Response of soil respiration to nitrogen fertilization: Evidence from a 6-year field study of croplands

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ABSTRACT

Agroecosystems receive large amounts of nitrogen (N), which can have a profound impact on soil respiration (Rs). Although Rs is the largest carbon dioxide (CO_2) flux from terrestrial ecosystems to the atmosphere, the potential effects of N fertilization on Rs and its components (heterotrophic respiration (Rh) and autotrophic respiration (Ra)) remain controversial, and information on the impacts of N fertilization on C emission from long-term experiments is lacking. In this study, we conducted a field experiment during 6 growing seasons (2012–2018) in which we applied N fertilizer at three rates (0, 180 and 360 kg N ha^{-1}, referred to as N0, N180 and N360, respectively) to two wheat varieties (Changhan No. 58 (CH) and Zhengmai No. 9023 (ZM)) and investigated the effects of N fertilization on Rs and its components by continuous half-hourly observations. We found that over the 6 growing seasons, N180 and N360 increased the mean growing season soil C emission by 29.7% and 11.6%, respectively, due to the increased C emission through Ra under fertilization, which was related to an increased leaf area index (LAI) and canopy photosynthesis (Pn). Significant seasonal variations in the Rs rate were observed; both Rh and Ra contributed to these variations, and the variations were significantly correlated with both soil and air temperature and plant-related traits (LAI and canopy Pn). In addition, the C emission through Rs during the growing season increased throughout the experiment, mainly because of an increase in C emissions through Rh that was caused by increasing temperature during the observation period due to climate change at the experimental site. However, C emission through Rs increased relatively rapidly under the N180 treatment due to the increased Rh throughout the experiment. Furthermore, interaction effects of soil moisture and temperature on the Rs rate and its components were also observed. Overall, our findings highlight the significance of N fertilization in term of soil C emission and its feedback under global warming in agroecosystems.

1. Introduction

As a dominant component of carbon (C) emissions, soil respiration (Rs) is the largest source of C flux from terrestrial ecosystems to the atmosphere, releasing 98 ± 12 Pg C year^{-1} to the atmosphere (Bond-Lamberty and Thomson, 2010) and surpassing anthropogenic carbon dioxide (CO_2) emissions by an order of magnitude (Boden et al., 2009). Croplands, which can fix as much as ~8 Pg C (eq) year^{-1}, contain approximately 10% of the global soil organic carbon (SOC) (Lal, 2004) and play a key role in tackling climate change (Paustian et al., 2016). To improve soil nutrient conditions and promote crop yields, nitrogen (N)

fertilizer is applied to croplands, which also has a profound effect on the C cycle (Gruber and Galloway, 2008). However, Rs and its components (heterotrophic respiration (Rh) and autotrophic respiration (Ra)) in croplands under long-term N fertilization have not attracted as much attention as in forest ecosystems with respect to the C cycle, which showed negative, neutral and even positive effects on Rs (Deng et al., 2010; Sun et al., 2014; Yan et al., 2018). Therefore, addressing the effects of long-term N fertilization on Rs in croplands is vital for predicting the global C cycle and its feedback in response to climate change.

In recent years, a large number of field experiments have been conducted to address the effects of N enrichment on Rs in terrestrial

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ecosystems (Guo et al., 2017; Hasselquist et al., 2012; Jia et al., 2012; Liu et al., 2015; Tu et al., 2013; Wang et al., 2019). However, the effect of N enrichment on Rs remains controversial, both in terms of its magnitude and direction, mainly because of the various responses of Rh and Ra (Janssens et al., 2010; Zhong et al., 2016a). For example, Chen et al. (2018) reported that the increase in Rs under N fertilization was caused mainly by an increase in Rh, whereas Sun et al. (2014) observed a significant decrease in Ra in response to N enrichment but no change in Rh, leading to a negative response of Rs. However, Olsson et al. (2005) attributed a decrease in Rs to a decline in both Rh and Ra. Furthermore, both N fertilizer rates and experiment duration had inconsistent effects on Rs and its components (Burton et al., 2004; Chen et al., 2018; Fierer et al., 2011; Hasselquist et al., 2012; Treseder, 2008; Zhong et al., 2016a). Hasselquist et al. (2012) reported that additions of 20 kg N ha^{-1} year^{-1} promoted Rs by increasing Ra, but additions of 100 kg N ha^{-1} year^{-1} had no effect, and a meta-analysis by Zhong et al. (2016a) revealed a threshold effect of the N rate on Rs. Moreover, Rs was found to increase during the first year of N fertilization but to decrease in subsequent years (Burton et al., 2004; Yan et al., 2010). However, previous studies have mainly focused on the effects of N fertilization on the Rs rate and its components, and few studies, particularly few long-term N fertilization studies, have assessed the effects of N application on seasonal C emissions through Rh or Ra. Therefore, the effects of long-term N application on Rs and its components should be further evaluated to provide vital information for predicting C emissions from ecosystems under conditions of long-term N input.

In general, soil temperature (ST) and soil water content (SWC) are considered the main factors that drive Rs via their direct effects, which control the growth of soil microbes and plant roots (Tu et al., 2013; Yan et al., 2010), and their indirect effects, which alter nutrient availability and plant productivity (Chen et al., 2017; Zhong et al., 2016b). For example, Matteucci et al. (2015) reported that ST and SWC drive Rs and Rh but that Ra is mostly driven by photosynthesis, and Chen et al. (2018) reported an interaction effect between SWC and ST on Rs. However, little is known about the threshold effect of SWC and ST on Rs and its components under long-term N application or about the change in temperature sensitivity of Rs (the Q10 value, the factor by which Rs increases with a 10 °C increase in temperature). Thus, determining the effects of abiotic and biotic factors under N input on Rs and its components could help predict the Rs under various environmental conditions under N fertilization (Hopkins et al., 2013).

