4 a	4.3 ± 0.2 a	4.2 ± 0.4 a	4.2 ± 0.1 a
	2015	4.2 ± 1.0 a	3.5 ± 0.6 a	3.7 ± 0.1 a	3.3 ± 0.1 a
Na ²⁺ (mmol·kg ⁻¹)	2014	1.5 ± 0.2 a	1.4 ± 0.04 a	1.3 ± 0.3 a	1.3 ± 0.2 a
	2015	1.2 ± 0.2 a	1.3 ± 0.4 a	1.2 ± 0.1 a	1.2 ± 0.1 a
Plant					
Fine root biomass (g·m ⁻²)	2014	172.2 ± 1.3 a	147.4 ± 4.0 c	161.9 ± 0.7 b	152.2 ± 1.5 c
	2015	178.9 ± 1.9 a	142.6 ± 1.7 d	159.7 ± 1.4 b	148.6 ± 1.2 c

Notes: Values are means with SE. The different lowercase letters in the same row indicate significant differences among the four treatments at $P < 0.05$ level. CK, control; N, nitrogen addition; P, phosphorus addition; NP, nitrogen and phosphorus addition

Soil respiration and its components

Soil respiration and its components exhibited strong seasonal patterns in both 2014 and 2015, with high values in July and August (Fig. 1). N or P addition significantly affected R_s , R_h and R_a in 2014 ($P < 0.01$), with the exception of R_h under N addition ($P > 0.05$, Table 2). However, there were no significant changes in R_s , R_h or R_a under N or P addition in 2015 ($P > 0.05$, Table 2). R_s and its components were significantly affected by the interaction of N addition and P addition in both 2014 and 2015 ($P < 0.01$). Mean R_s and R_h under N addition were reduced by 15.2% and 11.7%, respectively, and mean R_a was decreased by 15.0% relative to the control across the 2 years. P addition had an inconsistent effect on R_a , with R_a increasing by 50.5% in 2014 and decreasing by 26.6% in 2015. Moreover, P addition on average decreased the mean R_s and R_h by 11.1% and 19.9%, respectively during the experimental period (Fig. 1). NP addition on average increased R_s , R_a , and R_h by 1.9%, 7.9%, and 2.1%, respectively ($P > 0.05$) across 2 years. There were significant

correlations between soil respiration rates and soil temperature (Fig. S2). N and/or P addition did not alter Q_{10} values of R_s ($P > 0.05$). The Q_{10} values of R_h were significantly decreased under P and NP addition in 2014 ($P < 0.05$), but did not change by any treatment in 2015 ($P > 0.05$, Table 3).

Soil microbial community composition and enzyme activities

Compared to the control, N addition, P addition, and NP addition did not affect bacterial, actinobacterial, fungal PLFA and fungi: bacterial ratio in 2014 (all $P > 0.05$) (Fig. 2a–d), whereas the bacterial PLFA was significantly increased by 29.4% ($P < 0.05$) under NP addition in 2015 (Fig. 2e). Meanwhile, P addition increased fungal PLFA by 23.5% ($P < 0.05$), but fungi: bacterial ratio was not affected by any treatments in 2015 ($P > 0.05$) (Figs. 2g–h). Soil α -Glucosidase (AG) activity did not change significantly under any treatment in either years ($P > 0.05$) (Fig. 3a, e), whereas P addition significantly enhanced β -

