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Differential response of soil respiration to nitrogen and phosphorus addition in a highly phosphorus-limited subtropical forest, China

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ABSTRACT

Understanding feedback between terrestrial carbon (C) cycle and climate change is linked to the effects of nitrogen (N) and phosphorus (P) on soil respiration (*Rs*). However, the individual and interactive effects of N and P additions on soil respiration and its components (autotrophic [*Ra*] and heterotrophic respiration [*Rh*]) are not fully understood, especially in highly P limited subtropical forests. In this study, both field experiment and laboratory incubation (at 15 °C and 25 °C temperatures) were undertaken to examine the effects of N, P and N + P additions on *Rs* and *Rh*. Our results showed that N addition significantly increased *Rs* by 21.09%, but P and N + P additions exhibited no effects on *Rs* under field conditions. Under laboratory condition, N addition significantly suppressed *Rh* whereas P and N + P additions increased *Rh* compared with control. Meanwhile, N and P additions exhibited an antagonistic interaction on *Rs*, but N and P additions synergistically affected *Rh* under laboratory incubations at both incubation temperatures of 15 °C and 25 °C. Cumulative *Rh* was negatively correlated with fine root biomass, but was positively correlated with microbial biomass carbon regardless of incubation temperatures. Our findings indicated that both individual and interactive effects of N and P additions on *Rs* and *Rh* were required to be considered to improve prediction of N and P effects on forest C dynamics in the highly P limited subtropical forests.

1. Introduction

Anthropogenic activities, such as fossil fuel combustion and fertilizer application, have significantly increased atmospheric nitrogen (N) and phosphorus (P) depositions by threefold to fivefold over the past century (Liu and Greaver, 2010; Treseder, 2010). Therefore, both N and P depositions have been recognized as major contributors to global climate change (Galloway et al., 2004; IPCC, 2013). Increased N and P inputs to terrestrial ecosystems could significantly alter the biogeochemical cycle of carbon (C) leading to affect plant growth and soil biochemistry (Niu et al., 2016; Bai et al., 2015; Zhou et al., 2017a, 2017b). The altered C cycle induced by N and P depositions may lead to a positive or negative climate-biosphere C feedback, which in turn amplify or diminish their effects on ecosystem services and functions (Chapin et al., 2002; Zhou et al., 2014).

Soil respiration (*Rs*) is the second largest C flux between the atmosphere and terrestrial ecosystems varying between 68 and 90 Pg C yr^{-1} (Luo and Zhou, 2006). *Rs* consists of two components including autotrophic respiration (*Ra*), generated from live roots and their symbionts; and microbial respiration (*Rh*) generated from microbial decomposition of soil organic matter (Luo and Zhou, 2006; Zhou et al., 2016). A small change of *Rs* has a potential to significantly accelerate or mitigate the atmospheric carbon dioxide (CO_2) concentration (Gao et al., 2014; Zhou et al., 2019b). Therefore, understanding the responses of *Rs* and its components to N and P alterations is critical for us to predict terrestrial C cycle.

The effects of N and P additions on *Rs* have been widely studied in forest ecosystems (Gao et al., 2014; Niu et al., 2016). However, contradictory results with an increase (Gao et al., 2014), decrease (Illeris et al., 2003), and no effect (Mo et al., 2008) of N and P additions on *Rs*

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exist. The contradictory responses may be associated with the differences in initial soil nutrient condition, forest types and measurement methods (Janssens et al., 2010; Niu et al., 2016). For example, the response of *Rs* to N or P additions in temperate forests may differ from those in boreal forests since plant productivity and microbial diversity in warmer regions are usually higher than those in colder climates (Janssens et al., 2010; Niu et al., 2016). The N addition could initially increase plant N uptake, productivity and foliar N concentration in N-limited forests (Niu et al., 2016). However, N addition may gradually result in negative effects on plant growth as a result of N saturation (Galloway et al., 2004; Bai et al., 2015). Two meta-analyses have been used to address the inconsistencies exist among studies with respect to the effects of N and P additions on *Rs* and its components (Zhou et al. 2014; Feng and Zhu, 2019). It has been found that N addition significantly decreases *Rs* in forests globally, while P addition exhibits negative effects on *Rs* in forests (Feng *et al.,* 2019). A comprehensive review has also indicated that P addition significantly increases plant and soil C pools, but no effects of P addition on soil microbial biomass carbon (MBC) have been found (Yue et al., 2017). However, the effects of N and P additions on *Rs* and *Rh* in subtropical forests have been less studied where forests experience N depositions and have P-limited soil (Reay et al., 2008). These knowledge gaps may impede us to develop models of C cycle in response to future global change to some degree.

The relationship between N and P additions is coupled and may interactively affect *Rs* and its components (Niu et al., 2016; Zhou et al., 2017a, 2017b). Previous studies have demonstrated that the interactive effects among global climate change factors on the terrestrial C cycle are common but complex (Crain et al., 2008). For example, the interaction between N and P additions on soil C pool and MBC are generally additive at both individual and community levels (Yue et al., 2017; Zhou et al., 2019a). The N and P additions may also exhibit contradictory effects on soil organic C deposition processes (i.e., microbial respiration), which could further regulate soil organic C (SOC) turnover (Poeplau et al., 2016). It has also been suggested that N and P additions could jointly alter the temperature sensitivity (Q_{10}) of *Rs* by modifying soil biochemical and plant physiological processes (Wang et al., 2016). However, to what extent N and P additions interact to affect *Rs* and *Rh* in highly P-limited subtropical forest remain unclear.

Subtropical forest ecosystems are one of the most important vegetation types in China and play a dominant role in the C cycle in China due to their huge capacity to store C and produce above ground biomass (Pan et al., 2011; Zhou et al., 2017a, 2017b). Meanwhile, the subtropical forests in China have also experienced high N deposition rate and are highly P limited (Gao et al., 2014; Jia et al., 2014). Therefore, in this study, we conducted a field experiment to explore the effects of N and P additions and their interactions on *Rs* in a highly P limited subtropical forest. We also carried out a laboratory incubation experiment to verify our field experiment findings. Specifically, our objectives were to (1) examine the effects of N and P additions and their interactions on *Rs* and *Rh*; and (2) assess the potential mechanisms regulating *Rs* and *Rh* in response to N and P additions.

