

Soil biochemical parameters in the rhizosphere contribute more to changes in soil respiration and its components than those in the bulk soil under nitrogen application in croplands

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Abstract

Aims Soil respiration (RS), which is the second largest carbon flux between the atmosphere and terrestrial ecosystems, has significant impact on atmospheric CO₂ concentration and climatic dynamics. Nitrogen (N) fertilizer has been heavily applied in agroecosystems at the global scale for high crop yields, and plays a major role in regulating RS. Although the respective response of soil biochemical property and RS to N addition has been widely studied, the contributions of soil biochemical parameters especially in the rhizosphere to changes in RS and its components (soil heterotrophic (RH) and autotrophic (RA) respiration) under N application remain poorly understood. The present study aimed to examine whether the rhizosphere effect alters the

relationship between soil biochemical properties and RS under N addition in croplands.

Methods We conducted N application experiment in a wheat-maize rotation system in the North China Plain. N fertilizer was applied at four different levels during both wheat and maize growing seasons: 0, 120, 180 and 240 kg N ha⁻¹. Soil biochemical parameters (e.g. soil enzyme activities, available N, and glomalin contents), RS and its components were measured under all N treatments.

Results First, N addition only significantly enhanced RA in 2014 (the fifth year of N application) but increased both RA and RH in 2015 (the sixth year of N application) because RH had lower N sensitivity than RA or lower soil moisture in 2014 which weakened the

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effect of N on RH. Second, soil enzyme activities, glomalin and available N contents in both the rhizosphere and bulk soil were significantly improved by N addition. Third, soil biochemical parameters in the rhizosphere explained the changes in RS and its components than those in the bulk soil. Specifically, soil enzyme activities in the bulk soil were more related to RS and its components than those in the rhizosphere, however, available N and glomalin contents showed contrary results.

Conclusions Our study indicated that the contributions of soil biochemical parameters to RS and its components under nitrogen application vary between the bulk and rhizosphere soil, which should be considered in Earth system models to improve their predictive abilities.

Keywords Nitrogen fertilization · Autotrophic respiration · Heterotrophic respiration · Soil enzyme activity · Rhizosphere · Cropland

Introduction

Nitrogen (N) fertilizer has been heavily applied in agroecosystems at the global scale because of its enormous contributions to soil nutrient conditions and crop yields. N addition in agricultural ecosystems and other anthropogenic activities have increased atmospheric N deposition by three- to fivefold over the past century (Reay et al. 2008). N deposition has been considered as one of important global change factors that can significantly affect the terrestrial carbon (C) cycle and is predicted to increase in the future (Janssens and Luysaert 2009; IPCC 2013; Chen et al. 2015). As the second largest C flux between the atmosphere and terrestrial ecosystems with 98 Pg C yr^{-1} (Bond-lamberty and Thomson 2010), soil respiration (RS) shows significant impact on atmospheric CO_2 concentration and climatic dynamics (Luo and Zhou 2006). Compared with other ecosystems, the responses of RS and its components to N addition have been less studied in agroecosystems (Chen et al. 2017b; Chen et al. 2018). Furthermore, the relationships between soil biochemical parameters especially in the rhizosphere with RS and its components under N application remain unclear.

RS is the product of respiration by roots (autotrophic respiration, RA) and soil decomposers (heterotrophic respiration, RH). Although numerous experiments have

been conducted, the effect of N addition on RS is still debated. Previous studies in cropland systems showed that N fertilizer could increase, decrease, or have no effect on RS (Liang et al. 2015; Liu et al. 2015; Zang et al. 2016). These large inconsistencies in the literature are primarily attributed to the various responses of RH and RA (Bond-Lamberty et al. 2004; Zhou et al. 2014). Meanwhile, different responses of RS components to N addition that are used in terrestrial ecosystem models lead to the difficulties in model result comparison. Therefore, exploring the respective response of RA and RH to N application is crucial because it not only can improve our mechanistic understanding of how N addition affects soil C efflux but also provide valuable information for improving model predictive ability. The partitioning of RS is another important issue in carbon cycling, plant physiology, soil science, and global climate change modelling (Bond-Lamberty et al. 2004). The ratio of RA to RS (RA/RS) usually varies in different ecosystems. Gong et al. (2014) reported that RA/RS in grassland ecosystem ranged from 10% to 62%, but the range was 39%–53% in forest ecosystem (Hinko-Najera et al. 2015). RA/RS under N addition has been less studied in cropland systems (Chen et al. 2017b; Chen et al. 2018). Furthermore, all these limited numbers of studies only used one crop growing season. However, Jeong et al. (2016) explored the effects of compound fertilizer on RS and its components in a forest soil, and found that RA/RS significantly changed between the first and second year because of the increased nutrient availability. Therefore, studies should be done in agroecosystem to assess if RA/RS under N addition also changes in different growing seasons and explore the factors that result in this change.

Besides the impact on RS, N fertilizer plays a vital role in regulating soil biochemical properties. For example, N addition significantly increased soil available N contents in the bulk soil in croplands (Ai et al. 2012; Chen et al. 2017b). As a good indicator of soil biological condition, soil enzyme's response to nitrogen application has been widely studied because of its role in controlling soil C cycles (Hu et al. 2014; Liang et al. 2015). Recently, one meta-analysis about the effect of N addition on soil enzyme activities has been conducted (Chen et al. 2017a), and the result showed that N addition in agroecosystems significantly stimulated glycosidase activities such as β -glucosidase (BG), β -xylosidase (BXYL) and Cellobiohydrolase (CBH) in the bulk soil. As a critical zone of interactions among

plants, soils, and microorganisms, rhizosphere plays a great role in C and nutrient cycling (Zhang et al. 2010). However, compared to vast studies on enzyme activities and available N contents in the bulk soil, a limited number of experiments have been conducted to assess how different N application rates affect them in the rhizosphere (Ai et al. 2012). In order to achieve a comprehensive understanding of N application effects, soil biochemical parameters in both the bulk and rhizosphere soil should be simultaneously determined.

Although the respective responses of soil biochemical properties and RS to N addition have been extensively studied, the relationships between soil biochemical parameters especially in the rhizosphere with RS and its components under N application remain poorly understood. Many previous studies found that RS was significantly related to both available N contents and soil enzyme activities (e.g. BG, BXYL and CBH) in the bulk soil (Liang et al. 2015; Chen et al. 2018). Soil enzyme activities are usually lower in the bulk soil than in the rhizosphere, as a result of microbial activity induced by root exudates, or because of the release of enzymes from roots (Badalucco and Kuikman 2001). In addition, rhizosphere effect resulted in the different values of soil available N contents in the bulk and rhizosphere soil (Ai et al. 2012). It is, therefore, important to examine whether rhizosphere effect changes the relationships between RS and soil biochemical parameters.

Cropland ecosystem, which accounts for about 12% of the global landscape and stores large amounts of soil C, is considered to have a large potential for C sequestration and climate change mitigation (Lal 2004; Alexandratos and Bruinsma 2012). As one of main farmland regions all over the world, North China Plain (NCP) covers about 300,000 km² of farmland that is largely devoted to grain production. The wheat and maize rotation system in the NCP has been applied an extraordinary high N fertilizer rate (329 kg ha⁻¹) as compared with North America (155 kg ha⁻¹) (Pei et al. 2015), which not only induces low fertilizer use efficiency but also results in the degradation of the environment, through increased greenhouse gas emissions, nutrient run-off and significant acidification (Guo et al. 2010; Liang et al. 2015). Zhang et al. (2013) found that reduced N use on croplands in China could cut N fertilizer-related emissions by 54%, and such reduction would decrease China's total greenhouse gas emissions by 5%, which is significant on a global scale. Therefore,

examining the responses of RS and its components to different N addition levels is critical to recommend an appropriate N application rate in NCP which not only leads to relative low C loss but also improves soil biochemical properties (e.g. increasing soil enzyme activities and available N contents). In the present study, we conducted a field experiment to test the following hypotheses: (1) N addition enhances RA and RH simultaneously and then results in the increase of RS; (2) soil biochemical properties in the rhizosphere and the bulk soil show different responses to N addition; (3) soil biochemical parameters in the rhizosphere have stronger relationships with RS and its components than those in the bulk soil. To the best of our knowledge, this is the first study to examine the role of rhizosphere effect in regulating the contributions of soil biochemical parameters to RS and its components under N addition in two successive growing seasons of agroecosystem.