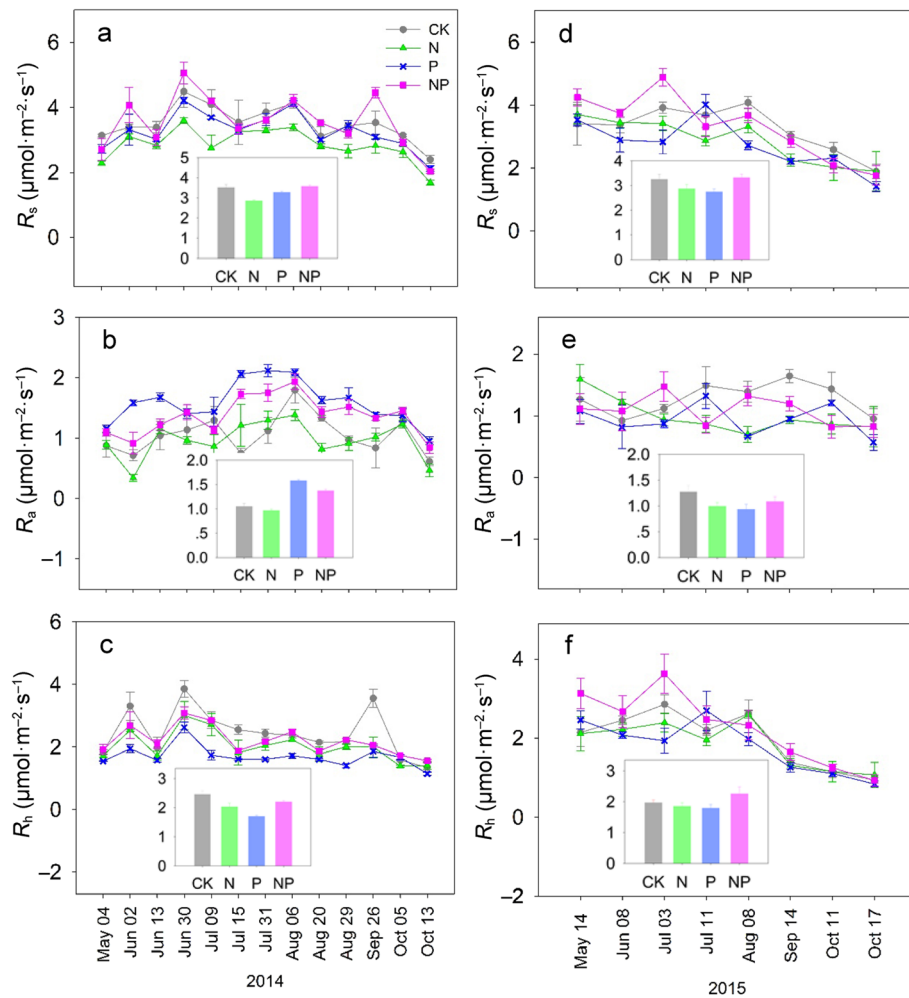


Fig. 1 Seasonal variability of soil respiration (R_s), autotrophic respiration (R_a) and heterotrophic respiration (R_h) under four treatments from 2014 to 2015. Vertical bars show standard errors of the means ($n = 4$). CK, control; N, nitrogen addition; P, phosphorus addition; NP, both nitrogen and phosphorus addition

Glucosidase (BG) activity in 2014 ($P < 0.05$) (Fig. 3b). Neither N nor P addition changed BG activity in 2015 (Fig. 3f). β -D-Cellubiosidase (CB) and β -Xylosidase (XYL) activity were significantly decreased under N addition and affected by the interaction of N and P addition in 2014 (Fig. 3c, d), while the significant treatment effect was only found for the interaction of N and P addition in 2015 ($P < 0.001$) (Fig. 3g, h).

Controlling factors of R_s and its components

R_a was negatively correlated with soil Al^{3+} ($P < 0.05$), $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ ($P < 0.001$), but positively related with DOC ($P < 0.05$), XYL and CB activities ($P < 0.001$) (Fig. 4). R_h was positively related to bacteria PLFA, Fungi PLFA, CB and XYL activities ($P < 0.01$). The random forest also showed that R_a was mainly controlled by XYL activity, followed by $\text{NO}_3^-\text{-N}$, CB activity, Al^{3+} , DOC,

and $\text{NH}_4^+\text{-N}$ (Fig. 5). However, the variation in R_h was primarily regulated by bacterial PLFA and CB activity, followed by fungal PLFA and XYL activity. Overall, R_s had significantly positive correlations with fungal PLFA, XYL and CB activities (Fig. S3). In addition, R_s increased significantly with soil MBC ($P < 0.05$) (Table S2).