2. Materials and methods

2.1. Field study

2.1.1. Site description

The field study is located in Tiantong National Forest Ecosystem Observation and Research Station (29°48′ N, 121°47′ E), Zhejiang Province, China. This area is characterized as a subtropical monsoon climate with humid hot summers and dry cold winters (Zhou et al., 2017a, 2017b). Mean annual temperature is 16.2 °C, ranging from 4.2 °C in January to 28.2 °C in July and mean annual precipitation is 1374 mm, which mainly occurs from May to August (data from China Climatological Survey in Tiantong, Zhejiang). The soil type is Acrisol, with medium-heavy loam texture and the organic layer is approximately 5 cm thick, and pH is ranging from 4.4 to 5.1 (Zhou et al., 2017a, 2017b). The dominant tree species in this region include *Castanopsis fargesii*, *Lithocarpus glaber* and *Schima superba.* Soil and vegetation properties of the experimental sites have been comprehensively described by Gao et al. (2014) and Zheng et al. (2017). Nitrogen and phosphorus deposition of this region is 36.02 kg N ha/y and 0.75 kg P ha/y, respectively (Zhu et al., 2016).

2.1.2. Field experimental design

The experimental design was a randomized complete block with three replicates. Each block had four 20 m × 20 m plots. The plots were at least 10 m apart. Each plot was enclosed with PVC boards (3 mm in thickness) inserted into the soil up to a depth of 60 cm to isolate lateral water movement. The treatments were randomly applied to the plots within each block. The treatments included control (CK) with no N and P additions; N treatment with 100 kg N ha/y addition; P treatment with 15 kg P ha/y addition; and N + P treatment with 100 kg N ha/y + 15 kg P ha/y. Fertilizer (NH_4NO_3 or NaH_2PO_3 in 20 L of water) was monthly applied over the litter layer from January 2011 to December 2018. Meanwhile, control plots (CK) received 20 L of water to avoid throughfall differences among different treatments.

2.1.3. Field soil respiration measurements

To measure soil respiration (*Rs*), two collars (50 cm × 50 cm × 10 cm) were randomly inserted into the soil permanently to the depth of 10 cm in each plot. Small aboveground seedling and grass inside the collars were removed manually without soil disturbance, 24 h prior to *Rs* measurement to eliminate aboveground plant respiration. Four gas samples were collected by 100 ml plastic syringes with intervals of 0, 10, 20 and 30 min after fitting the chamber (50 cm × 50 cm × 50 cm) tightly to the collar. *Rs* was measured monthly between 9:00 am and 11:00 am (local time) (Zheng et al., 2009). Gas samples were analyzed using the gas chromatography (Agilent Technologies, Palo Alto, CA, USA). Air temperatures, soil temperatures and soil water contents at the depth of 10 cm were simultaneously measured when gas samples were collected. Hourly *Rs* rates were calculated using the following equation:

$$Rs = \frac{M}{V_0} * \frac{P}{P_0} * \frac{T_0}{T} * \frac{dC_t}{dt} * h \quad (1)$$

where *Rs* is the soil respiration rate (mg C/m^2/h); M is the molecular weight of C (g/mol); V_0 is the gas volume (22.41 × 10^{-3} m^3/mol) at the standard condition (273 K, 1013 hPa); T_0 and P_0 are the absolute temperature (K) and gas pressure (hPa) at the standard condition, respectively; T and P are the air temperature (K) and air pressure (hPa) at the sampling time, respectively; dC_t/d_t is the slope of linear regression between CO_2 concentration and sampling time, and *h* is the height of chamber (m).

2.2. Laboratory study

2.2.1. Laboratory incubation and microbial respiration measurements

Soil samples were collected from the experimental site to establish a laboratory incubation in December 2013. Five samples were randomly collected using a soil corer (inner diameter 9 cm) at depths of 0–20 cm and then mixed to constitute one sample in each plot. Moist soil samples were carefully sieved to remove rocks and coarse plant debris (e.g., fresh and dead roots, stem materials and litter fractions) and then stored in the refrigerator at 4 °C less than one week before incubation.

Before the experiment, a sub-sample of each fresh soil (30 g) was placed in 250 ml incubation bottles, with 12 replicates for each treatment. Then, all soil samples were rewetted to 60% WHC using a sprayer to stimulate precipitation under an incubator with a constant temperature (25 °C). Each incubation bottle had a beaker containing 10 ml distilled water and a glass beaker containing 10 ml of NaOH solution (0.1 mol/l). The NaOH solution was simultaneously placed inside the

bottle and then the lid was immediately closed. All incubation bottles were placed into an incubator. Half of the incubation bottles were kept at the 15 °C and the other half kept under 25 °C. Six-replicated glass beakers were also put inside jars without soil as the blank treatment. Soil moisture was maintained constant throughout the incubation period by weekly weighing and replacing the lost moisture with distilled-water. Microbial respiration (*Rh*) were measured with titration method as descripted in Li (2015) on days 3, 7, 12, 18, 24, 29, 36, 43, 51, 59, 64, 70, 76, 83, 93, 103 following the incubation commencement. Other incubation details of this experiment can be found in Li (2015). Microbial respiration (*Rh*) was determined using the following equation:

$$Rh = \frac{(V_1 - V_2) * C_{\mathrm{HCl}} * 6}{M_s * T} \quad (2)$$

where *Rh* is the microbial respiration rate (mg C/g soil); V_1 is the volume of consumed HCl solution for blank treatment (mL); V_2 is the volume of consumed HCl solution for fertilization treatments (mL); C_{HCl} is the concentration of HCl solution (mg/L), M_s is the soil mass (g); T is the sealed time during per incubation period (d).

2.2.2. Fine root biomass, microbial biomass carbon and litterfall productivity

Five soil cores were randomly collected using a soil corer (6.5 cm in diameter) at two depths of 0–10 cm and 10–20 cm in each plot just before sample collection for the incubation study in October 2012. Soil fine root biomass (FRB, < 2 mm diameters) and microbial biomass C (MBC) were measured. Fine roots were hand-picked and all attached residues (e.g., soil, dead roots, stem materials and litter fractions) were carefully removed with tweezers. After collecting visible fine roots, the remaining soil was sieved through a 0.15 mm mesh sieve and was gently rinsed to collect fine root segments. FRB was oven dried at 70 °C for 48 h to reach a constant weight. MBC content was determined by fumigation extraction technique as descripted in Zhou et al. (2017a), (2017b). Four 1 m × 1 m traps in each plot were also established for litterfall measurement in September 2011. Litter samples were collected monthly during the period of study. Litter samples were oven dried at 70 °C for 48 h to calculate the litter biomass.

2.3. Data analyses

2.3.1. Temperature sensitivity

We assessed the sensitivity of mean soil CO_2 efflux to soil temperature by fitting exponential functions to the data from CK, N, P and N + P treatments.

$$R = ae^{bT} \quad (3)$$

where *R* is the mean value of *Rs* (mg C m^{-2} h^{-1}); *a* is the intercept of *Rs* when the temperature is zero (basal respiration rate), and T is the soil temperature (°C) at the 5 cm depth. The temperature sensitivity (Q_{10}) of microbial respiration (*Rh*) was then determined using the following equation.

$$Q_{10} = \frac{Rh_{25}}{Rh_{15}} \quad (4)$$

where Rh_{25} and Rh_{15} are the microbial respiration (mg C/g soil/d) under 25 °C and 15 °C, respectively.