Materials and methods

Experimental site and design

This experiment was established in a wheat-maize rotation system at Agricultural Station of Chinese Academy of Agricultural Sciences (39°36'N, 116°36'E) in 2010. This site is at an elevation of about 18 m above sea level, and is located in Hebei Province, China. The climate is typical temperate continental monsoon and the annual average temperature is 11.9 °C, with the mean monthly lowest and highest values occurring in January and July, respectively. The mean annual precipitation is 550 mm, with 80% falling between June and September. Soil properties (0–20 cm) before the experiment were 6.38 g kg⁻¹ soil organic C (SOC), 0.85 g kg⁻¹ total nitrogen content, 12.75 mg kg⁻¹ available P (Olsen method), 93.7 mg kg⁻¹ available K (NH₄AC extraction, atomic absorption spectrophotometer (AAS) method), and 8.0 pH (H₂O).

Twelve 10.4 × 6.4 m² plots, containing four treatments with three replicates, were initiated in 2010 by using randomized block design. According to numerous on-farm investigations from 1997 to 2007 (Cui et al. 2010), the typical N rate for farmers was about 240 kg N ha⁻¹ crop⁻¹ for the intensive wheat-maize system in the NCP. However, the regional mean optimal N rate is recommended to be 150–180 kg N ha⁻¹ crop⁻¹ for the intensive wheat-maize system in China (Zhang

et al. 2002). Therefore, this experiment included four different N fertilizer application rates: 0 (control), 120 (low N rate), 180 (optimal N rate) and 240 kg N ha⁻¹ (traditional N rate) for every crop growing season, and will henceforth be denoted as N0, N120, N180, and N240, respectively. Maize was sowed in June and harvested in October, and wheat was sowed in October and harvested in June of the following year. Crop straws in every plot were smashed by a harvester and then directly incorporated into the soil (20 cm depth) after crop harvesting. Nitrogen fertilizer was applied to the soil in the form of urea, 50% of N was applied as basal fertilizers just prior to sowing the crops, while the remaining half was applied at jointing and heading stages during wheat and maize growing season, respectively. Calcium superphosphate (150 kg P₂O₅ ha⁻¹) and Potassium sulphate (75 kg K₂O ha⁻¹) were also applied before sowing the crop.

A modified root exclusion method was used to partition RS (total soil respiration) into RH (heterotrophic respiration) and RA (autotrophic respiration). In each plot, we selected one pair of patches (0.5 × 0.5 m²) which were in the same furrow and next to each other to conduct comparative experiments (Fig. S1a), namely, the control treatment (with roots) and the comparative treatment (without roots). Specifically, for each comparative treatment, we dug a hole which was about 50 cm deep and 50 cm square 10 days after sowing crop. Soil at every 10 cm depth from the hole was placed separately. Nylon mesh (size 0.038 mm) was put on all internal surfaces of the holes to prevent root growth into the patches and allow the movement of water, bacteria, organic matter, and minerals, thus reducing disturbance to natural soil conditions (Moyano et al. 2007). After that, we refilled all soils into the hole according to former different depth (Fig. S1b). Therefore, all roots in each comparative treatment were excluded during the whole maize growing season. In contrast, we did not do anything for each control treatment. It was assumed that the CO₂ efflux measured in the comparative treatment was composed of only RH, while that measured in the control treatment was composed of both RH and RA. To eliminate the effect of digging hole on RH, we set up three pairs of comparative experiments (digging hole is “disturbed RH”, no-digging hole is “undisturbed RH”) on the bare soil which was close to our experimental field (Fig. S1c). Since there were no plants around the comparative treatment, the difference between “disturbed RH” and “no-disturbed RH” resulted from

digging hole. The CO₂ efflux measured in the comparative treatment at five growth stages in 2014 and 2015 were used to perform the linear regression analysis, respectively. We found that there were significant linear correlations between “disturbed RH” and “undisturbed RH” in both 2014 and 2015 (Fig. S2), and digging hole increased RH. We put measured RH (disturbed RH) under all N treatments in 2014 and 2015 into the linear regression in 2014 and 2015, respectively, to get the new RH (undisturbed RH) which were used in data analysis.

Soil sampling and analyses

Soil samples were collected at seedling stage in 2014 maize growing season, when both N addition and rhizosphere effects tend to be most pronounced. Rhizosphere soil was operationally defined as soil adhering to the total roots after gentle shaking, while bulk soil was defined as unvegetated soil adjacent to the plants (Ai et al. 2013). The whole plant and its roots were extracted from the soil, after shaking off the loosely adhering soil, we sampled the remaining soil attached to the plant roots (rhizosphere soil) by brushing it off with a toothbrush. The unvegetated soil cores (5 cm diameter) adjacent to the plants (bulk soil) were sampled at depth 0–20 cm. In order to obtain the enough rhizosphere soil for multiple assays, three plants were randomly selected from each plot, and these rhizosphere soils were pooled to form one composite sample. Correspondingly, one composite bulk soil consisting of three cores was randomly taken from each plot. Thus, three composite samples of each treatment were collected at seedling stage. The fresh samples were stored immediately in sealed plastic bags and transported to the laboratory in an insulated container. After the visible stones, roots and other litters were removed by hand, soil samples were sieved (<2 mm). Aliquots of the samples were then stored at room temperature until glomalin analysis, at 4 °C until soil enzyme activities, NH₄⁺-N, and NO₃⁻-N concentrations analysis (within 1 week). During the harvest, 10 maize plants in each plot were randomly collected and oven-dried at 60 °C until a constant weight. After measurement, we multiplied the mean of dried weight of these 10 plants by the number of plants in each plot to get aboveground biomass for every plot.

Glomalin in soil was quantified as glomalin-related soil protein (GRSP). Rosier et al. (2006) found that Bradford method can be useful in determining glomalin content when organic matter concentrations are low. In

the cropland at Agricultural Station of Chinese Academy of Agricultural Sciences where we studied, SOC and nutrients are generally low (Liang et al. 2015). Bradford method was also used to measure glomalin content in many recent N fertilizer application studies (Avio et al. 2013; Zhang et al. 2015). Briefly, air-dry soil samples (<2 mm) were added to 8 ml of 20 mM trisodium citrate dehydrate solution at pH 7.0 in centrifuge tube of 50 ml and then autoclaved at 121 °C for 30 min. After each extraction, the sample was centrifuged at 10,000 g for 5 min and the supernatant containing glomalin was collected and stored at 4 °C to determine easily extractable glomalin-related soil protein (EEG). EEG content in the extract was determined by Bradford assay, using bovine serum albumin as a standard. Results were expressed as mg glomalin g⁻¹ soil. The activities of soil enzymes relating to soil C cycle such as β-glucosidase (BG), β-xylosidase (BXYL), and Cellobiohydrolase (CBH) were detected through Enzyme-labelled meter method described by Ai et al. (2012). Specifically, 1.0 g dry mass of fresh soil was homogenized by using 100 ml sterilized water. The sterilized water, sample suspension, references (10 μM) and substrates (200 μM) were dispensed into the wells of a black 96-well microplate and then the microplates were incubated in the dark at 25 °C for 4 h. Fluorescence was quantified using a microplate fluorometer (Scientific Fluoroskan Ascent FL, Thermo, USA) with 365 nm excitation and 450 nm emission filters. The enzyme activities were expressed in units of nmol h⁻¹ g⁻¹. NH₄⁺-N and NO₃⁻-N concentrations were measured by extracting the soil with 1 M KCl solution (1:5, w:v) for 60 min and then measuring the concentrations using a flow injection autoanalyzer (Seal Analytical GmbH, Noderstedt, Germany). Overall, soil biochemical properties we measured in our study consisted of soil enzyme activities (BG, BXYL and CBH), soil available N contents (soil NH₄⁺-N and NO₃⁻-N concentrations), and EEG, which were expected to be affected by N application.

Measurements of soil respiration and soil environmental factors

RS and RH were measured using a portable soil respiration system (LI-8100, Li-COR Inc., Lincoln, Nebraska, USA) during 2014 and 2015 maize growing seasons. The specific dates of soil respiration measurements and their corresponding crop growth stages are presented in Table S1. The soil respiration chamber was mounted on

a polyvinyl chloride (PVC) collar that was sharpened at the bottom. Each PVC collar (10 cm in height, 20 cm in inner diameter) was inserted into the soil surface at a depth of 8 cm in both comparative experiments of each plot, respectively, to measure RS and RH. Soil respiration was measured between 9:00 and 11:00 am (local time). Since abiotic factors (e.g. soil moisture and temperature) can significantly influence soil respiration (Davidson et al. 1998), we measured soil moisture and temperature to explore whether N application alters soil respiration by changing them. Soil temperature at 10 cm depth was simultaneously determined with soil respiration measurement using thermometer of Li-8100 and soil moisture at 10 cm depth was gravimetrically measured by oven-drying method.