To assess the effects of N fertilization on Rs, we investigated Rs and its components during 6 growing seasons in a field planted with two varieties of winter wheat (*Triticum aestivum* L.) under long-term N application (more than 10 years) using continuous half-hourly observations. The aims of this study were to 1) determine the response of the seasonal Rs rate and interannual C emission through Rs to long-term N fertilization and the sources of its variation; 2) ascertain whether N fertilizer application increases the growing season C emission as a function of the duration of N fertilization; and 3) identify the biotic and abiotic factors driving the dynamics of the seasonal and inter-annual Rs rate and its emission.

2. Materials and methods

2.1. Site description

The field study was conducted in Yangling, Shannxi (34°17′56″ N, 108°04′7″ E), at the southern boundary of the Loess Plateau. The study area has a typical semi-humid climate of the temperate zone. The regional average annual temperature is 13 °C, and the average annual precipitation is 632 mm, with approximately 60% of the precipitation occurring between July and September. The monthly precipitation and air temperature during the experiment from November 2012 to June 2018 are shown in Fig. S1. The soil is classified as a Udic Haplustalf in the United States Department of Agriculture (USDA) system and as a

Eum-Orthic Anthrosol in the Chinese Soil Taxonomy system. The physicochemical properties of the soil are shown in Table 1.

2.2. Experimental design

In October 2004, 18 plots with an area of 2 × 3 m^2 were established in accordance with a randomized block design. Two wheat varieties, Changhan No. 58 (CH), which is drought-tolerant, and Zhengmai No. 9023 (ZM), which is water-sensitive and poorly drought-tolerant, were planted, and three replications involving N application rates of 0, 180 and 360 kg N ha^{-1} (referred to as N0, N180 and N360, respectively) have been ongoing since 2004. Each plot consisted of 20 rows (15 cm spacing) of wheat, which was sown at a density of 90 seeds per row in middle or late October and harvest in late May or early June. N was applied in the form of urea, and phosphorus (P) was applied as superphosphate (33 kg P ha^{-1} year^{-1}). All fertilizers were mixed and broadcast simultaneously onto the soil surface and then incorporated into the whole soil layer (0–20 cm depth) with a rake before sowing at one time. During the study, no irrigation was applied, and weeds were removed regularly.

2.3. Soil respiration components

Currently, several primary methods for separating Rh or Ra are employed in field studies, including the inferred approach according to the root biomass, the root exclusion method, and stable or radioactive isotope application (Baumgartner et al., 2020; Chen et al., 2017; Jia et al., 2006; Li et al., 2018; Liu et al., 2017). Although the isotopic method could be used for in situ studies, and causes minimal disturbance to the environment, it requires the use of expensive equipment (mass spectrometers) and labeled C sources (Baumgartner et al., 2020). The inferred approach based on the root biomass measures the regressive relationship between Ra rate and root biomass and thus requires multiple root sampling and can be easily affected by the environment (Liu et al., 2017). Therefore, the root exclusion method was used to partition the Rs components in this study. Although certain drawbacks could not be avoided, such as the lack of data on respiration by rhizospheric microorganisms, the root exclusion method is still considered an effective and feasible method of partitioning Rs components and has been used in many previous studies (Barba et al., 2016; Li et al., 2018), especially in those involving cropland research (Chen et al., 2017; Gao et al., 2017). To distinguish between Rs and Rh, two types of polyvinyl chloride (PVC) collars with inner diameters of 20-cm and heights of 11 cm and 80 cm, respectively; a total of 18 deep and shallow collars, were inserted into the soil in each plot after fertilization but before sowing every year. Given that the roots in the top 50 cm of the soil accounted for more than 92% of the biomass within the 0–100 cm soil profile at our experimental site (Zhong et al., 2016b), the 80-cm long collar was long enough to exclude most of the living roots. These deep collars, which had several small holes covered with fine mesh (100 μm) on the surface to prevent root growth but allow the exchange of soil water between the inside of

Table 1
Properties of the 0–20 cm soil layer before fertilization (n = 10).

Property	Value
Taxonomy	Udic Haplustalf
Texture	
2000–50 μm (g kg^{-1})	64
50–2 μm (g kg^{-1})	694
<2 μm (g kg^{-1})	242
Bulk density (g cm^{-3})	1.23
Water-holding capacity (%)	23.6
Soil organic C (g kg^{-1})	8.79
Total N (g kg^{-1})	0.96
Soil C:N	9.16
Available N (mg kg^{-1})	25.1
Available P (mg kg^{-1})	7.9

the collar and the soil, were used for Rh measurements. The shallow collars, which allowed root growth, were used for Rs measurements. The soil collars remained in place throughout the growing season, and plants growing inside the collar were removed in a timely manner.

Rs and Rh data were recorded continuously during the 2012–2018 growing seasons by an LI-8100 automated soil CO_2 flux measurement system and an LI-8150 multiplexer with six 8100-104 long-term chambers (Li-Cor Inc., Lincoln, NE, USA). We measured three shallow and corresponding deep collars in growing seasons with measuring Rh or six shallow collars in growing seasons without measuring Rh per day, respectively, during consecutive periods of similar weather in the same order over the growing season, thereby guaranteeing that the interval between two consecutive measurements was the same for each plot. Thus, 3 to 6 days were used in one-time Rs and Rh measurement. The gas chamber was closed for 120 s, and the slope of the CO_2 concentration was used to calculate the respiration rate. The mean respiration rate was calculated from forty-eight measurements (once every 30 min for a total of 48 measurements on a given day), and the Rs components were measured 8, 10, 8, 8, 6, and 5 times throughout the growing season from October to June in 2013, 2014, 2015, 2016, 2017 and 2018, respectively. Root respiration (Ra) was calculated as the difference between Rs and Rh.