2.3.2. Interactive effects

Hedge's d was employed to calculate the effect size of interactions in two individual paired treatments for *Rs*, *Rh*, MBC, FRB and litterfall as described by Gurevitch and Hedges (2001). The main effects of N and P additions and their interactions were calculated using Eqs. (5), (6) and (7), respectively.

$$d_A = \frac{(\bar{X}_N + \bar{X}_{N+P}) - (\bar{X}_P + \bar{X}_{CK})}{2s} J(m) \quad (5)$$

$$d_B = \frac{(\bar{X}_N + \bar{X}_{N+P}) - (\bar{X}_N + \bar{X}_{CK})}{2s} J(m) \quad (6)$$

$$d_I = \frac{(\bar{X}_{N+P} - \bar{X}_N) - (\bar{X}_P - \bar{X}_{CK})}{2s} J(m) \quad (7)$$

where $\bar{X}_{CK}$ $\bar{X}_N$ $\bar{X}_P$ and $\bar{X}_{N+P}$ are means of a variable in the control (CK), N, P and N + P, respectively; *s* and *m* are the pooled standard deviation, and degree of freedom ($m = n_{CK} + n_N + n_P + n_{N+P} - 4$). The $J(m)$ is the pooled standard deviation and correction term for small sample bias (Hedges and Olkin 1985). The *s*, *m* and $J(m)$, were estimated using Eqs. (8)–(10), respectively

$$s = \sqrt{\frac{(n_{CK} - 1)(s_{CK})^2 + (n_N - 1)(s_N)^2 + (n_P - 1)(s_P)^2 + (n_{N+P} - 1)(s_{N+P})^2}{n_{CK} + n_N + n_P + n_{N+P} - 4}} \quad (8)$$

$$m = n_{CK} + n_N + n_P + n_{N+P} - 4 \quad (9)$$

$$J(m) = 1 - \frac{3}{4m - 1} \quad (10)$$

where n_N, n_P, n_{CK}, n_{N+P} are the sample sizes, and s_N, s_P, s_{CK} and s_{N+P} are the standard deviations in the N and P treatments (s_N, s_P), control (s_{CK}), and N + P treatment (s_{N+P}), respectively; The variance of d_I(v_2) of main effects and interactions as well as weighted mean d_I (d_{++}) were estimated using the following equations.

$$v_{2_i} = \left[\frac{1}{n_{CK}} + \frac{1}{n_N} + \frac{1}{n_P} + \frac{1}{n_{N+P}} + \frac{d_I^2}{2(n_{CK} + n_N + n_P + n_{N+P})}\right]/4 \quad (11)$$

$$d_{++} = \frac{\sum_{i=1}^{m}\sum_{j=1}^{k} w_{ij} d_{ij}}{\sum_{i=1}^{m}\sum_{j=1}^{k} w_{ij}} \quad (12)$$

where *I* is the number of groups, *k* is the number of comparisons in the i^{th} group and w is weight, which is also the reciprocal of the variance ($1/v_2$).

When the sample number was > 20, the 95% CI of RR_{++} and d_{++} was calculated as $RR_{++} \pm C_{a/2} \times s(RR_{++})$ and $d_{++} \pm C_{a/2} \times s(d_{++})$, respectively, where $C_{a/2}$ is the two-tailed critical value of the standard normal distribution. The bootstrapping method was conducted to resampling based on 2500 iterations which was consistent with the calculations of CI of individual and combined effects, when the sample number was larger than 20. The interaction types were classified as antagonistic, synergistic and additive according to the above calculations (Crain et al., 2008). Specifically, the interactive effect was considered to be additive if the 95% CI overlapped with zero. For factor groups whose individual effects were either both negative or exhibit opposite directions, the interactions < 0 were classified as synergistic and > 0 were antagonistic (Crain et al., 2008).

One-way analysis of variance (ANOVA) was used to determine the impacts of N, P and N + P additions on *Rs*, *Rh*, FRB, MBC, and litterfall productivity. Data were natural-log-transformed when normal distribution and homogeneity of variance assumptions were not met. All statistical analyses were conducted with SPSS 16.0 for Windows (SPSS. Inc., Chicago, IL, USA), and figures were prepared using Sigmaplot 10.0 (Systat Software Inc., CA, USA).

3. Results

3.1. Microclimate

A significant seasonal pattern was found in both air and soil temperatures with higher values during June-October period than the December-February period from 2011 to 2013 (Fig. 1). In contrast, no