Data and statistical analysis

The autotrophic respiration (RA) was calculated by the difference between total soil respiration (RS) and heterotrophic respiration (RH):

$$RA = RS - RH \quad (1)$$

All maize residues were incorporated into the soil after harvest. For estimating plant C input, it is assumed that the C concentration in maize plant was 0.45 g g⁻¹ (Bolinder et al. 2007). Meanwhile, the shoot-to-root ratio of maize showed no marked change under N application (Bonifas et al. 2005), we assumed that it was 5.6 (Bolinder et al. 2007). Then the plant C input to soil (C_{input}) was calculated as follow:

$$C_{input} = \text{Aboveground biomass} \times (1 + 1/5.6) \times 0.45 \quad (2)$$

The C output (C_{output}) was defined as cumulative CO₂ emission in the form of RH which was calculated using the following relationship (Shao et al. 2014):

$$C_{output} = CO_2 - C = \sum_i^n X_i + X_{i+1} \times K + X_{i+2} \times K + \dots + X_{i+n} \times K \quad (3)$$

In which *i* is the first RH measured at seedling stage, *n* is the last RH measured at maturity stage, *X* represents the average RH (kg C ha⁻¹ day⁻¹) between two consecutive RH measurements, and *K* is the number of days between the two measurements. Since the measurements taken between 9:00 and 11:00 am could represent the daily mean (Xu and Qi 2001), *X* is RH we measured.

The net SOC sequestration in the whole maize growing season was calculated by subtracting the C_{output} from C_{input} :

$$\text{SOC sequestration} = C_{\text{input}} - C_{\text{output}} \quad (4)$$

For each variable measured in the rhizosphere or bulk soil, the data was analyzed by one-way ANOVA using the Least Significant Difference (LSD) test ($P < 0.05$) to compare the treatment means. One-way ANOVA was also used to determine significant differences of SOC sequestration, RS and its components among N treatments. We also used one-way ANOVA to analyze the effects of N addition on annual means of soil temperature, moisture, RS, and its components. Two-way ANOVA was used to determine statistical differences of soil biochemical parameters caused by soil fractions (rhizosphere and bulk soil) and N addition treatments. All above-mentioned statistical analyses were conducted using SAS 9.2 for Windows (SAS Institute, Cary, NC). The RELATE (a non-parametric Mantel-type test) test was used to determine which broad soil characteristics were significantly correlated to RS and its components. We divided soil parameters into three groups (soil fraction, soil parameters, and their interactions) and then analyzed the correlation between broad soil characteristics and RS and its components in each group.

Results

Soil respiration and its components

Both soil moisture and temperature showed strong seasonal variations during 2014 and 2015 maize growing seasons (Fig. 1a and b). N addition did not significantly affect annual means of soil temperature and moisture in both 2014 and 2015 (Table 1). During two consecutive maize growing seasons, RS and soil temperature showed similar seasonal patterns (Fig. 1b and c). RS of N240 was significantly higher than that of N0 only on 10 July during 2014 growing season ($P < 0.05$), however, significant differences of RS between these two treatments were found at all growth stages during 2015 growing season (Fig. 1c). The annual mean of RS during the two consecutive growing seasons increased significantly with the N addition rate (e.g. N240 = N180 >

N120 = N0 in 2014; N240 > N180 = N120 > N0 in 2015, Table 1). The significant differences at $p < 0.05$ level between RA of N0 and N240 were found in both 2014 (15 August and 6 September) and 2015 (3 July, 24 July, 7 September and 2 October) growing seasons (Fig. 2a). N application significantly increased the annual mean of RA in two growing seasons (Table 1). The number of dates that showed significant difference between RH of N0 and N240 increased from 1 (2014 growing season) to 4 (2015 growing season) (Fig. 2b). Furthermore, the differences of the annual mean of RH among N addition levels became significant in 2015 (Table 1). The range of RA/RS was 14%–66% in 2014, and 8%–60% in 2015. In addition, the annual mean of RA/RS under all treatments was 41% and 30% in 2014 and 2015, respectively (Fig. 2c). No significant effect of N application on the annual mean of RA/RS was found in 2014, however, annual means of RA/RS under N240 and N180 were significantly higher than those under N0 and N120 in 2015 growing season (Table 1).

No significant effects of N addition on C_{input} and C_{output} were found in 2014 (Fig. 3a and b). Conversely, N fertilizer application markedly enhanced C_{input} and C_{output} , compared with N0 treatment, in 2015 growing season (Fig. 3d and e). Different responses of SOC sequestration to N application rates were found in 2014 and 2015. N120, N180 and N240 increased SOC sequestration by 50.31%, 47.22% and 67.92% than N0 in 2014 (Fig. 3c). N180 had the highest SOC sequestration among all N addition treatments, and the values of SOC sequestration under the other three treatments were lower than zero in 2015 (Fig. 3f).

Soil biochemical properties

Soil enzyme activities in the bulk soil showed similar trends with the increase of N application rate (BG: N240 > N180 > N120 = N0, BXYL and CBH: N240 = N180 > N120 = N0, Fig. 4a-c). Both BXYL and CBH activities in the rhizosphere under N180 and N240 had highest values, which were significantly higher than those under N0. In contrast, N application did not significantly affect BG activity in the rhizosphere. EEG content in both the rhizosphere and bulk soil had similar patterns with increasing N fertilization (bulk soil: N240 > N180 = N120 = N0, rhizosphere: N240 = N180 > N120 = N0, Fig. 4d). NH_4^+ -N concentration in both the rhizosphere and bulk soil under N180 treatment

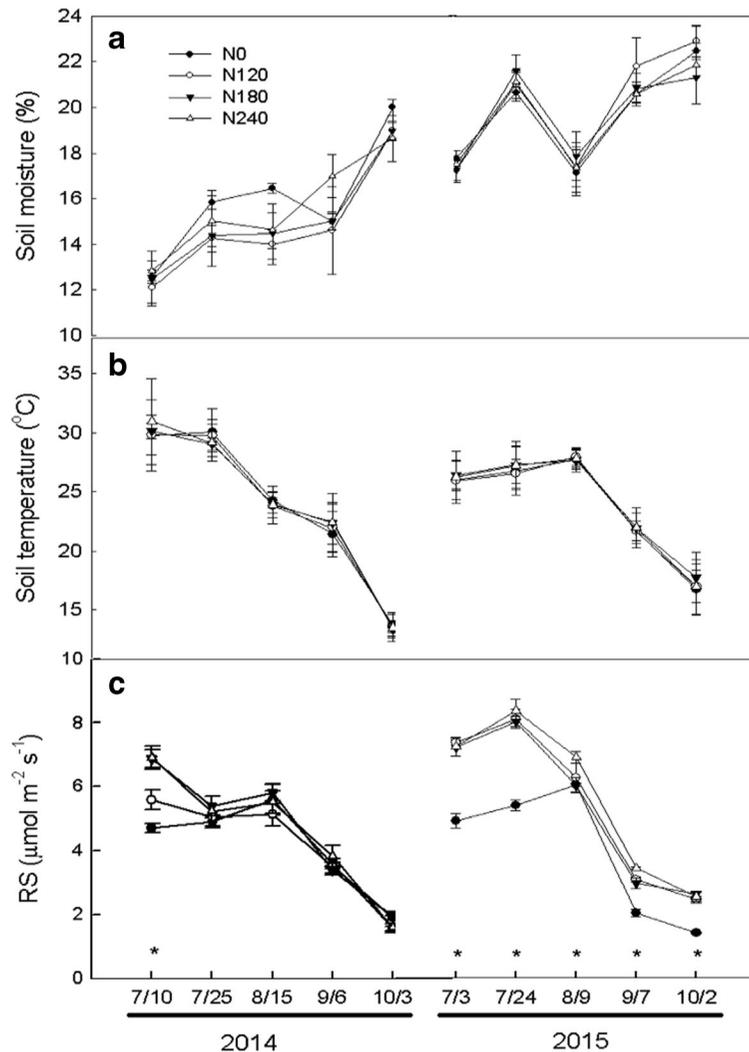


Fig. 1 Seasonal variations of soil moisture (a), temperature (b) and soil total respiration (c, RS) during 2014 and 2015 summer maize growing seasons. Asterisks indicate significant differences

between RS under N0 and N240 at $P < 0.05$. Thin bars are standard deviation. N0: 0 kg N ha⁻¹; N120: 120 kg N ha⁻¹; N180: 180 kg N ha⁻¹; N240: 240 kg N ha⁻¹

showed higher value compared to N0, N120 and N240 treatments. In addition, increasing trend of NO₃⁻-N content in both the rhizosphere and bulk soil with the increasing of N application rate was found (Fig. 4f). In general, all biochemical properties tended to be greater in the rhizosphere than in the bulk soil, except for NO₃⁻-N content, which was lower. Both the rhizosphere and N treatments had strong effects on all biochemical parameters (Table 2). Significant interactive effects of the rhizosphere and N treatments on BG activity, NH₄⁺-N and NO₃⁻-N contents were found, but the interactive effects on EEG content, BXYL and CBH activities were not significant.