Discussion

Contrasting responses of R_s to nitrogen and phosphorus addition

We found that N or P addition alone decreased R_s , while NP addition increased R_s during the 2-year field observation in this subtropical forest. This implies that soil respiration may be co-limited by N and P corroborating a previous study conducted in subtropical area (Wei et al. 2020). Both N and P addition alone inhibited R_s , which is in accordance with most of the previous studies in

Table 2 Results (F -values) of repeated-measures ANOVAs exploring the effects of measurement date (day) or year, nitrogen addition (N), phosphorus addition (P) and their interactions on soil respiration (R_s) and its components (R_a and R_h) ($n = 4$)

Year	Factor	R_s	R_a	R_h
2014	Day	25.26***	16.59***	22.73***
	N	8.02**	16.16***	0.96
	P	14.28***	147.78***	33.56***
	N × P	55.70***	2.43	81.09***
	Day × N	1.37	1.70	2.44**
	Day × P	1.24	2.74**	2.63**
	Day × N × P	1.53	1.61	1.74
	2015	Day	26.44***	2.06
N		0.89	0.97	3.45
P		0.06	3.26	1.54
N × P		21.39***	9.97**	9.63**
Day × N		2.94**	2.68*	0.82
Day × P		1.11	0.52	1.74
Day × N × P		2.12*	2.02	1.80

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

other forest ecosystems (Peng et al. 2017a; Li et al. 2018; Xiao et al. 2020; Zhang et al. 2020). It has been shown that the reduction of R_s was mainly due to the negative responses of R_h to fertilizer addition (Fig. 1). The positive effect of NP addition on R_s could be due to that soil labile C and microbial activities may be accelerated by P addition, which will cause the different impacts on soil microbial community and enzyme activities with N and P addition synchronously (Liu et al. 2019). This study demonstrated that the stimulation of fungal PLFA, CB and XYL activities induced by N and P addition in combination exceeded the inhibitory effects of N or P addition alone, accounting for the increased R_s under

Table 3 The Q_{10} values of soil total respiration (R_s) and heterotrophic respiration (R_h)

Year	Treatments	R_s	R_h
2014	CK	1.31 ± 0.07 a	1.44 ± 0.06 a
	N	1.44 ± 0.10 a	1.41 ± 0.13 a
	P	1.45 ± 0.08 a	1.10 ± 0.02 b
	NP	1.48 ± 0.12 a	1.31 ± 0.10 ab
2015	CK	1.79 ± 0.30 a	2.35 ± 0.67 a
	N	1.68 ± 0.31 a	2.47 ± 0.54 a
	P	1.39 ± 0.06 a	1.65 ± 0.14 a
	NP	1.60 ± 0.13 a	1.75 ± 0.35 a