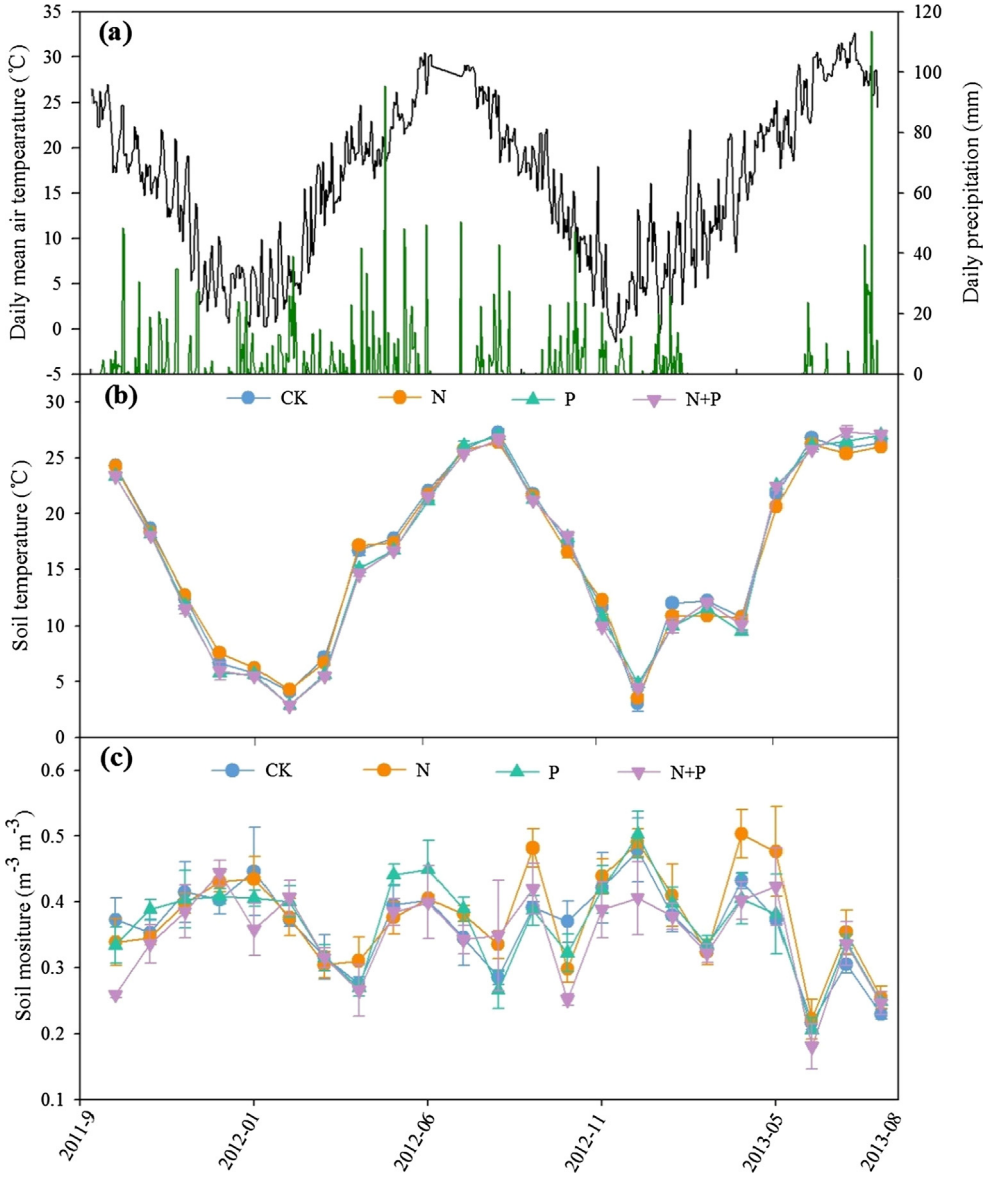

Fig. 1. Temporal variation of climate factors from September 2011 to August 2013. (a) Daily mean air temperature (gray line) and daily precipitation (black bar), (b) soil temperature at 5 cm, and (c) volumetric soil moisture to a depth of 5 cm (*v/v*%). Data of air temperature and precipitation were collected from a nearby weather station of Tiantong.

seasonal pattern of daily precipitation and soil moisture were observed throughout the study period. The N, P and N + P applications did not significanlty influence soil temperature and soil moisture at the depth of 0–10 cm compared with that of control (Fig. 1; $P > 0.05$).

3.2. Individual and interactive effects of N and P addition on Rs and Rh

The N and P additions had different effects on *Rs* and *Rh* under both field and laboratory conditions (Fig. 2,3). Specifically, N significantly increased *Rs* by 21.09%, while P and N + P treatments exhibited no effects on *Rs* compared with those in control treatment (CK) in the field condition (Fig. 2). However, N addition decreased *Rh* in soils incubated under 15 °C and 25 °C by 7.78% and 8.89%, respectively compared with CK (Fig. 3). P addition increased *Rh* by 5.76% and 14.28% under 15 °C and 25 °C in the laboratory condition (Fig. 3). The N + P did not affect *Rh* compared with CK at 15 °C but a significant increase of *Rh* was observed compared with CK at 25 °C in N + P treatment (Fig. 3).

The N and P additions interactively influenced *Rs* and *Rh* in Tiantong forest ecosystems. The interactive effect of N and P additions on *Rs* was antagonistic, while a synergistic interaction was observed on *Rh* under both 15 °C and 25 °C conditions (Fig. 4). Similar to *Rs*, N and P additions exhibited antagonistic interaction on FRB and MBC, while an additive interaction on litterfall was observed after N and P additions (Fig. 4).

3.3. Annual soil respiration and cumulative microbial respiration

Annual *Rs* was significantly higher in N treatment than those in CK, P and N + P (Fig. 5). Specifically, the average annual *Rs* under CK, N, P and N + P were 596.53, 854.11, 669.24, and 658.45 g C m^{-2} y^{-1}, respectively. Cumulative microbial respiration (*Rh*) throughout the incubation under P addition was higher than the other three treatments under both 15 °C and 25 °C (Fig. 5e, f). N addition also decreased the cumulative *Rh* under 15 °C and 25 °C by 7.57%, and 9.56% compared with that in the CK (Fig. 5e, f). Cumulative *Rh* under 25 °C was significantly higher than those under 15 °C across in all treatments.

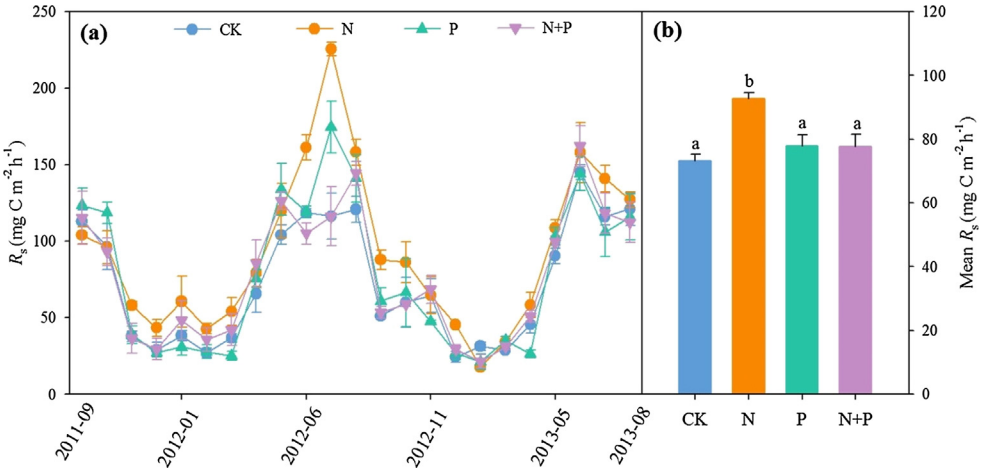

Fig. 2. Seasonal variability of soil respiration (*Rs*, a) under control, N addition, phosphorous addition and their combination (N + P). Average and standard error values of soil respiration (*Rs*, d) over the whole studies time under different treatments are shown as a histogram in the right part of each figure. Symbols *a* represents the significant differences among three application levels for the responses of selected variables to nitrogen, phosphorous and their combinations.

3.4. Temperature sensitivity of soil and microbial respiration

Soil temperature at 0–10 cm accounted for 65–76% variations of *Rs* among four treatments, with highest under CK and lowest under N + P (Fig. 6a). Soil moisture exhibited a significantly negative correlation with *Rs* but showed different slops under four treatments (Fig. 6b). The apparent Q_{10} value of *Rs* in the P treatment was significantly higher than that under the other three treatments, and Q_{10} did not differ among CK, N and N + P treatments (Fig. 6c). The Q_{10} value of *Rh* in the P and N + P additions were significantly higher than those in the CK and N addition (Fig. 6d).