2.4. Plant and soil physical properties

Temporal changes in ST and SWC were measured at the same time as the Rs and Rh were recorded with the LI-8100. To measure the ST and SWC, a thermocouple probe and a moisture meter (EC5, Decagon, USA) were inserted into the soil at a depth of 10 cm.

To measure the canopy photosynthesis (Pn), a metal base (50 × 50 × 10 cm, length × width × height, respectively) was inserted into the soil. The canopy Pn was measured using an LI-8100 attached to a 125-L (50 × 50 × 50 cm, length × width × height, respectively) or a 250-L (50 × 50 × 100 cm) transparent Perspex chamber, and to prevent air leakage, the bottom rim of the chamber was sealed with a rubber strip (Yan et al., 2017). The canopy Pn was measured for 90 s after the chamber was placed onto the metal base. The biomass (dry weight) was determined by weighing the plant material after harvesting, and the leaf area index (LAI) was measured with a LAI-2000 Plant Canopy Analyzer (Li-Cor Biosciences Inc., USA) at the central square meter of every plot at the jointing (Jo.), heading (He.), and filling (Fi.) stages.

2.5. Calculations and statistical analyses

The daily Rs and Rh rates were taken as the mean of 48 measurements per day, and the canopy Pn was taken as the mean of at least 6 measurements from 00:00–24:00. The cumulative growing season C emissions (kg C m^{-2}) was calculated as AC $= \sum_{1}^{x}(R_i + R_{i+1})/2 \times (T_{i+1} - T_i) \times 12/44$, where x and R_i are the number of measurements and the daily respiration rate during the growing season, respectively; $(R_i + R_{i+1})/2$ and $(T_{i+1} - T_i)$ represent the mean respiration rate (μmol m^{-2} s^{-1}) and the time between two successive measurements (s), respectively, and 12 and 44 are the molecular weights of C and CO_2, respectively. The response ratios of the growing season C emissions based on the Rs components under the N180 and N360 treatments were calculated as follows: response ratio $= 100\% \times \frac{\text{C emission in N180 or N360} - \text{C emission in N0}}{\text{C emission in N0}}$. An exponential function (Rs $= \beta\, e^{bT}$) was used to analyze the response of the daily Rs and Rh rates (μmol m^{-2} s^{-1}) to ST, where T is the soil temperature at a depth of 10 cm (°C), β is the daily Rs or Rh rate at a reference temperature of 0 °C and b is the temperature reaction coefficient. Then, the temperature sensitivity of Rs and Rh under different N treatments during each growing season was calculated using the equation Q10 $= e^{10b}$.

The Shapiro-Wilk test was used to test the normality of the data. One-way ANOVA was used to determine the differences among the N

fertilizer rate treatments at the 95% confidence level (SPSS version 20.0 software; SPSS Inc., Chicago, USA), and post hoc comparisons (Tukey's honestly significant difference test) were used to test for multiple comparisons when significance was detected at the P < 0.05 level. Three-way ANOVA was performed to test the effects of N application rate, growing season and variety on Rs and its components. Mixed-effect models inclusive of all response variables were performed, with N fertilization, variety and growing season as fixed effects and block as a random effect. Three-dimensional (3-D) analysis was performed, and the equation Rs $= a \times \exp(-c \times (ST - d)^2 - f \times (SWC - g)^2$ was used to evaluate the interaction effects between ST and soil moisture on the Rs rate and its components, where a, c, d, f, and g are coefficients, and ST and SWC represent soil temperature and moisture, respectively (Chen et al., 2018). All graphs were constructed using the R software package (version 3.1.1) and SigmaPlot (version 14.0, Systat Software, Inc.).

3. Results

3.1. Dynamics of the C emission and Rs rates under different N fertilizer rates

In this field study, the N180 treatment resulted in the greatest C emission through Rs across all growing seasons, whereas positive, neutral and even negative effects of the N360 treatment on CH and ZM were observed, respectively (Fig. 1a and b), and both N fertilization and growing season had significant effects on growing season C emissions. The lowest total C emission occurred during the 2013 growing season, and the highest C emission was observed during the 2017 growing season. There was a significant increase in C emissions from 2013 to 2018 growing season under all N treatments in both wheat varieties (Fig. 1c and d) (P < 0.01), and the N180 treatment showed higher C emission slopes (0.048 and 0.025 for CH and ZM, respectively) compared with the N0 (0.018 and 0.015) and N360 (0.033 and 0.011) treatments. The Rs rate increased from approximately 0.3 μmol m^{-2} s^{-1} to 3.0 μmol m^{-2} s^{-1} between November and June in the growing season under all N treatments from 2012 to 2018 (Figs. S2a and b). The mean Rs rates during all six growing seasons under the N0, N180 and N360 treatments were 1.19 ± 0.08, 1.51 ± 0.10 and 1.32 ± 0.08 μmol m^{-2} s^{-1}, respectively, and both N fertilization and growing season had significant effects on the Rs rate. In addition, the Rh rate also increased with growing season (Fig. S2c) while the Ra rate decreased at the end of the growing season in both wheat varieties (Fig. S2d).

3.2. Growing season C emissions through Rh and Ra under N fertilization

The C emissions through Rh differed in different growing seasons (Fig. 2a), and N fertilization and wheat variety had no effect on C emissions through Rh. For both varieties, the highest growing season C emission through Rh occurred in 2017 (Fig. 2a). The percentages of respired soil C emission through Rh were greater than 50%, and a higher percentage of C emission through Rh was observed with the N0 treatment (Fig. 2b). For both varieties, the respired soil C emission through Ra and the percentage of C emission through Ra were higher in the N180 and N360 treatments than the N0 treatment (Figs. S3a and b), and the C emissions through Ra did not show a significant increase throughout the experiment for either variety (Figs. S3c and d). In addition, for the CH variety, the lowest growing season C emission through Rh was observed in 2013, and N application significantly increased the respired soil C emission through Rh and the percentage of C emission through Rh to Rs from 2013 to 2017 (Fig. 2c and e); however, the percentage of C emission through Ra to Rs decreased significantly under the N0 and N180 treatments throughout the experiment under CH (Fig. S3e).