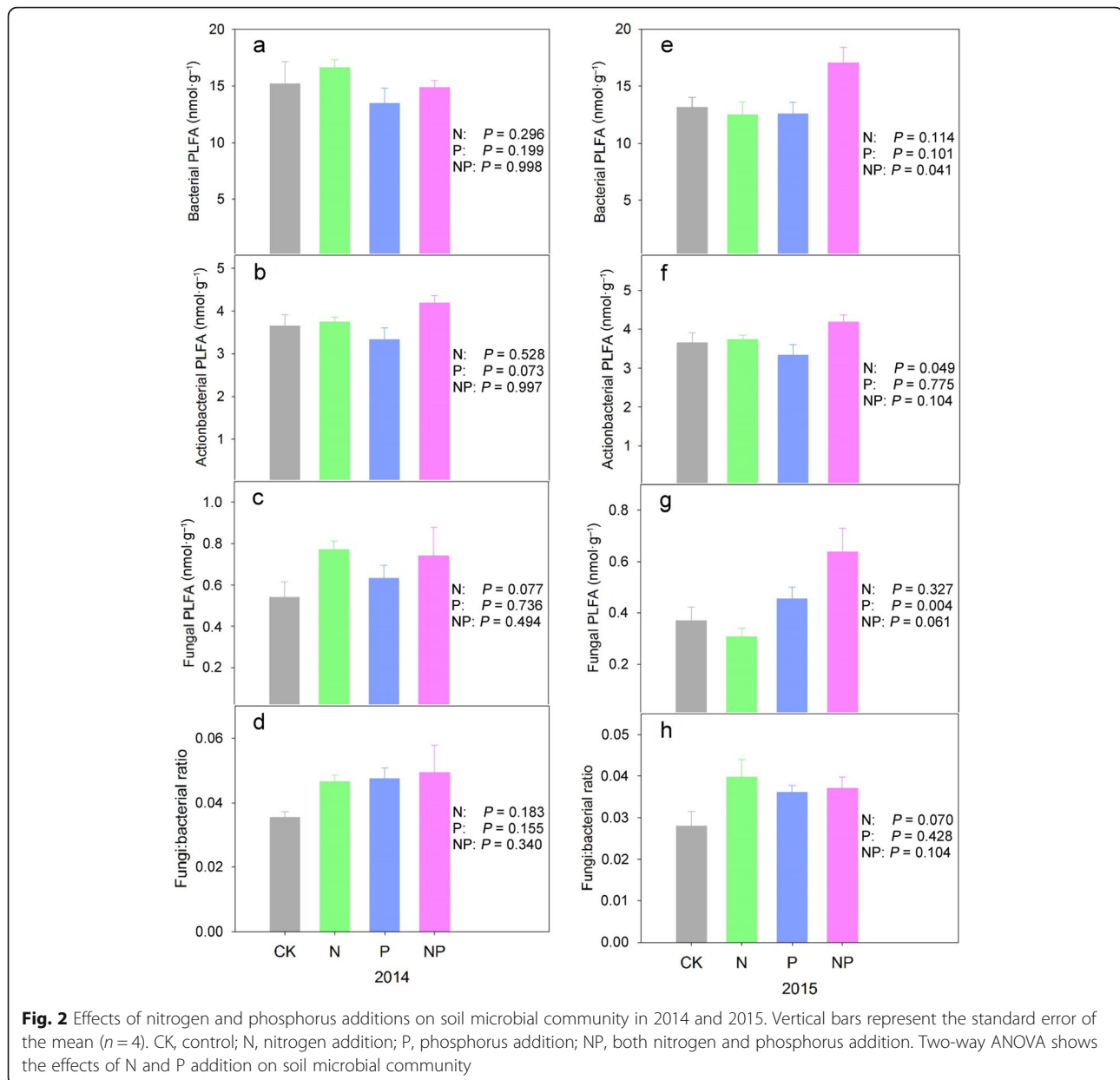
Note: Different letters in the column represent significant differences among the four treatments at $P < 0.05$ level

NP addition. In view of few studies on the interactive effect, the response of soil respiration to the interaction of N and P addition is critical to understand ecological dynamics in the forest management. Further work is still necessary to explore the potential mechanisms for R_s under N and P enrichment in combination (Li et al. 2016).

Differential responses of R_a and R_h to nitrogen and phosphorus addition

We demonstrated that the two components of soil respiration— R_a and R_h —responded differently to N and P addition. Nitrogen addition decreased R_a and R_h in our study in both years (Fig. 1). Previous studies have suggested that the reduction of R_a was closely related to the decrease in fine root biomass (Wang et al. 2015; Wang et al. 2017a; Liu et al. 2019). Although we detected a lower R_a accompanied with decreasing fine root biomass under N addition (Table 1), R_a had no significant correlation with fine root biomass (Fig. 5). The reduction of R_a could have resulted from soil acidification and ion imbalance by decreasing soil pH and base cations (Ca^{2+} , Na^+ and Mg^{2+}) (Table 1). Meanwhile, increasing Al/Ca ratios and decreasing base cations under excessive N addition indicate that the forest plants might be stressed by metal ion (Mao et al. 2017) and consequently affect R_a . The inhibitory of N enrichment on R_h was closely associated with changes in soil microbial activity and enzymes (Fig. 5). The global meta-analysis and field experiments have suggested that excessive N inputs exhibit negative effects on soil microbes (Tian et al. 2017; Zhang et al. 2018). It has also revealed that increasing soil available nutrients can reduce the production of soil enzymes (Cusack et al. 2010) and impede the formation of soil microbial C (Widdig et al. 2020). Moreover, soil acidification with N addition can affect soil microbial communities, which is closely associated with soil microbial respiration (Li et al. 2018). Hence, the lower soil pH, changes in microbial community composition, and reducing soil enzymes may explain the decrease in soil R_h under N addition in this study.