3.5. Effects of N and P addition on biotic factors

The N significantly stimulated FRB by 46.05%, while no significant differences in FRB were observed among CK, P and N + P treatments (Fig. 7a). The P addition increased MBC by 27.82%, but N and N + P addition had no effects on MBC compared with CK (Fig. 7b). The N + P addition significantly increased litterfall (+14.19%) compared with that of other treatments. However, no significant effects of N or P treatments on litterfall biomass were observed compared with CK treatment (Fig. 7c). The FRB exhibited significant negative correlations with cumulative *Rh* under both 15 °C and 25 °C but with different slopes (Fig. 7d). The MBC was positively correlated with cumulative *Rh* (Fig. 7e). In addition, no significant correlation between litterfall and cumulative *Rh* under 15 °C or 25 °C was observed (Fig. 7f).

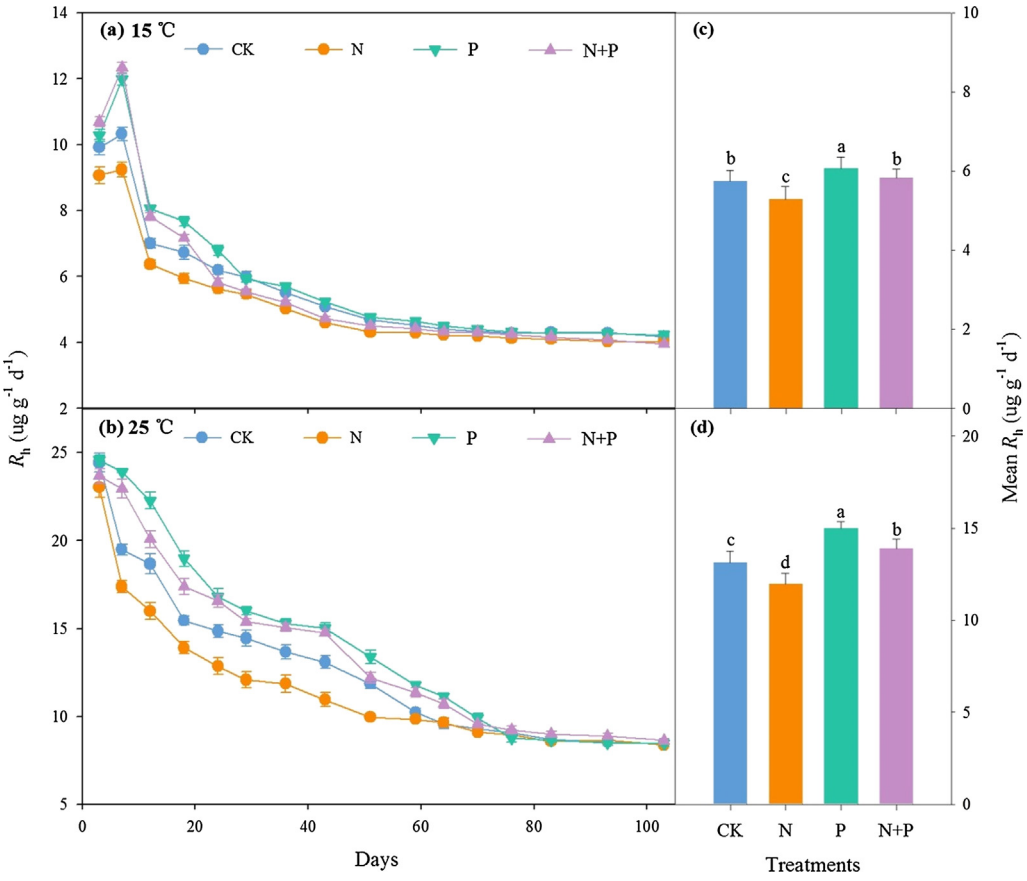

Fig. 3. Temporary variability of microbial respiration (*Rh*) incubated at 15 °C (a) and 25 °C (b), respectively under control, N addition, phosphorous addition and their combination (N + P). Average and standard error values of microbial respiration (*Rh*) over the whole incubating time under four treatments are shown as a histogram in the right part of each figure (c, d). Symbols *a* represents the significant differences among three application levels for the responses of selected variables to nitrogen, phosphorous and their combinations.

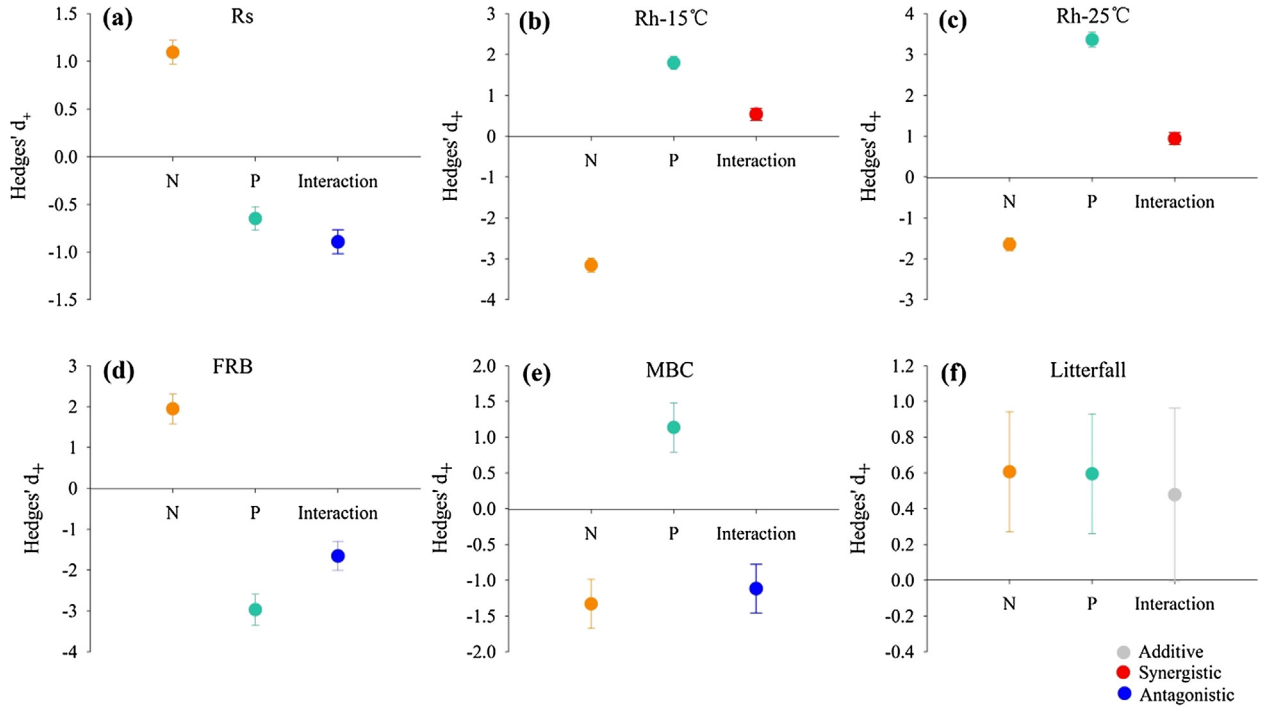

Fig. 4. The main and interactive effects of N addition, phosphorous addition and their combination on soil respiration, microbial respiration (*Rh*), FRB, MBC and litterfall. Values represent means with 95% bootstrap confidence intervals (CIs). If the 95% CIs do not overlap with zero, a response is considered to be significant ($P < 0.05$). FRB, fine root biomass; MBC, microbial biomass carbon.