3.3. Effects of N fertilization on Rs and other factors across all seasons

To determine the effects of N fertilization on the components of Rs
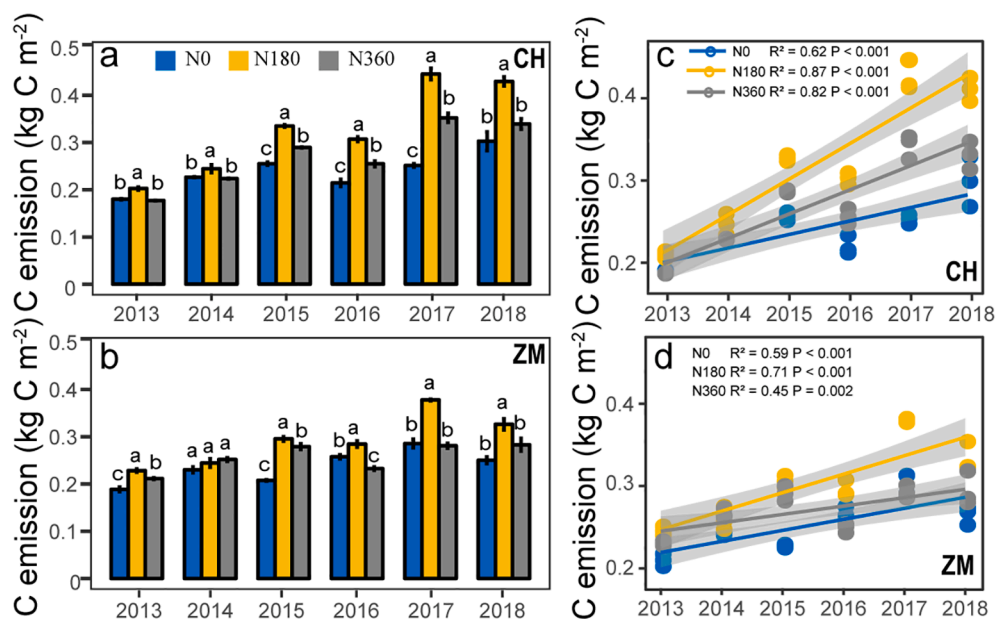
Fig. 1. Growing season carbon (C) emissions through soil respiration (Rs) from 2013 to 2018 for Changhan No. 58 (CH) (a) and Zhengmai No. 9023 (ZM) (b) and its trend throughout the experiment (c and d) under three different N fertilizer rates (N0: no N application; N180: 180 kg N ha⁻¹ year⁻¹; and N360: 360 kg N ha⁻¹ year⁻¹). The data represent the means ± SE of three replicates; the lowercase letters above indicate significant differences (P < 0.05) among the different N treatments.
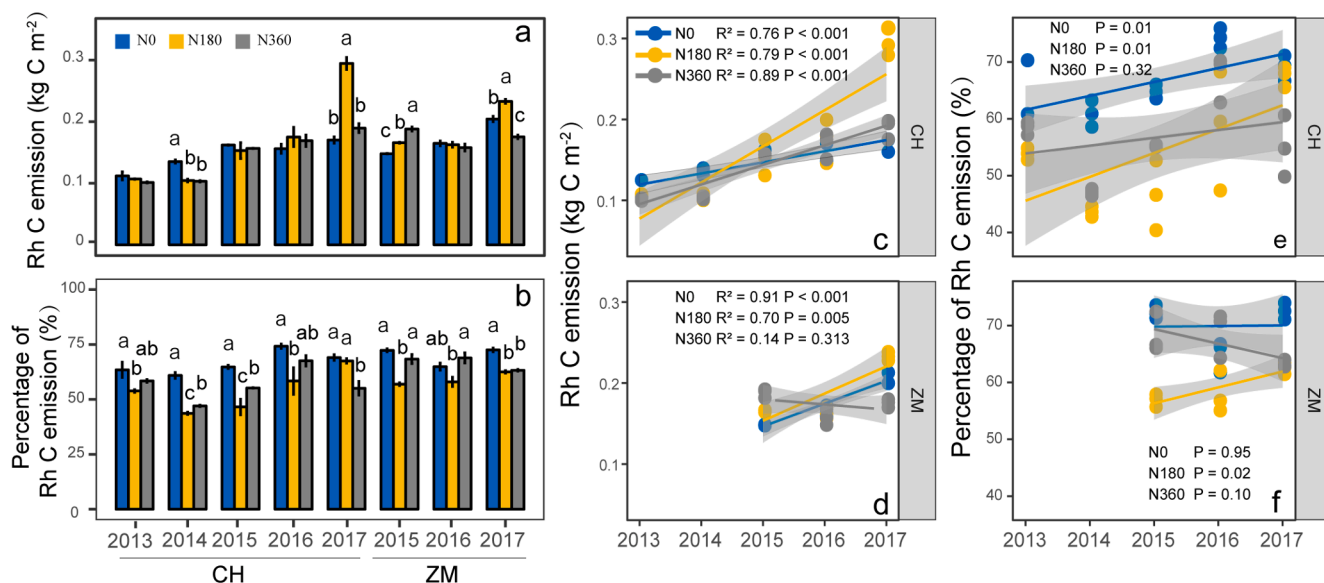

Fig. 2. Growing season C emissions through heterotrophic respiration (Rh) (a), percentage of C emissions through Rh to Rs (b), C emissions through Rh (c and d) and percentage of C emissions through Rh to Rs (e and f) throughout the experiment. The lowercase letters above indicate significant differences (P < 0.05) among the different N treatments.

across all growing seasons, we combined the data from the two varieties, as variety had no effect on C emissions (Fig. 1b). The results showed that, compared with the N0 treatment, the N180 and N360 treatments significantly increased the respired growing season soil C emission through Rs and Ra but had no effect on the C emission through Rh (Fig. 3a). During the growing season, N fertilization significantly increased the LAI and canopy Pn from the jointing to filling stages but had no influence at the tillering stage in December (Fig. 3b and c). N fertilization did not affect the mean annual ST or the SWC (Fig. 3d and e) but increased the biomass (Fig. 3f). In addition, the response ratios of growing season C emissions through Rs under N fertilization were positively correlated with the mean air temperature (Fig. 3g) and negatively correlated with the precipitation throughout the experiment (Fig. 3h). Furthermore, the response ratios of growing season C emissions through Rh under N fertilization also increased significantly with

the increase in temperature (Fig. S4c).