We also found that P addition alone had inconsistent effects on R_a in this subtropical forest, with R_a increasing in the first year and decreasing in the following year. The contrasting response of R_a to P addition with time may be due to changes in the abiotic and biotic controlling factors. For example, soil pH was not altered by P addition in 2014, but was significantly reduced in 2015 suggesting a progressive soil acidification with experiment lengthens (Table 1). Similarly, soil Al^{3+} toxicity was more apparent in the second year than the initial stage of experiment. The varying responses of R_a to P addition alone suggest that long-term studies in the field are still imperative



to assess the consequence of P addition on R_a in this P-limited ecosystem. P addition reduced R_h across 2 years, which is in accordance with a previous study in temperate forests (Zeng and Wang 2015). The reduction of R_h was also reflected by the decrease in soil enzyme activities, microbial PLFAs, and soil microbial biomass carbon (Jing et al. 2016). In this study, soil enzyme activities of CB and XYL were suppressed by P addition (Fig. 3, Table 1). Moreover, the significant positive correlations between R_h and PLFAs of fungi and bacteria suggested that the microbial community composition may also cause the change in soil R_h under P addition (Guo et al. 2017).

Contrary to the negative or inconsistent effects of N addition or P addition on R_a and R_h , we found NP addition increased soil respiration components in the present study. To date, few efforts have been made to investigate the interaction of N and P addition on R_a and R_h . In line with our findings, a combination of N and P addition experiment in N-limited temperate forests also found an increase in R_a with N and P addition in combination (Zeng et al. 2018). Furthermore, the stimulation of R_h under NP addition has been reported in another subtropical forest (Liu et al. 2019). The stimulatory effect of N and P co-addition on R_h could be attributed to an increase in fungi/bacteria ratio under nutrient

