Nitrogen fertilization stimulated soil heterotrophic but not autotrophic respiration in cropland soils: A greater role of organic over inorganic fertilizer

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\section*{ABSTRACT}

Nitrogen (N) enrichment may have considerable effects on soil carbon (C) fluxes. However, the responses of soil respiration (Rs) and especially its heterotrophic (Rh) and autotrophic (Ra) components to N fertilization remain controversial, and evidence on the impacts of N form and addition rate is lacking. We conducted a field experiment in a maize cropland in northeast China to investigate the responses of Rs, Rh, and Ra to different inorganic (IN) and/or organic (ON) N fertilization regimes, including no N addition (CK) and five N-fertilized treatments with a gradient ratio of IN to ON at 4:0 (IN4), 3:1 (IN3), 2:2 (IN2), 1:3 (IN1), and 0:4 (IN0). Annual Rs was higher in the N-fertilized treatments than CK, but only significantly so for IN1. Fertilization increased Rh from 118 to 123–149 g C m\(^{-2}\) with significant effects observed in all ON-fertilized treatments. However, fertilization did not affect Ra which varied at a range of 63–71 g C m\(^{-2}\). Rh was suppressed by excessive supply of ammonium and nitrate which was more effectively increased by IN than ON fertilization, but always increased with increasing extractable organic N and dissolved organic C which were higher in the treatments applied with more ON. Accordingly, a greater role of ON over IN fertilization was found in stimulating Rh. Rs (2.76–3.81) and Rh (2.67–3.81) had higher \(Q_{10}\) values than Ra (1.51–2.05). Application of N fertilizer, especially IN, enhanced the \(Q_{10}\) value of Ra, but decreased those of Rs and Rh. Unexpectedly, grain yield and aboveground biomass were reduced by IN fertilization, but increased with increasing ON fertilizer application rate. Overall, our findings highlight the significance of the form and addition rate of N fertilizer on soil C cycling and its feedback to climate change under N enrichment.

\section*{1. Introduction}

Anthropogenic input of reactive nitrogen (N) has increased from ~15 Tg N yr\(^{-1}\) in 1860 to 187 Tg N yr\(^{-1}\) in 2005 on a global basis, mainly through fossil fuel combustion and N fertilizer application (Galloway et al., 2008). Global carbon (C) and N cycles are tightly coupled, and thus the widespread N enrichment can considerably alter the terrestrial C cycle (Gruber and Galloway, 2008). Soil respiration (Rs), i.e., soil carbon dioxide (CO\(_2\)) flux, is estimated at 98 ± 12 Pg C yr\(^{-1}\) and is the largest C flux from terrestrial ecosystems to the atmosphere (Bond-Lamberty and Thomson, 2010). The Rs can be considered composed by two components: heterotrophic respiration (Rh) from the microbial decomposition of soil organic matter and litter, and autotrophic respiration (Ra) from the maintenance and growth of living roots and rhizosphere microbes (Subke et al., 2006). Cropland contains ~10% of global soil organic C (SOC) and has a large potential in C sequestration (Lal, 2004). Meanwhile, 60% of global anthropogenic reactive N is input to agricultural soils through nitrogenous fertilizers application (Galloway et al., 2008). Therefore, addressing the effects of N supply on the dynamics of soil C flux in cropland is crucial to quantify and predict global C cycle and its feedback to climate change (Robertson et al., 2013). However, compared with other ecosystems, the effects of N addition on Rs have been less studied in agricultural ecosystems and much less for the Rs components (Ding et al., 2010;
Currently, a number of field experiments have been carried out to examine the impacts of N addition on Rs; however, large inconsistencies and variations exist in the literature, which can be largely attributed to the various responses of Rh and Ra (Janssens et al., 2010; Zhou et al., 2016). For example, Tu et al. (2013) found that N addition stimulated Rs as a result of increases in both Rh and Ra. Comeau et al. (2016) showed that N fertilizer application enhanced Rs due to the increase of Rh but not Ra. While, Yan et al. (2010) reported that N addition increased Ra but decreased or did not alter Rh, leading to a positive response of Rs. By contrast, both Rh and Ra, and thus Rs were shown to be reduced by fertilization in the study of Olsson et al. (2005). However, other researchers suggested that the decreased Rs by N supply was mainly because of the reduction of Rh (Burton et al., 2004) or Ra (Sun et al., 2014). Therefore, it is crucial to quantify the respective response of Rh and Ra to N fertilization to achieve a comprehensive perspective on the effects of N enrichment on soil C fluxes. Furthermore, Rs is regulated by a large set of environmental factors, which may complicate the effects of N supply on Rs and its components. Among these factors, concerns about the role of temperature have been increased considering that if warming stimulates Rs, a positive feedback to climate change would occur (Crowther et al., 2016). The temperature sensitivity of Rs is commonly assessed by the Q10 value, the factor by which Rs increase with a 10 °C temperature rise (Davidson and Janssens, 2006). Despite numerous studies, response of the Q10 value of Rs to N addition remains controversial, and little is known on the effect of N fertilization on the Q10 of Rs components (Sun et al., 2014).