3.4. Relationships between Rs and temperature and moisture

In the study, the Rs and Rh rates increased exponentially with increasing ST (Fig. 4a and b) among the three N treatments in each growing season, but parabolic relationships were observed between Ra and ST (Fig. S5; P < 0.01). We then calculated the Q10 values and found that the Q10 values of both Rs and Rh were significantly negatively related to the mean air temperature during the growing season (MGT) in the N180 and N360 treatments throughout the experiment (Fig. 4c and d). 3-D functions integrating both ST and SWC explained 77%, 69%, and 73% of the variation in Rs under the N0, N180 and N360 treatments, respectively (Fig. 5); these values are greater than those obtained using models based on ST (67%, 58%, and 60% in the N0, N180 and N360
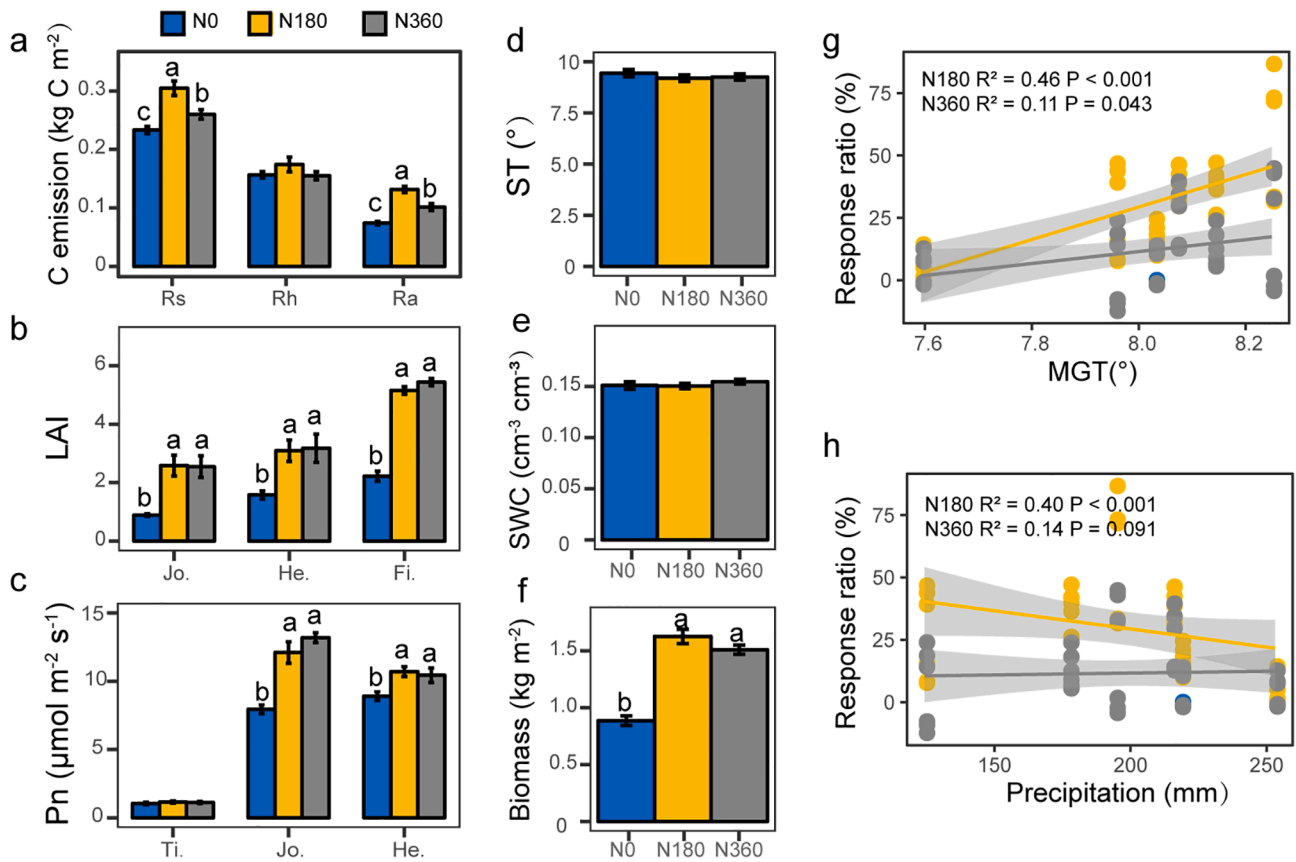
Fig. 3. Mean growing season C emissions (kg C m^{-2}) through Rs and its components (a) across six years, the leaf area index (LAI) (b), canopy photosynthesis (Pn) (c), soil temperature (ST) (d), soil water content (SWC) (e), and plant biomass (f) under three N application rates; and relationships between the response ratio of C emissions through Rs under N fertilization and the mean air temperature throughout the growing season (MGT) (g) and precipitation (h) during the growing season. The lowercase letters indicate significant differences (P < 0.05) among the different N treatments.
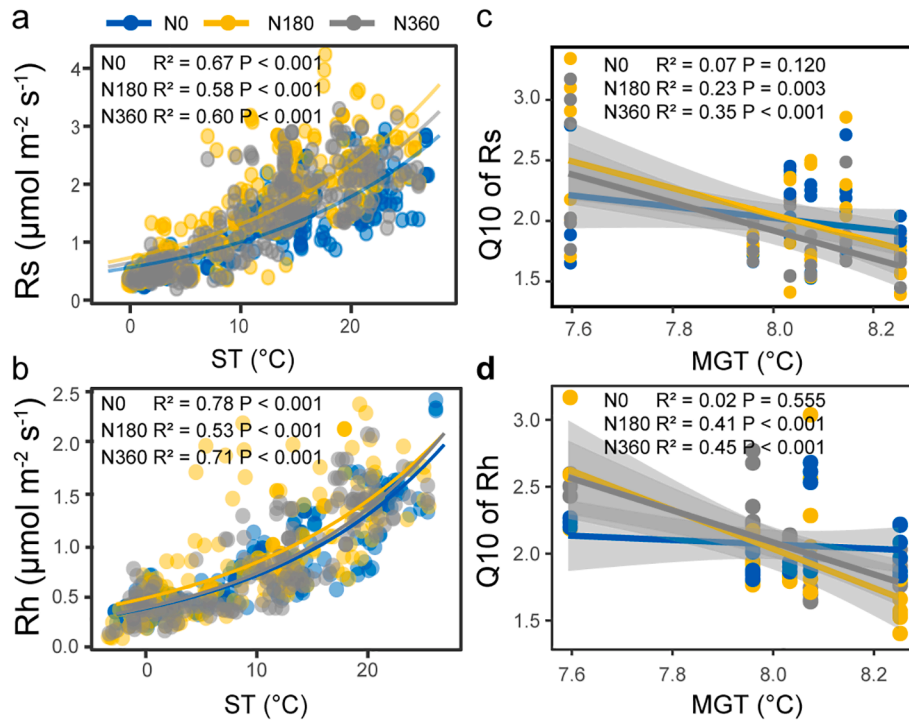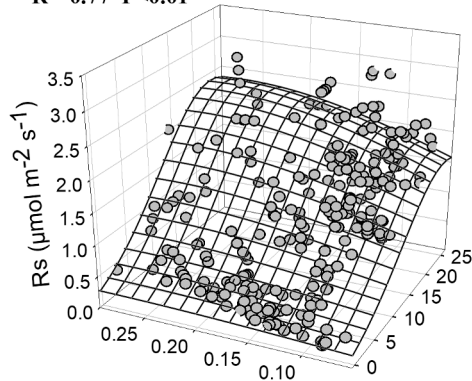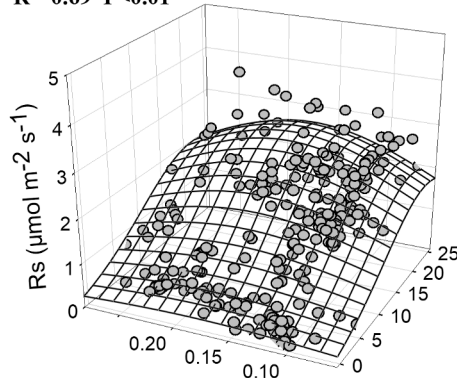