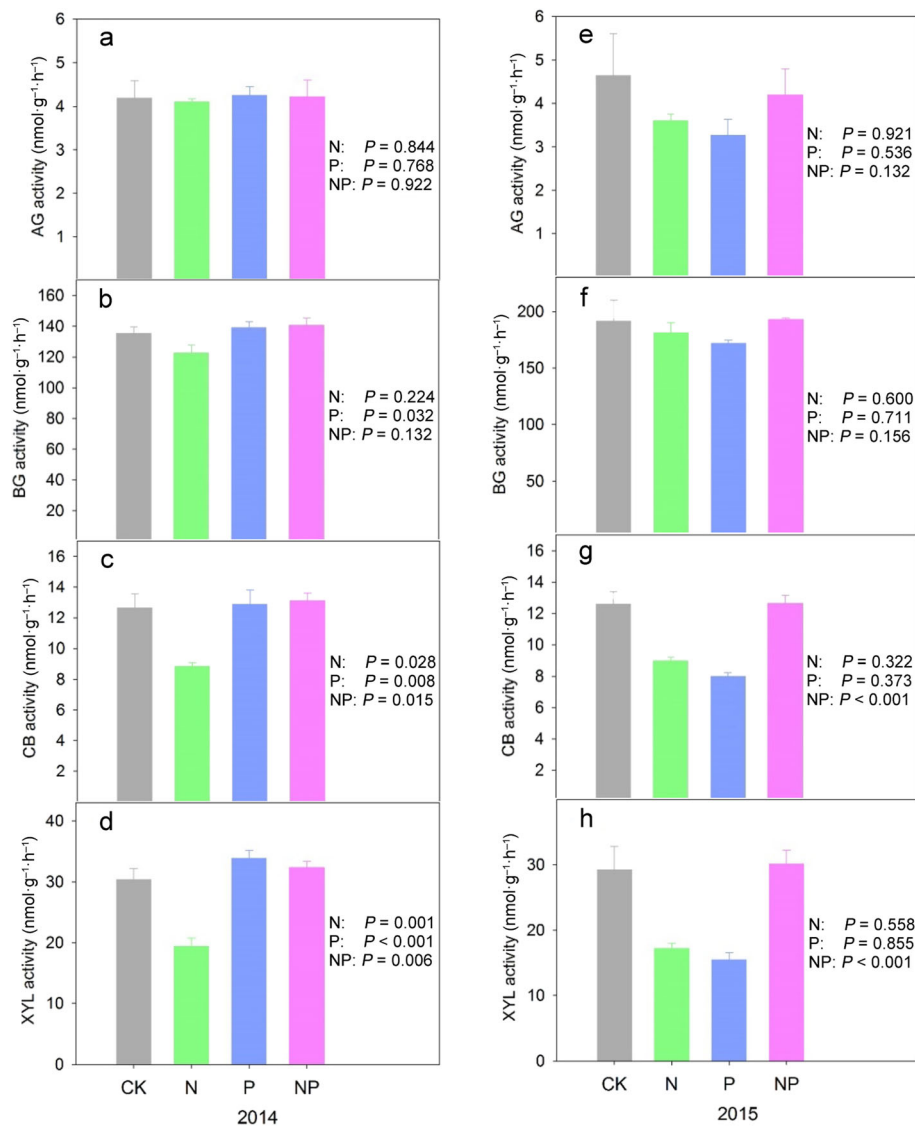


Fig. 3 Effects of nitrogen and phosphorus additions on soil enzyme activity in 2014 and 2015. Vertical bars represent the standard error of the mean ($n = 4$). CK, control; N, nitrogen addition; P, phosphorus addition; NP, both nitrogen and phosphorus addition. Two-way ANOVA shows the effects of N and P addition on soil enzymes activity

enrichment (Fig. 2), which might facilitate soil microbial carbon use efficiency (Riggs and Hobbie 2016). The increase of fungi/bacteria ratio in NP addition was also found in several other studies (Hagerberg et al. 2003; Liu et al. 2012; Zeng and Wang 2015). These results indicate that shifts in microbial community composition may play a vital role in regulating R_h changes following nutrient additions (Edwards et al. 2011). Accordingly, considering the interaction of N addition and P addition is necessary to evaluate responses of soil C processes in the scenario of aggravated N and P deposition. Our findings have additional implications for prediction of the impacts of N

and P enrichment on soil C dynamics in subtropical forests.

Responses of Q_{10} to nitrogen and phosphorus addition

Soil temperature is considered as one of the most important factors in regulating the changes of R_s (Zou et al. 2018). We found that Q_{10} values of R_s and R_h were not affected by fertilization with the exception of P addition significantly reducing Q_{10} of R_h in 2014. Similarly, Wei et al. (2020) also found that N and/or P addition did not significantly alter Q_{10} of R_s in a subtropical evergreen broad-leaved forest. The nonresponsiveness of temperature sensitivity of soil respiration