Relationship between soil respiration and soil properties in the rhizosphere and bulk soil

For soil fraction, rhizosphere (Rho = 0.694, $P = 0.001$) showed stronger relationship with RS and its components than bulk (Rho = 0.589, $P = 0.003$) (Table 3). For soil parameters, significant correlations between RS and soil enzyme (Rho = 0.605, $P = 0.002$), available N (Rho = 0.665, $P = 0.001$) and EEG (Rho = 0.695, $P = 0.001$) were found, and EEG had the best relationship with RS. For the interactions between soil fraction and parameters, all parameters except EEG in the bulk soil (Rho = 0.163, $P = 0.087$) were significantly related to

Table 1 One-way analysis of variance (ANOVA) on the effects of N application rate on annual means of soil respiration and its components ($\mu\text{mol m}^{-2} \text{s}^{-1}$), RA/RS (%), soil temperature ($^{\circ}\text{C}$)and moisture (%) during 2014 and 2015 summer maize growing season (Mean \pm SD, $n = 3$)

Growing season	Treatment	RS	RA	RH	RA/RS	T	M
2014 summer maize	N0	4.07 \pm 0.15 ^b	1.35 \pm 0.05 ^b	2.71 \pm 0.11 ^a	37.40 \pm 1.10 ^a	23.86 \pm 1.68 ^a	15.96 \pm 0.09 ^a
	N120	4.22 \pm 0.08 ^b	1.53 \pm 0.20 ^b	2.69 \pm 0.22 ^a	38.37 \pm 4.58 ^a	23.78 \pm 1.03 ^a	14.99 \pm 0.34 ^a
	N180	4.67 \pm 0.17 ^a	2.07 \pm 0.32 ^a	2.60 \pm 0.22 ^a	44.14 \pm 7.14 ^a	23.79 \pm 0.87 ^a	15.05 \pm 0.27 ^a
	N240	4.61 \pm 0.20 ^a	2.08 \pm 0.27 ^a	2.52 \pm 0.12 ^a	45.97 \pm 4.41 ^a	23.98 \pm 2.01 ^a	15.62 \pm 0.63 ^a
2015 summer maize	N0	3.75 \pm 0.08 ^c	1.03 \pm 0.04 ^c	2.72 \pm 0.07 ^c	28.36 \pm 0.48 ^b	24.23 \pm 1.80 ^a	19.73 \pm 0.14 ^a
	N120	5.46 \pm 0.16 ^b	1.49 \pm 0.02 ^b	3.97 \pm 0.14 ^{ab}	30.99 \pm 0.69 ^b	24.26 \pm 1.01 ^a	20.07 \pm 0.30 ^a
	N180	5.38 \pm 0.11 ^b	1.52 \pm 0.03 ^b	3.85 \pm 0.08 ^b	31.89 \pm 0.57 ^a	24.70 \pm 1.58 ^a	19.78 \pm 0.11 ^a
	N240	5.70 \pm 0.08 ^a	1.61 \pm 0.02 ^a	4.10 \pm 0.10 ^a	31.57 \pm 0.82 ^a	24.51 \pm 1.98 ^a	19.69 \pm 0.23 ^a

RS: annual mean of soil total respiration; RA: annual mean of soil autotrophic respiration; RH: annual mean of soil heterotrophic respiration; RA/RS: the ratio of RA to RS; T: annual mean of soil temperature at 10 cm; M: annual mean of soil moisture at 10 cm. N0: 0 kg N ha⁻¹; N120: 120 kg N ha⁻¹; N180: 180 kg N ha⁻¹; N240: 240 kg N ha⁻¹. Significant differences among treatments are indicated by different letters (one-way ANOVA with LSD test, $\alpha = 0.05$)

RS. Both EEG and available N in the rhizosphere had stronger correlations with RS than those in the bulk soil, however, soil enzymes in the bulk soil ($Rho = 0.643$, $P = 0.001$) explained the change in RS better than that in the rhizosphere ($Rho = 0.324$, $P = 0.02$).

Discussion

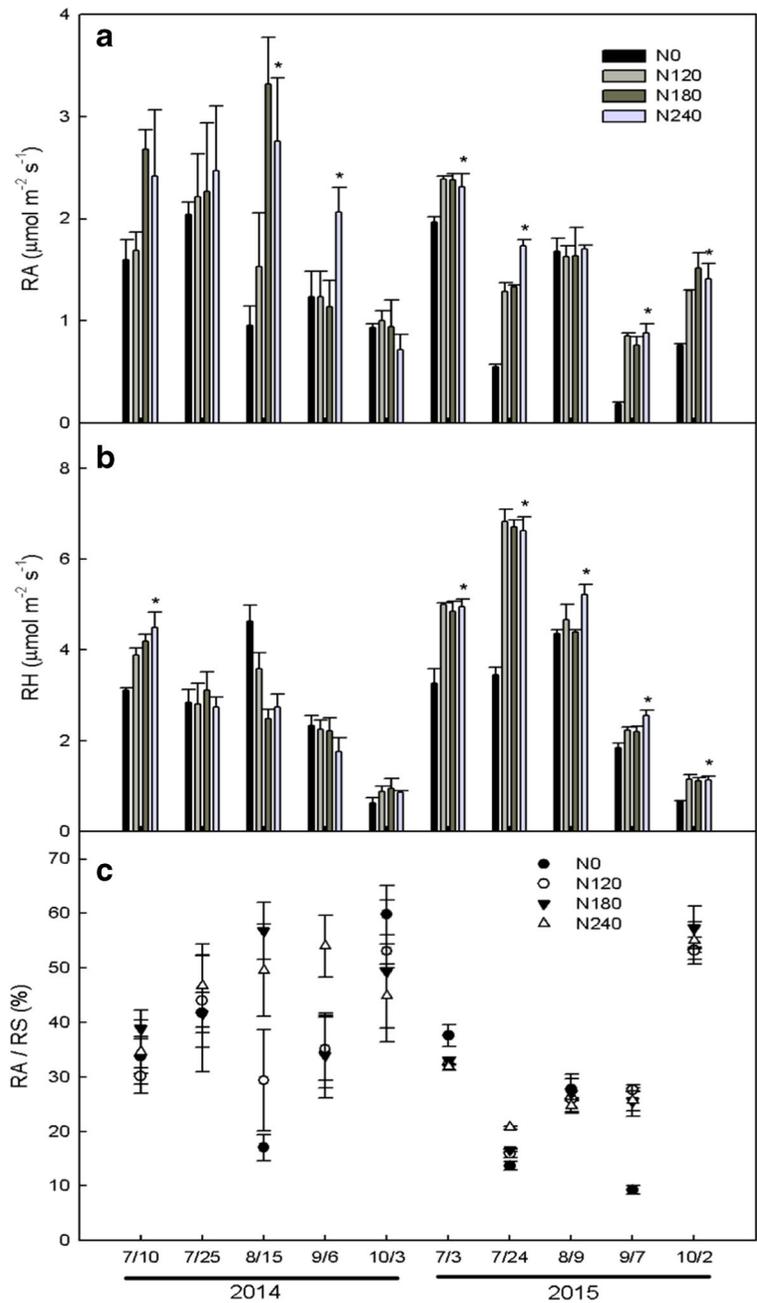
The responses of soil respiration and its components to N addition and microclimate

A meta-analysis study found that N addition significantly increased both RS and RA but had null effect on RH in croplands (Zhou et al. 2014). Our study showed the same result in 2014 (the fifth year of N application), however, the positive impact of N application on RH became significant in 2015 (the sixth year of N application) (Table 1). The reasons might be twofold: (1) RH had less N sensitivity than RA, which was also found in a previous study (Chen et al. 2017b): on one hand, soil microorganism can be strongly activated if soil nutrition content exceeds the threshold; on the other hand, it was a slow process for N addition to significantly improve soil nutrients (e.g. SOC and available N content) which are the main sources for soil microbial growth (Cai et al. 2018). For example, N addition markedly increased RH only after we applied N fertilizer at seedling stage in 2014, no significant effects of N addition on RH were found at other stages (Fig. 2b). However, after 6-years N

addition, the values of soil nutrients (e.g. SOC and available N content) exceeded the threshold levels that strongly stimulated soil microbial activity, and thus resulted in the significant effects of N addition on RH at all stages in 2015. (2) Soil water limitation: annual mean of soil moisture in 2014 (about 15%) was significantly lower than that in 2015 (about 20%) (Fig. 1a). Soil moisture was the dominant factor that influenced RH in 2014, therefore, the low soil moisture weakened the effect of N addition on RH. However, this restriction from soil moisture on the effect of N on RH weakened when the annual mean of soil moisture was high in 2015. Overall, our study suggested the different responses of RA and RH to N addition might result from different N sensitivities between RA and RH and/or water limitation.