Previous researches have demonstrated that N fertilization regimes, mainly including the form and rate of applied N, could exert large impacts on soil microbes and plant behaviors and thus Rs and its components (Liu and Greaver, 2010; Chen et al., 2017a). Zhai et al. (2017) reported that with the increasing N addition rate, Rs exhibited a linear increase in a cropland and plantation but exhibited a parabolic response in a grassland in eastern China. Low rate addition of N can increase the biomass of soil microbes and plant fine root, leading to an increase in Rh and Ra, respectively (Hasselquist et al., 2012). However, excessive N loading may inhibit soil extracellular enzyme activities and fungi biomass, resulting in a decrease in Rh (Maaroufi et al., 2015; Jian et al., 2016), and may reduce the belowground allocation of plant C, root biomass and thus Ra (Högberg et al., 2010; Hasselquist et al., 2012). Du et al. (2014) and Li et al. (2014) reported that the activities of glucosidase, cellulase, and polyphenol oxidase, and SOC decomposition were suppressed after the addition of inorganic N (IN) but stimulated by organic N (ON) fertilization. Chen et al. (2017a) conducted a meta-analysis and found that ON was more effective than IN in stimulating soil microbial respiration. Moreover, it has been shown that the form of IN, i.e., ammonium (NH4+) or nitrate (NO3−), can also affect the performance of soil microorganisms and plant, and accordingly the response of Rh and Ra to N addition (Gavrichkova and Kuzyakov, 2008; Jian et al., 2016). However, Ramirez et al. (2010) concluded that N inhibited Rh regardless of the form of applied N based on the results observed in a laboratory incubation. Therefore, the role of N forms and rates should be further addressed to provide an insight into the effects of N application on Rs and its components.

The cropland located in northeast China is characterized by its inherently high fertility and played a major role in the national crop production. However, soil in this area has experienced a gradual loss of SOC for several decades, and combined application of inorganic and organic fertilizer is recommended to enhance the SOC content as well as ensure N supply to crops (Chen et al., 2014). In this study, a field experiment was conducted in a maize-planted cropland in northeast China, and Rs was measured throughout the year from soils with different IN and/or ON fertilization treatments. The Rh and Ra were separated by the commonly used root exclusion method (Subke et al., 2006). The main objective of this study was to examine the responses of Rs, Rh, and Ra and their temperature sensitivities to fertilization with different N forms and application rates.

2. Material and methods

2.1. Study site

The experimental site was located in the Hailun National Agro-ecological Experimental Station, Heilongjiang Province, China (47°26′N, 126°38′E) at an altitude of 240 m above sea level. The climate is temperate semi-humid with a short hot summer and long cold winter. The long-term (from 1953 to 2013) mean annual air temperature is 1.9 °C. The mean monthly air temperature varies from −21.6 °C in January to 21.6 °C in July. The long-term mean annual precipitation is 556 mm. The precipitation distribution is irregular, with 87% of annual precipitation occurs from May to October.