4. Discussion

4.1. Individual effects of N and P additions on Rs and Rh

Predicting ecosystem response to N and P additions and assessing the C-climate cycle feedback strongly rely on our understanding the mechanisms that affect soil respiration and its components following N and P additions (Luo and Zhou, 2006; Jiang et al., 2017). In this study, we found that N addition significantly increased *Rs* under the field condition, but decreased *Rh* under laboratory incubation (Figs. 2, 3). Increased *Rs* by N addition may be explained through different mechanisms. One possible mechanism is that N addition significantly stimulated fine root biomass (FRB). It has been shown that N addition increases photosynthetically fixed C inputs to roots, leading to increase FRB as well as soil C pools which partly explains increased *Rs* (Gao et al., 2014; Zhou et al., 2014). The increased plant N uptake may stimulate the plant N content in leaves and roots, resulting in increased net primary productivity (NPP) and increased root respiration (Xia and Wan, 2008; Zhou et al., 2014). However, in contrast to field observations, N addition significantly decreased microbial respiration (*Rh*) in the laboratory incubation under both 15 °C and 25 °C, indicating that microbial activities might have been inhibited by N addition. High N inputs to soil may cause soil acidification and ion imbalance, leading to decreased soil microbial biomass and diversity which in turn reduce microbial respiration (Wallace et al., 2007; Zhou et al., 2014; Jiang et al., 2017). In our study, stimulation of FRB induced by N addition outweighed the inhibition effects of N addition on microbial biomass, explaining increased *Rs* under field condition after N addition.

Interestingly, P addition exhibited no significant effects on *Rs* during the 2-year field study (Fig. 2). The non-significant effect of P on *Rs* may be attributed to the delayed response of plants to P addition (Chapin et al., 2002). Our results also confirmed that P addition had no

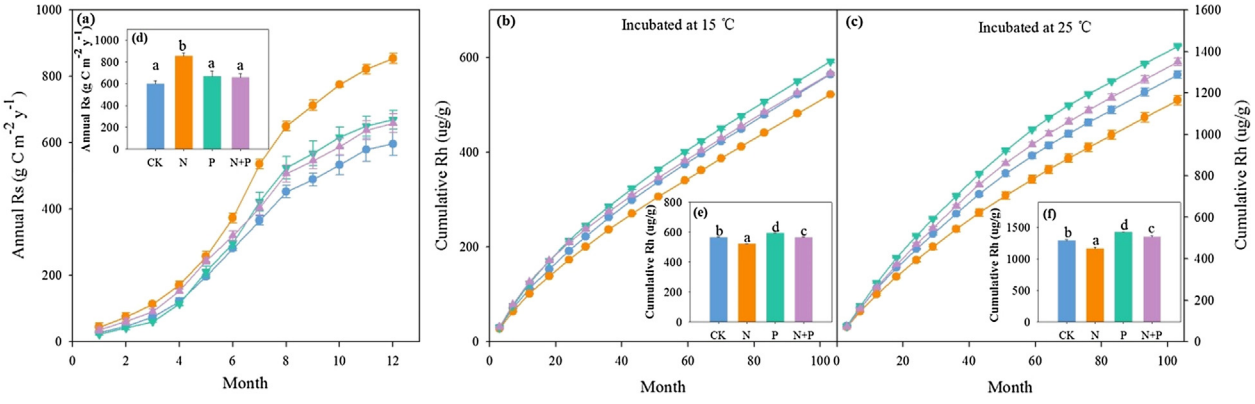

Fig. 5. Cumulative soil respiration (*Rs*) under four treatments (a). Temporary cumulative microbial respiration (*Rh*) incubated at 15 °C (b) and 25 °C (c), respectively under control, N addition, phosphorous addition and their combination (N + P). Annual soil respiration (d) and cumulative microbial respiration (*Rh*) at 15 °C (e) and 25 °C (f) under four treatments during the whole concerned study period. Symbols *a* represents the significant differences among three application levels for the responses of selected variables to nitrogen, phosphorous and their combinations.