Besides N addition, environmental factors such as soil temperature and moisture showed different effects on RS in a wide range of ecosystems (Gong et al. 2014; Sun et al. 2014; Liang et al. 2018). These inconsistent results are likely caused by the various responses of RA and RH. Chen et al. (2017b), for example, reported that RA was more sensitive to soil temperature than RH, but RH was more closely related to soil moisture than RA in croplands. Therefore, assessing the responses of both RA and RH to environmental factors and N addition is crucial to improve our mechanistic understanding and the accuracy of model prediction. The decreased RA/RS in 2015 compared to 2014 was caused by increased RH but decreased RA in 2015 (Fig. 2). A previous study

Fig. 2 Seasonal variations of RA (a), RH (b) and RA/RS (c) during 2014 and 2015 summer maize growing seasons. Asterisks indicate significant differences between RS under N0 and N240 at $P < 0.05$. Thin bars are standard deviation. RA: soil autotrophic; RH: soil heterotrophic respiration; RS: soil total respiration; RA/RS: the ratio of RA to RS. N0: 0 kg N ha⁻¹; N120: 120 kg N ha⁻¹; N180: 180 kg N ha⁻¹; N240: 240 kg N ha⁻¹



showed that root biomass increased with the increase of soil moisture but started to decrease after soil moisture was higher than 90% soil field capacity (Zhang et al. 2017). In our study, on one hand, higher annual mean of soil moisture in 2015 (about 20%) (Table 1) might decrease RA by inhibiting root growth (Fig. 2a) because of decreased root requirement for absorbing water. On the other hand, this high annual mean of soil moisture in

2015 which was appropriate for microbial growth, together with improved nutrient conditions, dramatically enhanced RH by stimulating soil microbial activity (Fig. 2b). Overall, the opposite responses of root growth (negative) and microbial activity (positive) to the increase of soil moisture led to decreased RA but increased RH in 2015, respectively, and then caused the interannual change of RA/RS.

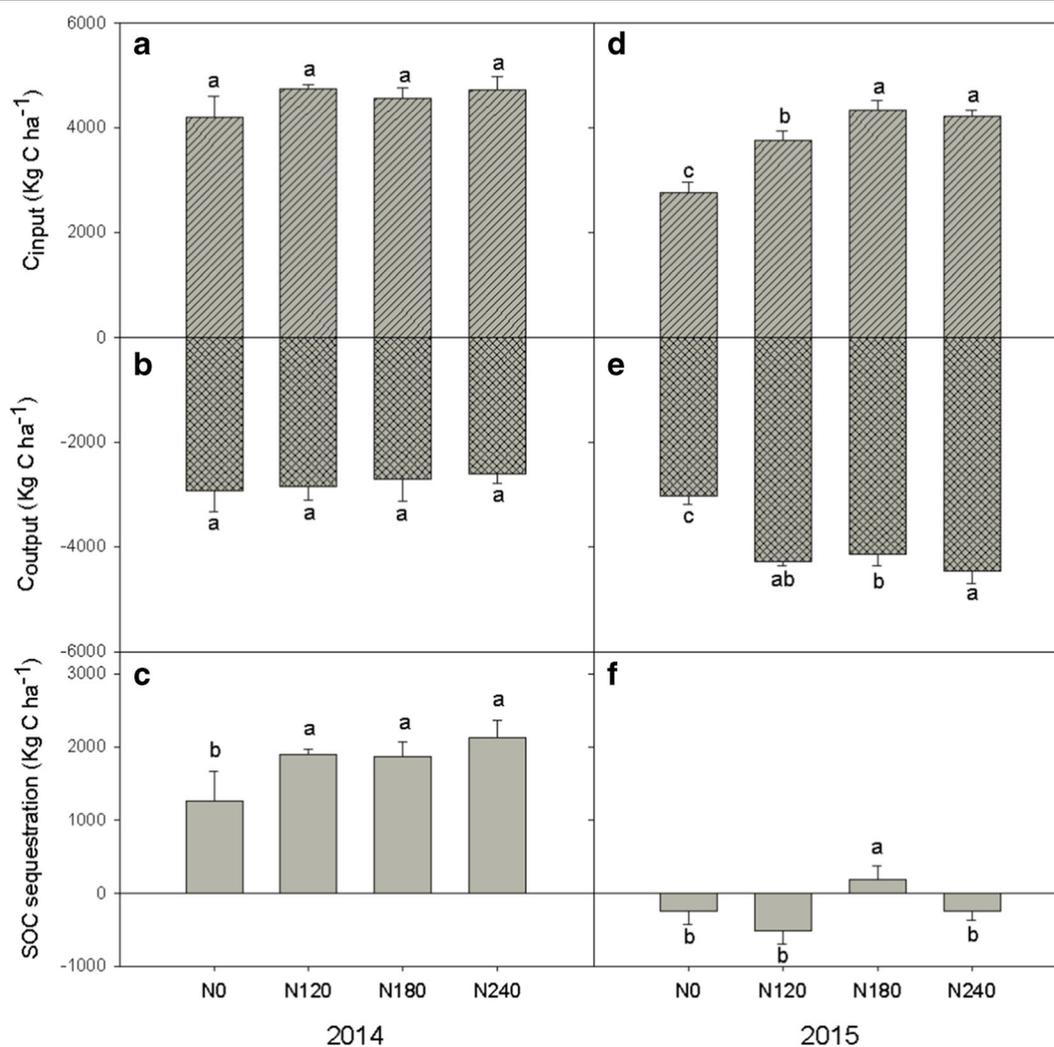


Fig. 3 C_{input} (a and d), C_{output} (b and e) and SOC sequestration (c and f) under different N fertilization treatments in 2014 and 2015 summer maize growing seasons. Vertical bars are the standard deviations of the means ($n=3$). Significant differences between

treatments at $P < 0.05$ are indicated by different letters. N0: 0 kg N ha⁻¹; N120: 120 kg N ha⁻¹; N180: 180 kg N ha⁻¹; N240: 240 kg N ha⁻¹

The soils under all N treatments were net C sinks in 2014 but became sources except for N180 treatment in 2015 (Fig. 3c and f). The negative effect of no N addition on plant growth (C_{input}) did not appear, and RH (C_{output}) under all treatments were low because of low soil moisture in 2014 (Fig. 3a and b), therefore, SOC sequestration values under all treatments were positive. However, N application levels showed different impacts on plant growth, and high soil moisture made a dramatic increase in RH in 2015, thus, SOC sequestration values under all treatments except N180 became lower than zero. The different effects of N120 (negative), N180 (positive) and

N240 (negative) on SOC sequestration in 2015 were resulted from their different respective effects on C_{input} and C_{output} . Specifically, N180 had highest C_{input} but lowest C_{output} among these three treatments. However, N120 had lower C_{input} than N180, but the same C_{output} with N180. In addition, N240 had the same C_{input} with N180, but C_{output} under N240 was significantly higher than that under N180. According to our 2-year experiment, N180 (180 kg N ha⁻¹) was recommended for local farming practice because it increased SOC sequestration by having the same C_{input} but lower C_{output} than N240 (240 kg N ha⁻¹).