Fig. 4. Relationships between Rs (a) and Rh (b) rate and soil temperature (ST) at a soil depth of 10 cm, and between the Q10 values of Rs (c) and Rh (d) and the mean air temperature during the growing season (MGT).

a $Rs=2.4\times\exp[-0.004\times(ST-23.54)^2-8.1\times(SWC-0.27)^2]$
$R^2=0.77$ P<0.01

b $Rs=2.7\times\exp[-0.004\times(ST-22.10)^2-28.2\times(SWC-0.18)^2]$
$R^2=0.69$ P<0.01

c $Rs=2.4\times\exp[-0.005\times(ST-20.79)^2-17.1\times(SWC-0.21)^2]$
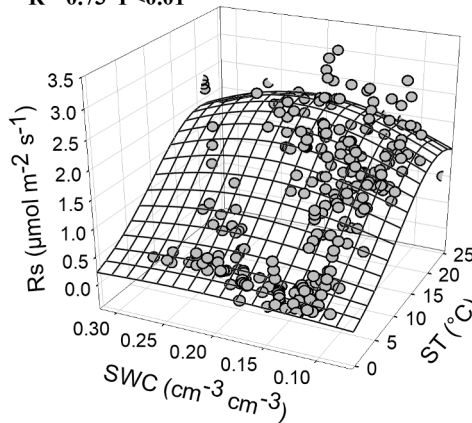$R^2=0.73$ P<0.01

Fig. 5. Three-dimensional (3-D) images of the relationships between Rs rate and ST and SWC for the N0 (a), N180 (b) and N360 (c) treatments.

treatments, respectively) or SWC (a weak correlation between SWC and Rs) alone. The optimum ST was 23.54 °C, 22.10 °C and 20.79 °C, and the optimum SWC was 0.27, 0.18 and 0.21 cm⁻³ cm⁻³ in the N0, N180 and N360 treatments, respectively. In addition, Rh increased with increasing ST, but SWC had a threshold effect on Rh, whereas both ST and SWC had a threshold effect on Ra (Fig. S6)