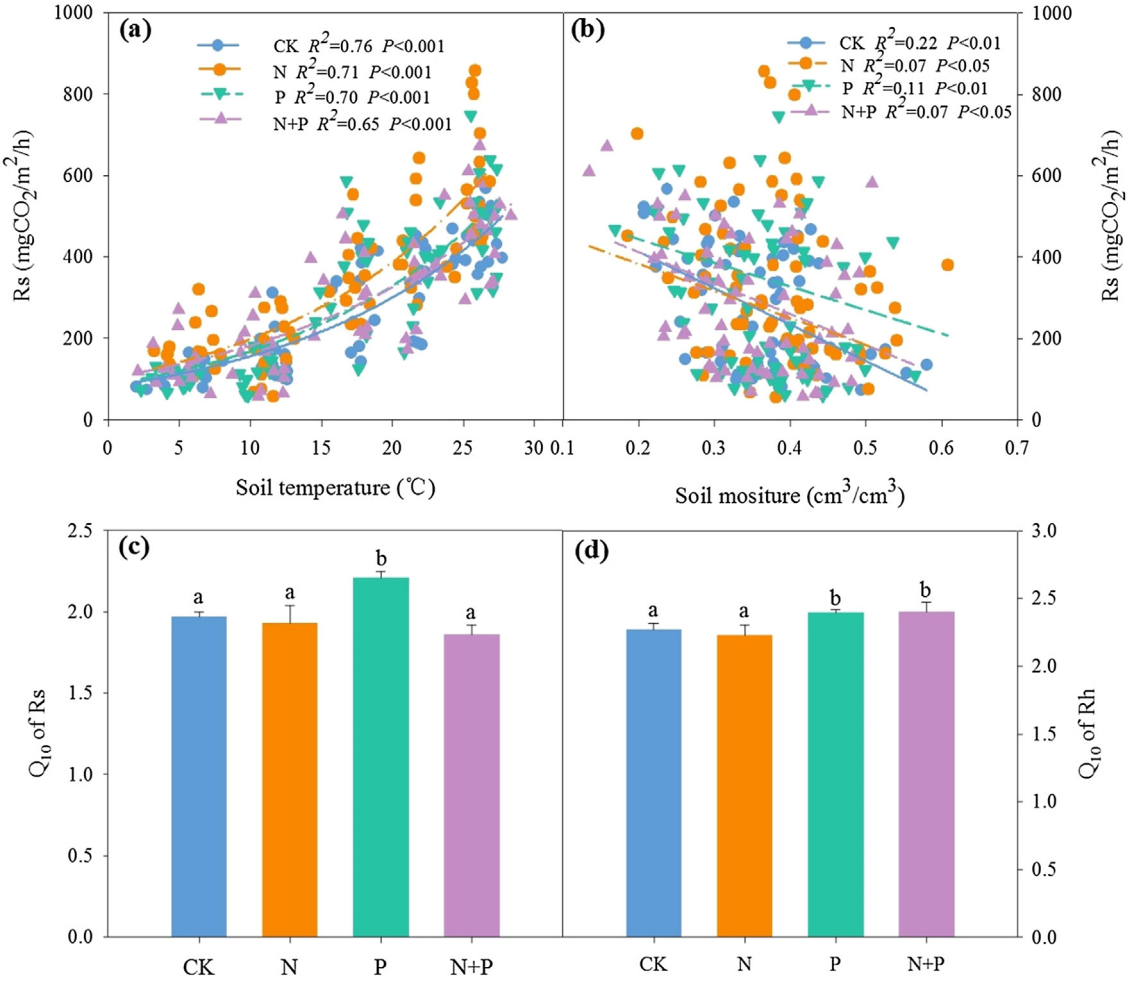

Fig. 6. Fine root biomass (a), microbial biomass carbon (b) and litterfall (c) under control, N addition, phosphorous addition and their combination (N + P). Relationships between cumulative microbial respiration (*Rh*) with fine root biomass (d), microbial biomass carbon (e) and litterfall (f). Symbols *a* represents the significant differences among three application levels for the responses of selected variables to nitrogen, phosphorous and their combinations.

significant effect on FRB in the field condition ($P > 0.05$, Fig. 7). Similarly, no effects on root growth, microbial biomass and *Rs* have been reported following P addition in the first two years in another study (Yang, 2014). However, increases in root growth, microbial biomass and *Rs* in year three following the P addition have been reported (Yang, 2014). In contrast to our field observation, P addition significantly enhanced *Rh* under both 15 °C and 25 °C laboratory incubation temperatures (Fig. 3). The increase of soil P availability eliminates the nutrient limitation for microbial growth, leading to increased microbial biomass, diversity and then microbial respiration (Chapin et al., 2002).

4.2. Combined and interactive effects of N and P (N + P) addition on Rs and Rh

Global climate change usually involves simultaneous changes in multiple environmental factors (e.g., N and P additions), which may interactively affect *Rs* and its components (Yuan and Chen, 2015). In this study, N + P treatment significantly increased *Rh* and the stimulations were stronger than those under N addition but weaker than those under P addition at the 25 °C incubation temperature (Figs. 3, 4). When no plant competes with microbes for N uptake under laboratory conditions, continuous N addition is likely to outride soil N saturation thresholds to some degree. N addition may thus exhibit negative effects on soil microorganisms thorough increase in soil acidification or metal ion imbalance (Wallace et al., 2007; Niu et al., 2016), thereby decreasing *Rh*. The P addition may stimulate microbial biomass, however, the inhibited microbial activity and diversity induced by N addition may mask the effects of P addition on soil microbes when N and P additions occur concurrently (Chapin et al., 2002; Yue et al., 2017). A study undertaken in a forest ecosystem has suggested that N or P alone exhibits no effects on *Ra* and *Rh* whereas N + P application significantly increases *Ra* but decreases *Rh* (Zeng and Wang, 2015). This difference may have resulted from the contrast effects of N and P on fine root biomass and fine root N concentration, but N + P had larger stimulation on root biomass and microbial C-use efficiency than N or P alone (CUE, Bekele et al., 2003; Sinsabaugh et al., 2013).

Interestingly, the interactive effects of N and P additions on *Rs* were antagonistic in the field condition, but showed a synergistic effect on *Rh* under both 15 °C and 25 °C incubation temperatures (Fig. 4). Plants are more tolerant to increased soil N content than microbes, increased N inputs may stimulate root growth but inhibit soil microbial activity in the field condition (Niu et al., 2016). Soil microbial activities and organic matter decomposition rates may be impeded by competition for nutrients among different soil communities (e.g., bacteria and fungi) when N and P additions occur concurrently (Yang, 2014; Niu et al., 2016; Yue et al., 2017). Therefore, increased microbial biomass stimulated by P addition may not be well translated into increased microbial activity. N and P showed a synergistic interaction under both 15 °C and 25 °C incubation temperatures (Fig. 4). The suppressed 'microbial N mining' process and shifted microbial community composition induced by N addition, largely protect more labile substrates from decomposition (Gallo et al., 2004; Craine et al., 2007). P addition may