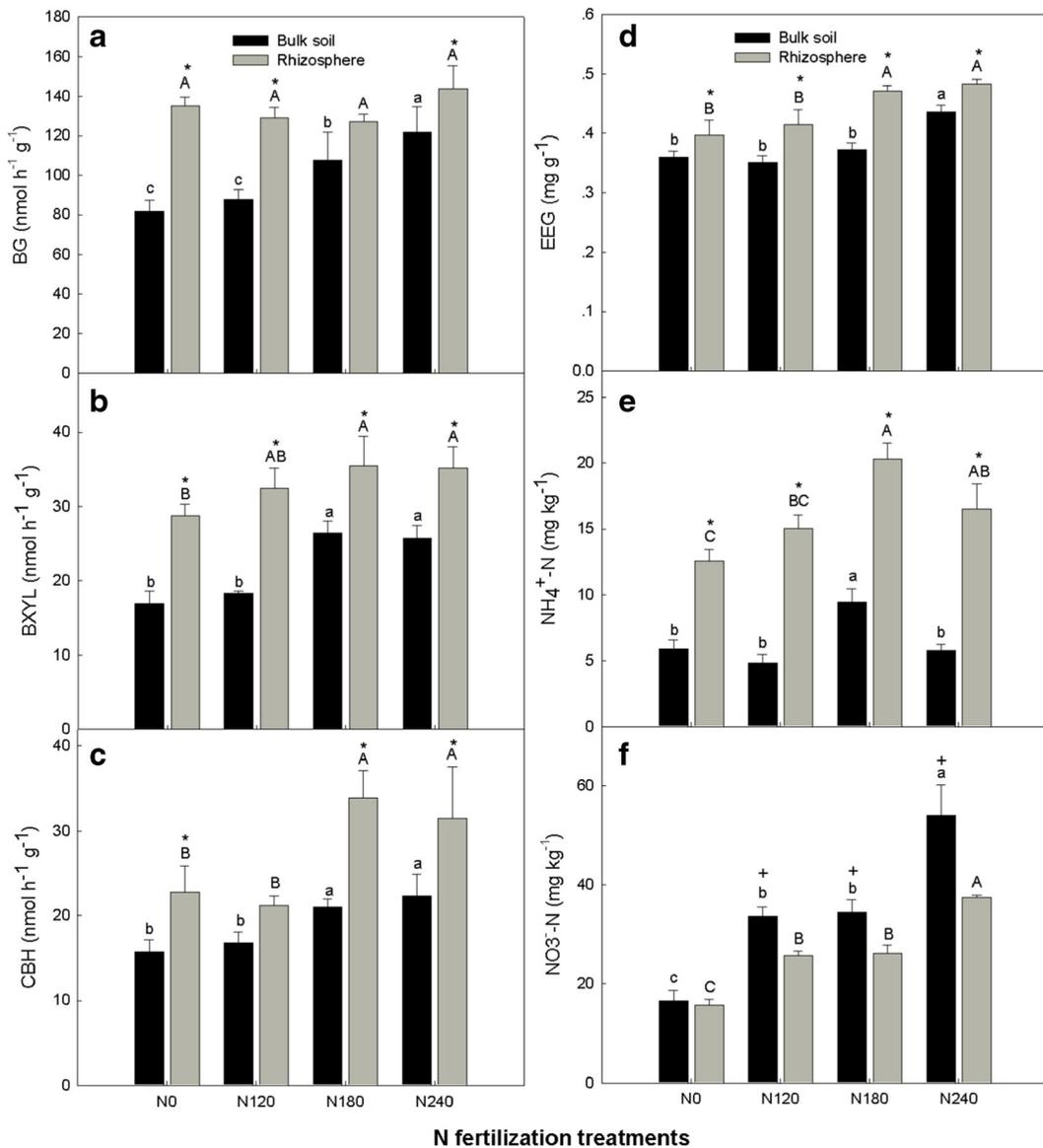


Fig. 4 Soil biochemical properties (BG: a; BXYL: b; CBH: c; EEG: d; NH₄⁺-N: e; NO₃⁻-N: f) in both the rhizosphere and bulk soil under different N fertilization treatments at seedling stage of 2014 summer maize growing season. Vertical bars represent the standard deviations (n = 3) and different letters indicate significant differences among N treatments in the rhizosphere or bulk soil at P < 0.05. Asterisks indicate soil properties in the rhizosphere soil are significantly higher than those in the bulk soil at P < 0.05. Pluses

indicate soil properties in the bulk soil are significantly higher than those in the rhizosphere soil at P < 0.05. β -glucosidase activity (BG), β -xylosidase activity (BXYL), Cellobiohydrolase activity (CBH), Easily extractable glomalin-related soil protein (EEG), Ammonium N content (NH₄⁺-N), Nitrate N content (NO₃⁻-N). N0: 0 kg N ha⁻¹; N120: 120 kg N ha⁻¹; N180: 180 kg N ha⁻¹; N240: 240 kg N ha⁻¹

The effects of N fertilizer on soil biochemical parameters in the rhizosphere and bulk soil

All soil enzyme activities in both the rhizosphere and bulk soil increased with the increase of N application rate except BG activity in the rhizosphere (Fig. 4a-c). A

previous study also found that soil enzyme activities in the rhizosphere and bulk soil were changed by N addition, but not in the same way (Ai et al. 2012). This might be because the amounts of soil microbes in bulk soil (one source of soil enzymes) were enough to produce BG, there was no need for plant root (another source of

Table 2 Two-way ANOVA analysis of soil biochemical properties in 2 soil fractions (the rhizosphere and bulk soil) and 4 N fertilizer treatments each with 3 replicates ($n = 24$)

	Soil fractions (Bulk or rhizosphere)		N treatments		Soil fractions \times N treatments	
	F	P	F	P	F	P
BG	59.35	<0.0001	6.85	<0.01	3.26	<0.05
BXYL	93.13	<0.0001	11.89	<0.001	1.04	0.402
CBH	32.4	<0.0001	10.05	<0.001	1.47	0.261
EEG	61.77	<0.0001	23.53	<0.0001	2.98	0.063
NH ₄ ⁺ -N	336.4	<0.0001	22.74	<0.0001	3.55	<0.05
NO ₃ ⁻ -N	39.59	<0.0001	82.13	<0.0001	5.84	<0.01

The data in bold indicates that soil biochemical properties are not affected by soil fractions, fertilizer treatments or their interaction ($P < 0.05$). BG: β -glucosidase activity; BXYL: β -xylosidase activity; CBH: Cellobiohydrolase activity; EEG: Easily extractable glomalin-related soil protein; NH₄⁺-N: Ammonium N content; NO₃⁻-N: Nitrate N content

soil enzymes) to secrete BG. Furthermore, our result showed that the magnitude of increased BXYL in the bulk soil was higher than that in the rhizosphere, however, contrary result was found in CBH (Fig. 4b and c). Both the amount of soil microbes in the bulk soil which produced specific enzymes (mentioned above) and the types of soil enzymes might lead to this inconsistent result. Our findings indicated that soil enzymes in the rhizosphere and bulk soil had different sensitivities to N addition, which is needed to be taken into consideration in future experimental, meta-analysis and model studies.

As one of the most important nutrients that affect plant and microbial growth, it is not surprising that available N (NH₄⁺-N and NO₃⁻-N) contents in both the rhizosphere and bulk soil were significantly increased by N application (Fig. 4e and f). However, NH₄⁺-N contents in both the rhizosphere and bulk soil under N240 were lower than those under N180 treatment (Fig. 4e). One study in cropland found that ammonia volatilization under 225 kg N ha⁻¹ application rate only increased 2.45 kg N ha⁻¹ than that under 150 kg N ha⁻¹ application rate, however, ammonia volatilization under 300 kg N ha⁻¹ application rate increased 8.65 kg N ha⁻¹ than that under 225 kg N ha⁻¹ application rate (Li et al. 2017). This result indicated that the same amount of increase of N application rate in high application range resulted in higher ammonia volatilization than in low application range. Therefore, as the source of ammonia volatilization, much more NH₄⁺-

Table 3 Correlations of broad characteristics to soil respiration and its components by RELATE

Characteristic ^a	Rho	P
Soil fraction		
Bulk	0.589	0.003
Rhizosphere	0.694	0.001
Soil parameters		
Soil enzyme	0.605	0.002
Available N	0.665	0.001
EEG	0.695	0.001
Soil fraction \times parameters		
Soil enzyme in the bulk soil	0.643	0.001
Soil enzyme in the rhizosphere soil	0.324	0.02
Available N in the bulk soil	0.511	0.002
Available N in the rhizosphere soil	0.708	0.001
EEG in the bulk soil	0.163	0.087
EEG in the rhizosphere soil	0.695	0.001

^a Individual factors that are grouped into each characteristic:

Bulk: all soil biochemical properties in the bulk soil; Rhizosphere: all soil biochemical properties in the rhizosphere soil

Soil enzyme: BG, BXYL and CBH activities in both the bulk and rhizosphere soil; Available N: NH₄⁺-N and NO₃⁻-N contents in both the bulk and rhizosphere soil; EEG: EEG content in both the bulk and rhizosphere soil

Soil enzyme in the bulk soil: BG, BXYL and CBH activities in the bulk soil; Soil enzyme in the rhizosphere soil: BG, BXYL and CBH activities in the rhizosphere soil; Available N in the bulk soil: NH₄⁺-N and NO₃⁻-N contents in the bulk soil; Available N in the rhizosphere soil: NH₄⁺-N and NO₃⁻-N contents in the rhizosphere soil; EEG in the bulk soil: EEG content in the bulk soil; EEG in the rhizosphere soil: EEG content in the rhizosphere soil

N was released as ammonia volatilization under N240 than N180 treatment, and then lower NH₄⁺-N content under N240 treatment was found in our study. Inselsbacher et al. (2013) found that plant root preferentially absorbed NO₃⁻-N rather than NH₄⁺-N for growth. This mechanism led to higher NH₄⁺-N but lower NO₃⁻-N content in the rhizosphere than in the bulk soil in our study (Fig. 4e and f).