4. Discussion

4.1. Effects of N fertilization on Rs rates and C emissions

The impact of N fertilization on Rs was related to the response of its components caused by direct and indirect effects of N on the tradeoffs

between plant growth and the soil substrate (Chen et al., 2019). During the six growing seasons, the mean growing season C emissions through Rs, Rh and Ra increased by 20.7%, 5.4% and 60.0% under N fertilization, respectively, compared with those in the N0 treatment. These results are consistent with the results of previous works that reported increased Rs after N fertilization in cropland ecosystems (Liang et al., 2019; Prolingheuer et al., 2014; Zhang et al., 2013). The increase in C emission through Rs occurred mainly because N fertilization promoted the increase in C emission through Ra in this study, and this resulted in a reduction in the percentage of respired soil C emission through Rh under the N fertilization treatment (Fig. 2b). In this study, N180 and N360 treatment slightly decreased the soil C:N ratio (the soil C:N ratios were 10.31, 9.95 and 10.19 in the N0, N180 and N360 treatments, respectively, in the 2013 growing season), which might mitigate the N limitations on soil and enhance plant growth, as indicated by increased LAI, canopy Pn and biomass (Fig. 3), and promoted the allocation of carbohydrates belowground, thus resulting in the increase in growing season C emissions through Ra (Fig. S3a) (Zhang et al., 2013; Zhou et al., 2016). Moreover, the slight increases in Rh under N fertilization are also consistent with the results of previous studies (Chen et al., 2019, 2018). N fertilization increased soil N availability, which might mitigate the N limitations on soil microbes (Zhong et al., 2015) and thereby increase the Rh, and an increase in plant growth under N fertilization would increase the input of soluble organic compounds (e.g., exudates) exuded by roots, which could also cause an increase in Rh under N fertilization (Chen et al., 2019). However, the ST slightly decreased under N fertilization compared with that under the N0 treatment because of the significant increase in the LAI (Fig. 3d), which would decrease the Rh; thus, the direct and indirect effects of N fertilization led to negative or weakly positive Rh under N fertilization.

Our results showed that the stimulatory effect of the N180 treatment on the growing season C emissions through Rs, Rh and Ra (29.7%, 9.7% and 81.3%, respectively) was greater than that of the N360 treatment (11.6%, −1.0% and 38.7%, respectively), indicating the occurrence of N threshold effects on C emissions through Rs and its components. This is consistent with previous studies (Peng et al., 2017; Zhong et al., 2016a; Zhu et al., 2016) that reported nonlinear responses of Rs to increasing N addition, and excessive N input might lead to a decrease in root biomass because under such conditions plants do not need to develop more roots to absorb soil nutrients (Zhong et al., 2016b). This may have resulted in a smaller increase in Ra in the N360 treatment compared with the N180 treatment. In addition, excessive N input can alter microbial biomass and composition (Jia et al., 2020; Zhang et al., 2018; Zhong et al., 2015), reduce the mineralization activity of soil microbes by forming recalcitrant compounds (Guo et al., 2017) and suppress the activity of enzymes related to C decomposition (Jia et al., 2020); all of these effects would reduce soil decomposition rates and eventually decrease Rh. Importantly, with increasing temperature, the growing season C emissions through both Rs and Rh increased more rapidly under the N180 and N360 treatments than under the N0 treatment (Fig. S7), indicating that N fertilization can increase C emissions with increasing temperature, a finding that is consistent with previous results (Jia et al., 2012; Zhong et al., 2016a), and is probably because both rising temperature and N fertilization increased the soil microbial activity and plant growth. These results indicate that C emissions from the soil would be expected to increase in cropland ecosystems in response to global warming.

4.2. Seasonal and interannual variation in Rs and C emissions

In this study, the Rs and Rh rates were relatively low at the beginning of the growing season and then steadily increased until they peaked in May when the daily temperature was close to 20 °C (Fig. S1), for all treatments across all growing seasons (Fig. S2); in contrast, the Ra rate decreased at the end of the growing season (Fig. S2d). These results are consistent with those of previous studies of wheat croplands (Prolingheuer et al., 2014; Zhang et al., 2013) because the increases in both ST

and root exudates due to photosynthates would enhance the Rh rate. However, the Ra was influenced by processes related to plant growth, including the seasonality of the daily total gross primary productivity and the living root biomass (Chen et al., 2016; Zhang et al., 2013; Zhong et al., 2016b); thus, both Rh and Ra contributed to the seasonal variation in Rs in this study. In addition, the mean Ra to Rs ratio was 38% (ranging from 26% to 55%), similar to the results of Zhang et al. (2013), who reported a mean Ra/Rs of 36% in wheat croplands. However, our result was lower than that of Janssens et al. (2010), who reported that the Ra explained 67.3% of the Rs (the Ra/Rs ranged from 22.3% to 86.6%) in *Setaria italica* croplands. The lower mean annual precipitation (382 mm) in that study compared with the present study is likely responsible for the higher Ra/Rs because plants tend to develop more roots to absorb soil water in areas with low precipitation, resulting in increased Ra.

In the present study, the total C emissions ranged from 173 to 436 g C m^{-2} per growing season (Fig. 1a and b), which was at the low end of the range of 120 to1500 g C m^{-2} year^{-1} for croplands reported by Chen et al. (2014) on the basis of a global analysis, mainly due to the fact that our study calculated the C emissions only during the growing season. The average contribution of Ra to Rs emissions was 38.6%, which was very close to the global percentage of the Ra to Rs (38.3%) reported for croplands (Chen et al., 2014). Although the deep-collar method has several drawbacks (Zhou et al., 2007), our results are very similar to the C emissions through Ra to Rs reported for wheat croplands by Zhang et al. (2013) (36%) using the root exclusion method.

Interestingly, over the duration of the experiment, the C emissions through Rs increased significantly (Fig. 1c and d); the interannual mean air temperature (P < 0.01) and precipitation (P < 0.05) in growing season and plant biomass (P < 0.01) may account for the interannual changes in C emissions (Deng et al., 2012; Hui et al., 2003). Our observations consistent with the results reported by Epron et al. (2004), who observed an increase in Rs due to direct climatic effects or through an effect on annual productivity during a six-year study conducted from 1996 to 2001 in beech forest ecosystems. The growing season C emissions through Rh also increased significantly throughout the experiment (Fig. 2c; P < 0.01), as did the percentage of C emission through Rh to Rs in CH (Fig. 2), whereas the C emissions through Ra showed no increase (Fig. S3; P ≥ 0.05). Therefore, the variation in C emissions through Rs was caused mainly by increased Rh over the duration of the experiment, and it was related to the increasing air temperature at the experimental site (Fig. S7).