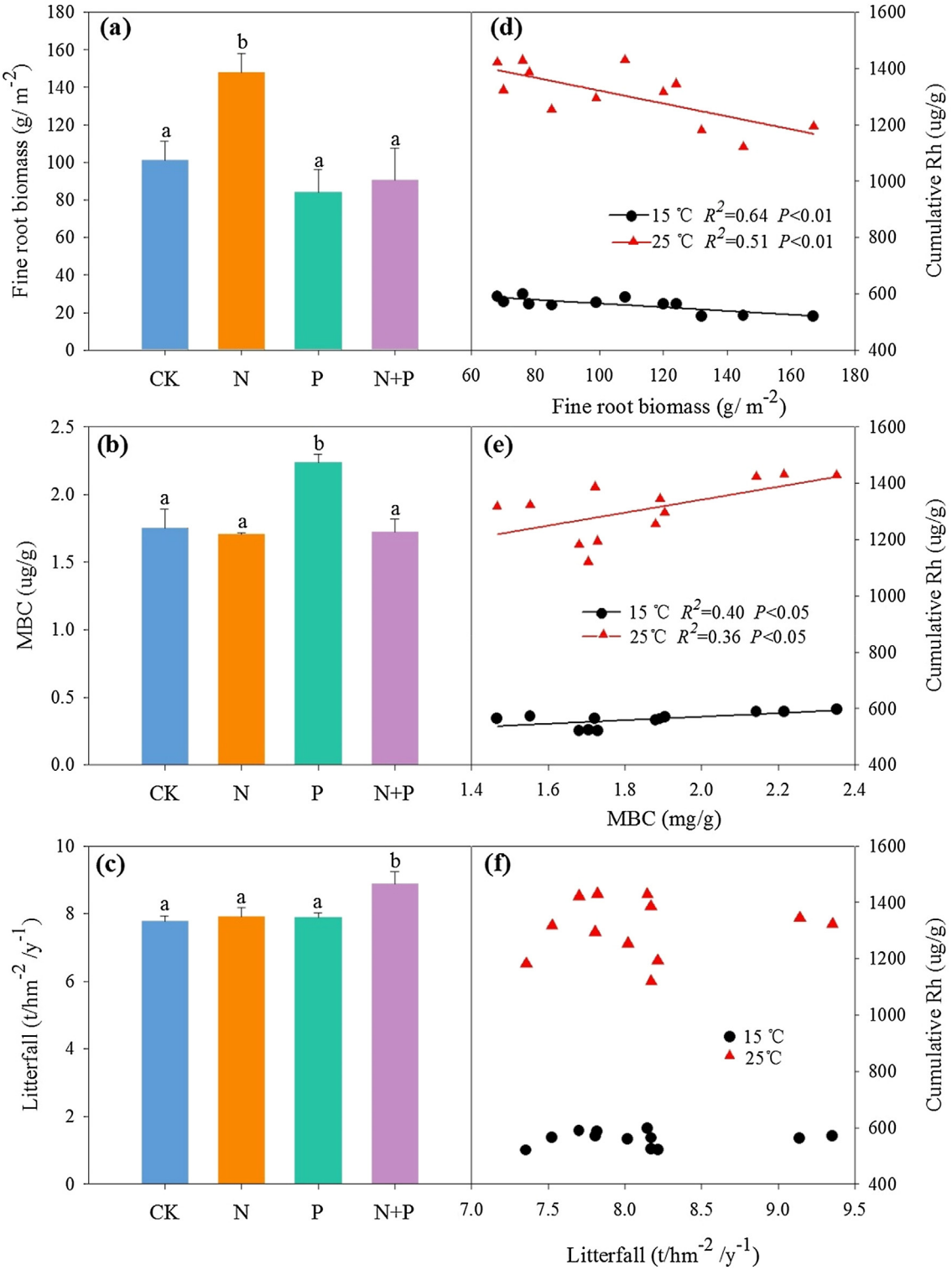

Fig. 7. Relationships between soil respiration (*Rs*) with soil temperature (a) and soil moisture (b) at 0–10 cm under control, N addition, phosphorous addition and their combination (N + P). Temperature sensitivity (Q_{10}) of soil respiration (c) and microbial respiration (*Rh*) under the above four treatments. Symbols *a* represents the significant differences among three application levels for the responses of selected variables to nitrogen, phosphorous and their combinations.

significantly enhance microbial biomass and activity by stimulating microbial C assimilation leading to increase the decomposition of labile substrates and then increase C release (Luo and Zhou, 2006). Therefore, the synergic effects of N + P addition on soil microbial activities may be explained by the different microbial response to N and P addition.

4.3. Effects of initial FRB, MBC and litterfall on Rh under laboratory condition

Substrate availability has been recognized as one of the major factors in controlling microbial respiration (Luo and Zhou, 2006; Janssens et al., 2010). In this study, we found that the cumulative microbial respiration (*Rh*) exhibited a significant negative correlation with initial

FRB, but positively correlated with MBC under both incubation temperatures (Fig. 7). Root usually has a competitive advantage for nutrient uptake than microbes under P-limited condition, leading to a decrease in microbial biomass when FRB increases (Chapin et al., 2002; Yang, 2014). It has been shown that abundant root exudates (e.g., oxalic acid and glucose) may stimulate the decomposition of native soil C with "priming effect" or by interrupting mineral protection of soil C (Kuzyakov et al., 2000; Keiluweit et al., 2015). The net loss of soil C induced by FRB decreases substrate availabilities, leading to reduce cumulative microbial respiration in the laboratory condition (Luo and Zhou, 2006; Keiluweit et al., 2015). In addition, P addition significantly increased microbial diversity and then MBC in the P-limited forest, causing more substrates being decomposed by soil microbes and then stimulating microbial respiration under laboratory incubation (Fig. 7; Luo and Zhou, 2006). Therefore, we concluded that the difference in substrate availability may explain the difference observed in microbial respiration between field and laboratory conditions.

4.4. Implications for future experiments design and model development

Global climate changes usually have a drastic influence on biogeochemical cycles of C of Earth's ecosystems, which may lead to a positive or negative climate-biosphere feedback (Janssens et al., 2010; Yuan and Chen, 2015). In this study, we explored the individual and interactive effects of N and P additions on soil microbial respiration in a Chinese subtropical forest using both field and laboratory incubation methods. Thus, our findings would provide some suggestions for the design of future experiments as well as the development and improvement of land surface models in subtropical forest ecosystem as follows.

First, experimental duration may be crucial in evaluating the response of soil microbial respiration (*Rs* and *Rh*) to different global climate change drivers. The plant would have shown an increased N uptake until N demand is met and then levels off over time (Niu et al., 2016). Soil respiration has been shown to decrease in response to N addition with increased duration of N addition across the terrestrial ecosystems (Zhou et al., 2014). Meanwhile, the delayed response of plant growth to P addition may cause different response patterns in soil respiration over time in forest ecosystems. Therefore, it is necessary to investigate the individual and combined effects of N and P on soil respiration from mid-term (decades) to long-term (centuries) for better understanding the climate-biosphere feedback.

Second, warming, elevated atmospheric CO_2, N addition, P addition, drought and changes in precipitation patterns may interact to modify the biogeochemical C cycle in terrestrial ecosystems (Crain et al., 2008; Yue et al., 2017; Zhou et al., 2018). Our study only investigated the interactions between N and P additions on soil microbial respiration in a subtropical forest ecosystem. How global climate change factors (at least 3) interact to influence *Rs* and *Rh* in forests remain a major gap of knowledge. Therefore, well-designed global change experiments with more factors (≥ 2) at large scale are necessary to better understand the individual and interactive of global change factors on the C cycle in forest ecosystems.

Third, our results showed that whilst N addition significantly increased *Rs* in the field experiment, a significant negative effect of N addition on microbial respiration (*Rh*) was observed in the laboratory condition. P addition, however, had no effects on *Rs* in the field but significantly stimulated microbial respiration in the laboratory condition. These results jointly demonstrated that microbial respiration may exhibit different performance under field and laboratory conditions following N and P additions. However, most of the current soil respiration models do not differentiate the C source of microbial respiration, which may under or over-estimate actual C flux (Herbst et al., 2008). Future land-surface models may need to differentiate the C source of *Rh* in order to develop more precise process-based mechanisms to forecast the feedback of forest ecosystems to climate change.

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Appendix A. Supplementary material

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