Different contributions of soil parameters in the rhizosphere and bulk soil to soil respiration

Rhizosphere has a vital role in plant growth and soil microbial activity, and the different responses of soil enzyme activities in the rhizosphere and bulk soil to N addition were found in a previous study (Ai et al. 2012).

Our result indicated soil biochemical properties in the rhizosphere had a stronger correlation with RS and its component than those in the bulk soil (Table 3), which meant that soil biochemical properties in the rhizosphere was a better indicator for RS and its components.

Soil enzymes, which is an important indicator of microbial activity, can significantly affect RH. In addition, as the main sources of N, available N significantly influences both plant growth and microbial activity which contributes to RA and RH, respectively. Glomalin, which is produced by arbuscular mycorrhizal fungi (AMF), contributes to the preservation of organic carbon in the soil and indirectly influences RH (Rillig et al. 2001). Therefore, all three parameters (soil enzyme activities, available N, and glomalin contents) showed significant correlations with RS and its components in our study (Table 3).

Both EEG and available N in the rhizosphere showed stronger correlations with RS than in the bulk soil, however, contrary result was found in soil enzyme activity (Table 3). This might be explained as follows: (1) N application might preferentially stimulate the large amount of dormant soil microbes in the bulk soil to secrete soil enzymes to participate in N fertilizer decomposition; (2) soil microbes in the rhizosphere did not need to produce redundant soil enzymes because the amount of enzymes in the bulk soil was nearly sufficient for nutrient cycling which was stimulated by N addition. Generally speaking, more soil enzyme sensitivity to N addition and partition in nutrients cycling in the bulk soil led to lower correlation between RS and soil enzyme activity in the rhizosphere than in the bulk soil. The different effects of soil fraction (bulk and rhizosphere) on the relationship between soil biochemical properties and RS should be considered in the microbial model to make it more accurate.

Conclusions

Nitrogen (N) fertilizer has been heavily applied in agroecosystems at the global scale for high crop yields. The responses of soil biochemical properties and soil respiration (RS) to N addition have been widely studied, respectively, however, the contributions of soil biochemical parameters in both the rhizosphere and bulk soil to changes in RS and its components (soil heterotrophic (RH) and autotrophic (RA) respiration) under N application remain poorly understood. Our study found

that N addition only significantly enhanced RA in 2014 but increased both RA and RH in 2015 because RH had lower N sensitivity than RA, or lower soil moisture in 2014 weakened the effect of N on RH. Soil biochemical properties (e.g. soil enzyme activities, glomalin and available N contents) in both the rhizosphere and bulk soil were significantly improved by N application. Both glomalin and available N contents in the rhizosphere were more related to RS and its components than those in the bulk soil, but soil enzyme activities showed opposite result. Our study suggested that soil biochemical parameters in the rhizosphere and bulk soil show different contributions to RS and its components under N addition, respectively, which varies with the types of soil biochemical property.

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References

- Ai C, Liang G, Sun J, Wang X, Zhou W (2012) Responses of extracellular enzyme activities and microbial community in both the rhizosphere and bulk soil to long-term fertilization practices in a fluvo-aquic soil. *Geoderma* 173–174:330–338. <https://doi.org/10.1016/j.geoderma.2011.07.020>
- Ai C, Liang G, Sun J, Wang X, He P, Zhou W (2013) Different roles of rhizosphere effect and long-term fertilization in the activity and community structure of ammonia oxidizers in a calcareous fluvo-aquic soil. *Soil Biol Biochem* 57:30–42. <https://doi.org/10.1016/j.soilbio.2012.08.003>
- Alexandratos N, Bruinsma J (2012) World agriculture towards 2030/2050. *Land Use Policy* 20:375. [https://doi.org/10.1016/S0264-8377\(03\)00047-4](https://doi.org/10.1016/S0264-8377(03)00047-4)
- Avio L, Castaldini M, Fabiani A, Bedini S, Sbrana C, Turrini A, Giovannetti M (2013) Impact of nitrogen fertilization and soil tillage on arbuscular mycorrhizal fungal communities in a Mediterranean agroecosystem. *Soil Biol Biochem* 67:285–294. <https://doi.org/10.1016/j.soilbio.2013.09.005>
- Badaluco L, Kuikman PJ (2001) Mineralization and immobilization in the rhizosphere. *The Rhizosphere. Biochemistry and Organic Substances at the Soil-Plant Interface*. Marcel Dekker, New York, pp 141–196
- Bolinder MA, Janzen HH, Gregorich EG, Angers DA, VandenBygaart AJ (2007) An approach for estimating net primary productivity and annual carbon inputs to soil for common agricultural crops in Canada. *Agric Ecosyst*