The study field is rainfed upland and was cultivated with maize (Zea mays L.). The soil is derived from loamy loess and classified as Typic Hapludoll according to the USDA soil taxonomy. Prior to the establishment of our experiment, the soil (0–20 cm) had a clay loam texture (8% sand, 72% silt, and 20% clay), a pH value of 6.1, and a bulk density of 1.0 g cm−3, and contained 28.3 g kg−1 organic C, 2.1 g kg−1 total N (TN), 52.6 mg kg−1 dissolved organic C (DOC), 3.2 mg N kg−1 NH4+, and 8.7 mg N kg−1 NO3−. The particle size was determined by a laser particle size analyzer (LS13320, Beckman Coulter, Brea, USA). Soil pH was analyzed in a 1:2.5 soil-water ratio and bulk density was measured with the intact core method. The SOC and TN content were analyzed on a CN analyzer (Vario Max CN, Elementar, Hanau, Germany). Soil NH4+, NO3−, and DOC concentrations were measured as described below.

2.2. Experimental design

The experiment used a completely random block design with six treatments replicated four times. Twenty-four 4.2 × 4 m2 plots were established in May 2013. One of the six plots in each block was randomly assigned to one treatment, including no N addition as control (CK) and five N-fertilized treatments with a ratio of inorganic (IN) to organic (ON) N at 4:0 (IN4), 3:1 (IN3), 2:2 (IN2), 1:3 (IN1), and 0:4 (IN0). The IN3, IN2, IN1, and IN0 treatments were defined as the ON-fertilized treatments. IN fertilizer was applied as urea, and ON fertilizer was pelleted chicken manure obtained from commercial company. The manure had a pH of 7.9 and a C/N ratio of 11.0, and contained 113.9 g kg−1 organic C, 10.3 g kg−1 TN, 8.01 g kg−1 DOC, 90.9 mg N kg−1 NH4+, and 5.1 mg N kg−1 NO3−. The manure properties were analyzed using the same methods as soil, with the exception of NH4+ and NO3− that were analyzed with the MgO-Devarda alloy method.

The N application rate was 150 kg N ha−1 in each N-fertilized treatment. ON fertilizer was applied as starter fertilizer before planting at a rate of 37.5, 75, 112.5, and 150 kg N ha−1 for IN3, IN2, IN1, and IN0, respectively. IN fertilizer was applied in two splits: 75 and 37.5 kg N ha−1 for IN4 and IN3, respectively, as starter fertilizer; and 75, 75, 75, and 37.5 kg N ha−1 for IN4, IN3, IN2, and IN1, respectively, as sidedress fertilizer. The field is split into ridges and furrows at a distance of 70 cm. Starter fertilizers were banded in the ridges, and maize seeds were then sown at a plant spacing of 25 cm on 19 May 2013. An area of 1.4 × 2.0 m2 in each plot was left unplanted. Pre-emergence herbicide was used for weed control. Sidedress fertilizers were applied on 28 June 2013 at the V6–V8 stage. Maize was harvested on 5 October 2013. Samples of maize grain and straw in each plot were oven-dried at 60 °C and weighed to obtain the grain yield and above-ground biomass. All crop residues were removed from the plots and then the field was tilled manually.

2.3. Measurement protocols

Soil CO2 fluxes were measured over the experimental period from 21 May 2013 to 24 April 2014 using the static closed chamber method.
A polyvinyl chloride (PVC) tube (10 cm height and 10 cm inner diameter) was inserted 5 cm into soil, including one maize plant, at the center of one ridge in each plot. A PVC base frame (25 cm length, 70 cm width, and 20 cm height) was driven into soils at a depth of 10 cm, making the PVC tube located at its center. The tube and base frame were installed before sowing and left undisturbed throughout the course of the experiment. For the base frame area, fertilizers were weighed and applied separately to ensure accurate application rates. When collecting gas samples, a PVC pipe (30 cm height and 10 cm outer diameter) was embedded to the PVC tube. A stainless-steel chamber (25 cm length, 70 cm width, and 25 cm height) was fitted to the PVC base frame by inserting into the groove around the upper edge of the base frame. The chamber was custom-made and consisted of two separate parts which were linked by two hinges. In the middle of the chamber top, a circular opening (10 cm diameter) was made to fit the PVC pipe with sealing strips for airtightness. In the unplanted subplot, a circular opening (10 cm diameter) was embedded to the PVC tube. A stainless-steel chamber and placed into pre-evacuated 20-mL glass vials from the chamber headspace at an interval of 10 min after closing the fitting CO2 concentration was recorded on 12 January 2014; while the maximum AT (26.2 °C) was recorded on 31 July to 27 August 2013, when rain storm caused soil waterlogging, were removed when calculating the Q10 values.