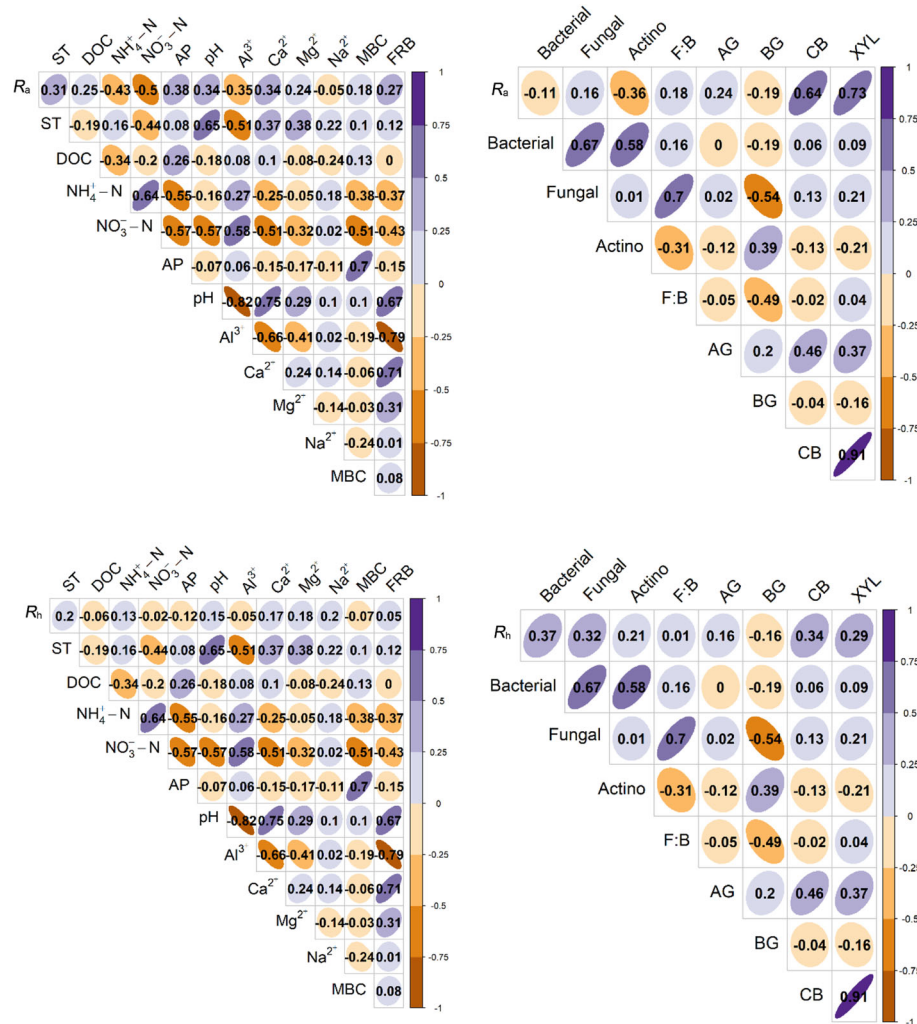


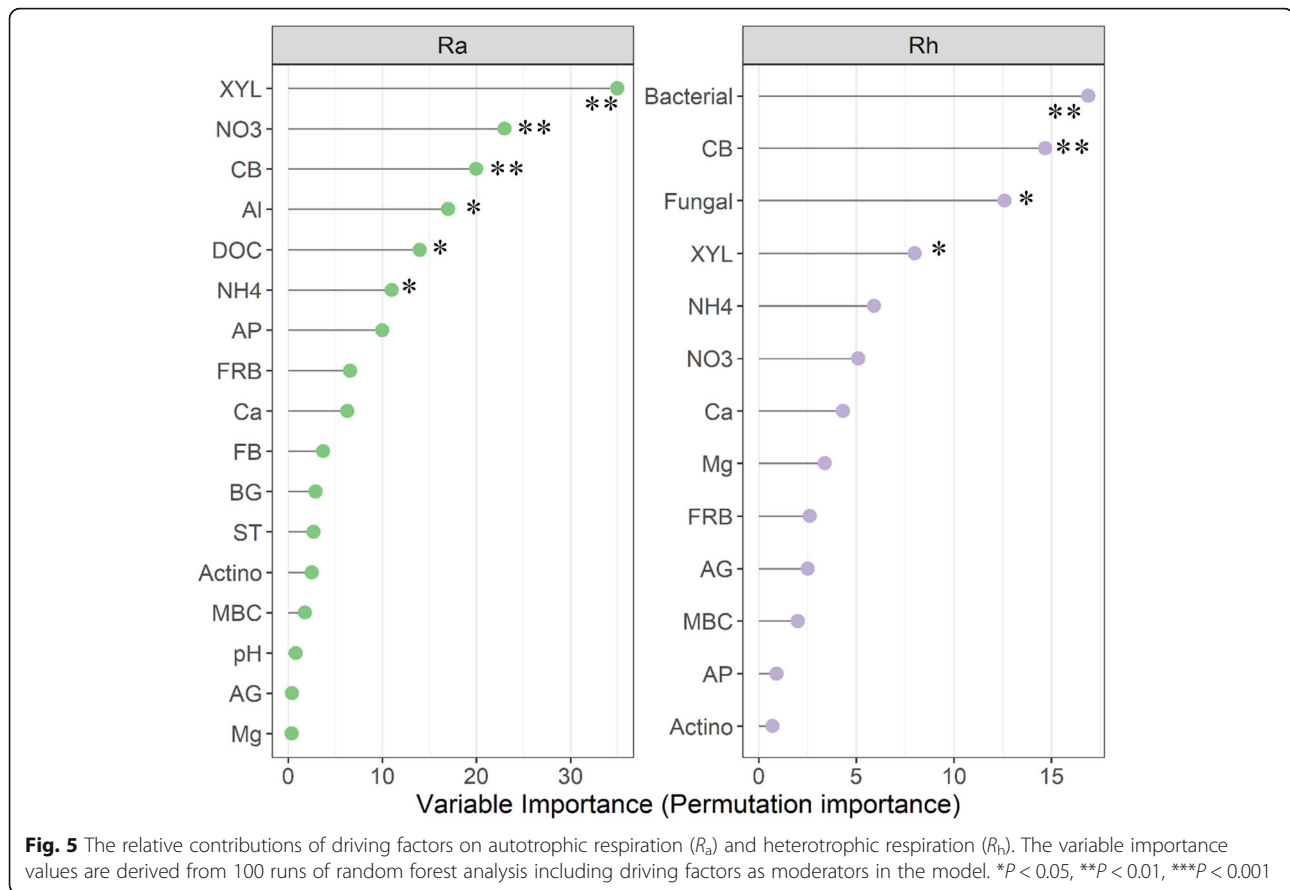
Fig. 4 Soil autotrophic respiration (R_a) and heterotrophic respiration (R_h) in relation to soil properties, soil microbial community, and enzymes activities. The colors show Spearman's rank correlation coefficient and indicate a positive or negative correlation