- Environ 118:29–42. <https://doi.org/10.1016/j.agee.2006.05.013>
- Bond-lamberty B, Thomson A (2010) Temperature-associated increases in the global soil respiration record. *Nature* 464: 579–582. <https://doi.org/10.1038/nature08930>
- Bond-Lamberty B, Wang C, Gower ST (2004) A global relationship between the heterotrophic and autotrophic components of soil respiration? *Glob Chang Biol* 10:1756–1766. <https://doi.org/10.1111/j.1365-2486.2004.00816.x>
- Bonifas KD, Walters DT, Cassman KG, Lindquist JL (2005) Nitrogen supply affects root:shoot ratio in corn and velvetleaf (*Abutilon theophrasti*). *Weed Sci* 53:670–675. <https://doi.org/10.1614/WS-05-002R.1>
- Cai A, Zhang W, Xu M, Wang B, Wen S, Shah SAA (2018) Soil fertility and crop yield after manure addition to acidic soils in South China. *Nutr Cycl Agroecosystems* 111:61–72. <https://doi.org/10.1007/s10705-018-9918-6>
- Chen H, Li D, Gurmessa GA, Yu G, Li L, Zhang W, Fang H, Mo J (2015) Effects of nitrogen deposition on carbon cycle in terrestrial ecosystems of China: a meta-analysis. *Environ Pollut* 206:352–360. <https://doi.org/10.1016/j.envpol.2015.07.033>
- Chen J, Luo Y, Li J, Zhou X, Cao J, Wang RW, Wang Y, Shelton S, Jin Z, Walker LM, Feng Z, Niu S, Feng W, Jian S, Zhou L (2017a) Costimulation of soil glycosidase activity and soil respiration by nitrogen addition. *Glob Chang Biol* 23:1328–1337. <https://doi.org/10.1111/gcb.13402>
- Chen Z, Xu Y, Fan J, Yu H, Ding W (2017b) Soil autotrophic and heterotrophic respiration in response to different N fertilization and environmental conditions from a cropland in Northeast China. *Soil Biol Biochem* 110:103–115. <https://doi.org/10.1016/j.soilbio.2017.03.011>
- Chen Z, Xu Y, He Y, Zhou X, Fan J, Yu H, Ding W (2018) Nitrogen fertilization stimulated soil heterotrophic but not autotrophic respiration in cropland soils: a greater role of organic over inorganic fertilizer. *Soil Biol Biochem* 116: 253–264. <https://doi.org/10.1016/j.soilbio.2017.10.029>
- Cui Z, Chen X, Zhang F (2010) Current nitrogen management status and measures to improve the intensive wheat-maize system in China. *Ambio* 39:376–384. <https://doi.org/10.1007/s13280-010-0076-6>
- Davidson EA, Belk E, Boone RD (1998) Soil water content and temperature as independent or confounded factors controlling soil respiration in a temperate mixed hardwood forest. *Glob Chang Biol* 4:217–227. <https://doi.org/10.1046/j.1365-2486.1998.00128.x>
- Gong J-R, Wang Y, Liu M, Huang Y, Yan X, Zhang Z, Zhang W (2014) Effects of land use on soil respiration in the temperate steppe of Inner Mongolia, China. *Soil Tillage Res* 144:20–31. <https://doi.org/10.1016/j.still.2014.06.002>
- Guo JH, Liu XJ, Zhang Y, Shen JL, Han WX, Zhang WF, Christie P, Goulding KWT, Vitousek PM, Zhang FS (2010) Significant acidification in major Chinese croplands. *Science* 327:1008–1010. <https://doi.org/10.1126/science.1182570>
- Hinko-Najera N, Fest B, Livesley SJ, Amdt SK (2015) Reduced throughfall decreases autotrophic respiration, but not heterotrophic respiration in a dry temperate broadleaved evergreen forest. *Agric For Meteorol* 200:66–77. <https://doi.org/10.1016/j.agrformet.2014.09.013>
- Hu W, Jiao Z, Wu F, Liu Y, Dong M, Ma X, Fan T, An L, Feng H (2014) Long-term effects of fertilizer on soil enzymatic activity of wheat field soil in loess plateau, China. *Ecotoxicology* 23:2069–2080. <https://doi.org/10.1007/s10646-014-1329-0>
- Inselsbacher E, Wanek W, Strauss J, Zechmeister-Boltenstern S, Müller C (2013) A novel ¹⁵N tracer model reveals: plant nitrate uptake governs nitrogen transformation rates in agricultural soils. *Soil Biol Biochem* 57:301–310. <https://doi.org/10.1016/j.soilbio.2012.10.010>
- IPCC (2013) *Climate change 2013: the physical science basis*. Cambridge University Press, Cambridge
- Janssens I a, Luyssaert S (2009) Carbon cycle: Nitrogen's carbon bonus. *Nat Geosci* 2:318–319. <https://doi.org/10.1038/ngeo505>
- Jeong J, Bolan N, Kim C (2016) Heterotrophic soil respiration affected by compound fertilizer types in red pine (*Pinus densiflora* S. et Z.) stands of Korea. *Forests* 7:309. <https://doi.org/10.3390/f7120309>
- Lal R (2004) Soil carbon sequestration impacts on global climate change and food security. *Science* 304(80):1623–1627. <https://doi.org/10.1126/science.1097396>
- Li Y, Huang L, Zhang H, Wang M, Liang Z (2017) Assessment of ammonia volatilization losses and nitrogen utilization during the rice growing season in alkaline salt-affected soils. *Sustain* 9. <https://doi.org/10.3390/su9010132>
- Liang G, Houssou AA, Wu H, Cai D, Wu X, Gao L, Li J, Wang B, Li S (2015) Seasonal Patterns of Soil Respiration and Related Soil Biochemical Properties under Nitrogen Addition in Winter Wheat Field. *PLoS One*:1–15. <https://doi.org/10.1371/journal.pone.0144115>
- Liang G, Wu H, Houssou AA, Cai D, Wu X, Gao L, Wang B, Li S (2018) Soil respiration, glomalin content, and enzymatic activity response to straw application in a wheat-maize rotation system. *J Soils Sediments* 18:697–707. <https://doi.org/10.1007/s11368-017-1817-y>
- Liu L, Hu C, Yang P, Ju Z, Olesen JE, Tang J (2015) Effects of experimental warming and nitrogen addition on soil respiration and CH₄ fluxes from crop rotations of winter wheat-soybean/fallow. *Agric For Meteorol* 207:38–47. <https://doi.org/10.1016/j.agrformet.2015.03.013>
- Luo Y, Zhou X (2006). *Soil respiration and the environment*. Academic Press/Elsevier, San Diego, CA
- Moyano FE, Kutsch WL, Schulze ED (2007) Response of mycorrhizal, rhizosphere and soil basal respiration to temperature and photosynthesis in a barley field. *Soil Biol Biochem* 39: 843–853. <https://doi.org/10.1016/j.soilbio.2006.10.001>
- Pei H, Scanlon BR, Shen Y, Reedy RC, Long D, Liu C (2015) Impacts of varying agricultural intensification on crop yield and groundwater resources: comparison of the North China plain and US High Plains. *Environ Res Lett* 10. <https://doi.org/10.1088/1748-9326/10/4/044013>
- Reay DS, Dentener FJ, Smith P, Grace J, Feely RA (2008) Global nitrogen deposition and carbon sinks. *Nat Geosci* 1:430–437. <https://doi.org/10.1038/ngeo230>
- Rillig MC, Wright SF, Nichols KA, Schmidt WF, Tom MS (2001) Large contribution of arbuscular mycorrhizal fungi to soil carbon pools in tropical forest soils. *Plant Soil* 233:167–177. <https://doi.org/10.1023/A:1010364221169>
- Rosier CL, Hoyer AT, Rillig MC (2006) Glomalin-related soil protein: assessment of current detection and quantification

- tools. *Soil Biol Biochem* 38:2205–2211. <https://doi.org/10.1016/j.soilbio.2006.01.021>
- Shao R, Deng L, Yang Q, Shangguan Z (2014) Nitrogen fertilization increase soil carbon dioxide efflux of winter wheat field: a case study in Northwest China. *Soil Tillage Res* 143:164–171. <https://doi.org/10.1016/j.still.2014.07.003>
- Sun Z, Liu L, Ma Y, Yin G, Zhao C, Zhang Y, Piao S (2014) The effect of nitrogen addition on soil respiration from a nitrogen-limited forest soil. *Agric For Meteorol* 197:103–110. <https://doi.org/10.1016/j.agrformet.2014.06.010>
- Xu M, Qi Y (2001) Soil-surface CO₂ efflux and its spatial and temporal variations in a young ponderosa pine plantation in northern California. *Glob Chang Biol* 7:667–677. <https://doi.org/10.1046/j.1354-1013.2001.00435.x>
- Zang H, Wang J, Kuzyakov Y (2016) N fertilization decreases soil organic matter decomposition in the rhizosphere. *Appl Soil Ecol* 108:47–53. <https://doi.org/10.1016/j.apsoil.2016.07.021>
- Zhang F, Wang X, Ju X, Ma W, Wang Q, Kou C (2002) Spatial and temporal variation of nitrogen, phosphorus and potassium, and fertilization recommendation. In: Zhang F, Jiang R (eds) *Integrated nutrient management*. China Agricultural University Press, Beijing, pp 79–87 (in Chinese)
- Zhang F, Shen J, Zhang J, Zuo Y, Li L, Chen X (2010). *Nutrient use Efficiency and Crop Productivity. Implications for China*, 1st ed, advances in agronomy. Elsevier Inc. doi: [https://doi.org/10.1016/S0065-2113\(10\)07001-X](https://doi.org/10.1016/S0065-2113(10)07001-X)
- Zhang W, Dou Z, He P, Ju X-T, Powlson D, Chadwick D, Norse D, Lu Y-L, Zhang Y, Wu L, Chen X-P, Cassman KG, Zhang F-S (2013) New technologies reduce greenhouse gas emissions from nitrogenous fertilizer in China. *Proc Natl Acad Sci* 110: 8375–8380. <https://doi.org/10.1073/pnas.1210447110>
- Zhang J, Tang X, He X, Liu J (2015) Glomalin-related soil protein responses to elevated CO₂ and nitrogen addition in a subtropical forest: potential consequences for soil carbon accumulation. *Soil Biol Biochem* 83:142–149. <https://doi.org/10.1016/j.soilbio.2015.01.023>
- Zhang W, Cao Z, Xie Z, Lang D, Zhou L, Chu Y, Zhao Q, Zhang X, Zhao Y (2017) Effect of water stress on roots biomass and secondary metabolites in the medicinal plant *Stellaria dichotoma* L. var. *lanceolata* Bge. *Sci Hortic (Amsterdam)* 224:280–285. <https://doi.org/10.1016/j.scienta.2017.06.030>
- Zhou L, Zhou X, Zhang B, Lu M, Luo Y, Liu L, Li B (2014) Different responses of soil respiration and its components to nitrogen addition among biomes: a meta-analysis. *Glob Chang Biol* 20:2332–2343. <https://doi.org/10.1111/gcb.12490>