4.3. Biotic and abiotic factors affecting Rs and C emissions

In the present study, the variation in the Rs rate was significantly correlated with the air temperature, ST, LAI, and canopy Pn across all treatments (Tables 2 and S2). The C emissions through both Rs and Rh were significantly positively correlated with the plant biomass and air temperature but negatively correlated with precipitation during the growing season across all treatments (Table 3). Interestingly, the C emissions through Rs were weakly correlated with biomass under the N0 treatment, but significant correlations between C emissions through Rs or Rh and plant biomass and mean air temperature were observed in the N180 and N360 treatments (Table S3). Taken together, the above results indicate that both biotic and abiotic factors affect Rs and C emissions through their direct or indirect effects on plant growth and microbial

activity (Chen et al., 2017; Yan et al., 2020; Zhong et al., 2016b).

The Q10 values of Rs ranged from 1.4 to 3.2 in this study; these values are within the range of global values (mean: 2.4; range: 1.3–3.3) (Raich and Schlesinger, 1992), and significantly negative relationships between the Q10 values of both Rs with Rh and the MGT were observed (Fig. 4c and d). Moreover, the Q10 values decreased faster in the N180 (slope = −1.11 and −1.46 for Rs and Rh, respectively; N180 VS N0: P < 0.01) and N360 treatments (slope = −1.16 and −0.90 for Rs and Rh, respectively; N360 VS N0: P < 0.01) than under the N0 treatment (slope = −0.47 and −0.46 for Rs and Rh, respectively) with increasing air temperature, indicating that N fertilizer applications significantly decreased the temperature sensitivity of Rs with increasing temperature; this may be due to the increased N concentration in the plants and changes in soil microbial communities under N fertilizer application (Wang et al., 2019; Yan et al., 2015).

Exponential correlations between Rs and Rh rates and ST were observed in all N fertilization treatments across all growing seasons (Fig. 4a and b and S5; P < 0.01). However, negative parabolic relationships between Ra and ST were also observed in the study (Fig. S5), mainly due to the reduction in the allocation of photosynthates to belowground plant parts and the living root biomass at the end of the growing season (Prolingheuer et al., 2014; Vargas et al., 2011; Zhang et al., 2013). When we include both ST and SWC, we found that the model yielded higher R^2 values for Rs, Rh, and Ra compared with single-variable models (Fig. 5); this is consistent with the results of Chen et al. (2018), who reported that functions integrating both ST and SWC explained a greater percentage of the variation in Rs and its components than models that include ST or SWC alone, and that the resulting R^2 value was greater than the sum of the R^2 values of these two single-variable models. Thresholds of SWC on Rs and its components were observed in the present study, and above this threshold, an increase in SWC resulted in a decrease in Rs and its components, consistent with the results of previous works in croplands (Chen et al., 2018; Zhang et al., 2013). In general, an increase in the SWC promotes root growth and increases the supply of carbohydrates from aboveground plant parts (Kuzyakov and Gavrichkova, 2010), while a relatively high SWC can also reduce the soil oxygen availability, which could lead to a reduction in both Rs and Ra (Castellano et al., 2011; Moyano et al., 2013). These results suggest that the interaction effects of multiple environmental factors should be considered when evaluating soil C emissions in response to climate change (Zhou et al., 2016).

5. Conclusions

Our results emphasize the importance of partitioning the Rs components because Rh and Ra responded differently to N fertilization at the inner- and interannual scales. During the six growing seasons studied, N180 and N360 treatments increased the amount of growing season C emissions through Rs by 29.7% and 11.6%, respectively. Both Ra and Rh contributed to the seasonal variation in the Rs rate, and the increased Rs rate and total C emissions under the N180 and N360 treatments were mainly due to the increase in Ra, which was in turn related to the increase in the LAI and canopy Pn under N fertilization. More importantly, our results showed that the C emissions through Rs increased most rapidly under the N180 treatment throughout the experiment, and that this increase depended mainly on the increasing C emissions through Rh

Table 2
Relationships between the soil respiration (Rs) rate and its components (heterotrophic respiration (Rh), and autotrophic respiration (Ra)) and the soil temperature (ST), soil water content (SWC), leaf area index (LAI), canopy photosynthesis (Pn), and air temperature (AT).

Rate	Rh	Ra	ST	SWC	LAI	Pn	AT
Rs	0.83**	0.83**	0.81**	−0.08*	0.58**	0.37**	0.78**
Rh	1	0.38**	0.80**	−0.12**	0.16	0.05	0.76**
Ra		1	0.51**	−0.06	0.62**	0.56**	0.48**

The bold values marked with * indicate significant effects at P < 0.05, and those marked with ** indicate a significant effect at P < 0.01.

Table 3

Relationships between growing season C emissions through Rs and its components and biomass, mean soil temperature (MST), mean soil water capacity (MSWC), mean air temperature (MAT) and precipitation throughout the duration of the experiment.

C emissions	Rh	Ra	Biomass	MST	MSWC	MAT	Precipitation
Rs	**0.85****	**0.73****	**0.53****	0.05	0.01	**0.45****	−0.24*
Rh	1	**0.26***	**0.37****	**0.31****	−0.19	**0.63****	−0.30*
Ra	**0.26***	1	**0.71****	−0.148	0.041	−0.07	0.177

The bold values marked with * indicate significant effects at P < 0.05, and those marked with ** indicate a significant effect at P < 0.01.

in response to increasing temperature at the experimental site. These results suggest that N fertilization plays an important role in soil C cycling in response to increasing temperature.

Declaration of Competing Interest

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

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.geoderma.2020.114829.

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