rates may be largely owing to that N or P addition did not induce changes in soil temperature and soil moisture (Fig. S1), which have proven to be responsible for the decline in the Q_{10} values of R_s and R_h under fertilization (Zhou et al. 2006). Nonetheless, we detected that P addition significantly reduced the Q_{10} value of R_h in the first year, which also had a trend to decrease under P addition or NP addition in the second year. Given that there were no significant effects of nutrient addition on soil temperature and moisture, the variation of Q_{10} values was probably due to shifts in soil microbial community structure and metabolic pathways (Zhang et al. 2014). It is suggested that the decomposition of soil organic matter decreases due to the formation of recalcitrant compounds, and soil enzyme activities could be inhibited by nutrient additions (Sun et al. 2014). Moreover, fertilization could affect the diversity and

community structure of soil microorganisms (Zhang et al. 2018). All of which have a potential to decrease the temperature sensitivity of soil microbial respiration under fertilization.

Conclusions

Soil respiration (R_s) and heterotrophic respiration (R_h) decreased when N or P was added separately, but increased when N and P was added in combination during 2-year field study. Nitrogen addition reduced autotrophic respiration (R_a) across 2 years, while P addition had an inconsistent effect on R_a , and N and P co-addition increased R_a . R_a was predominantly controlled by soil nutrient availability, enzyme activities, and soil dissolved organic carbon, while the variation in R_h was mainly ascribed to the changes in soil microbial community composition and enzyme activities under



fertilization. Temperature sensitivities of R_s and R_h were not affected by nutrient addition except for P addition significantly reducing the temperature sensitivity of R_h in the first year. Our findings highlight the contrasting responses of soil respiration and its components to N or P addition against N and P co-addition, which should be incorporated into biogeochemical models to understand and project soil carbon dynamics in the scenarios of aggravated N and P enrichment in terrestrial ecosystems. It is also important to note that this experiment was conducted in a short duration, whereas the delayed response of soil respiration to N or P addition may lead to different patterns with time. We call for that long-term field experiments are still urgent to disentangle the temporal effects of fertilization on soil carbon cycle processes.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s40663-021-00313-z>.

Additional file 1.

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Authors' contributions

Shuli Niu and Jinsong Wang designed research, Junjun Zhang and Yong Li performed research, collected and analyzed data; all authors discussed the results and revised the manuscript. The authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed in this study are available from the corresponding author on request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

All authors have no conflict of interest.

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