The Kolmogorov-Smirnov test was applied for data normality assessment. If necessary, In-transformation was used to improve the normality of data distribution. The response ratio of a variable to N fertilizer application (RR) was calculated as the ln-ratio of the value in the N-fertilized treatment to that of CK. Significant difference among treatments was tested using the one-way analysis of variance followed by the least significant difference test. Pearson analysis was used to extensively investigate the correlations between the flux of Rs, Ra, and Rh and each climatic and soil environmental factor. Regression analysis was implemented to further explore the relationships between Rs, Rh, and plant biomass with soil NH4+, NO3−, EON, or DOC. Trivariate models were developed using the nonlinear response surface regression to examine the combined effects of soil temperature and moisture on the Rs, Rh, and Ra fluxes. Linear regression analysis was employed to assess the effects of IN and ON fertilizer application rates on NH4+, NO3−, EON, DOC, Rs, Rh, Ra and their Q10 values, and plant biomass. Statistical analyses were conducted with SPSS 18.0 (SPSS Inc., Chicago, USA) and Origin Pro 8.5 (OriginLab, Northampton, USA), with significance at P < 0.05.

3. Results

3.1. Climatic and soil environmental conditions

Air temperature (AT) had a clear seasonal pattern and averaged at 2.4 °C during the study period (Fig. 1a). The minimum AT (-29.6 °C) was recorded on 12 January 2014; while the maximum AT (26.2 °C)
was recorded on 24 June 2013. Soil temperature (ST) varied from −4.1 to 24.8 °C and followed the trend of AT except in winter. The relationship between ST and AT could be described by the functions of $ST = 1.00 \times AT - 0.84$ ($R^2 = 0.88$, $P < 0.0001$) under $AT > 0$ °C, and $ST = 0.15 \times AT + 0.38$ ($R^2 = 0.50$, $P < 0.0001$) under $AT < 0$ °C. A total of 831 mm precipitation fell over the experimental period, which was 1.5-fold higher than the long-term mean annual precipitation, largely due to a rain storm event (178 mm) occurring on 30 July 2013. There was no significant treatment effect on soil water content (SWC), and the mean SWC for all plots were presented in Fig. 1a. The SWC generally increased after rainfall, with a highest value (56.9%) observed after the rain storm in early August 2013.

The seasonal dynamics of soil NH$_4^+$, NO$_3^-$, EON, and DOC concentrations were similar among different treatments (Fig. S1). Annual mean soil NH$_4^+$ concentration was increased by N fertilization, however, only IN4 exerted a significant effect comparing with CK (Fig. 2a). All N-fertilized treatments had significantly higher annual mean NO$_3^-$ concentration than CK, especially for IN4 and IN3 (Fig. 2b). Likewise, fertilization significantly increased annual mean EON concentration which increased with increasing ON addition rate (Fig. 2c). Annual mean DOC concentration increased from 48.0 mg C kg$^{-1}$ for CK to 49.8–51.7 mg C kg$^{-1}$ in the N-fertilized treatments (Table 2). Rs was significantly higher in IN1 than CK and IN4, but did not significantly differ among the other treatments. All ON-fertilized treatments exhibited a significant increment in Rh in comparison with CK and IN4 (except IN0 vs. IN4). The highest Rh was measured in IN1 with a ratio of IN to ON application rate at 1:3. Regression analysis showed that annual Rh increased with the percent of applied N as ON, while the opposite was true for EON and DOC.

### 3.2. Soil respiration and its components

As shown in Fig. 1b, the fluxes of Rs gradually increased with increasing temperature after the onset of the experiment and peaked at 110–127 mg C m$^{-2}$ h$^{-1}$ in all treatments in early July 2013. A large reduction was observed in all plots when soil was nearly saturated by the rain storm. During this period, Rs fluxes were below 40 mg C m$^{-2}$ h$^{-1}$. As soil dried, high Rs fluxes (> 100 mg C m$^{-2}$ h$^{-1}$) were measured again on 27 August 2013. After that, Rs declined with decreasing soil moisture and temperature, and was below 46 mg C m$^{-2}$ h$^{-1}$ after maize harvest. No obvious fluxes were detected during the freezing period. The temporal patterns of the Rh (Fig. 1c) and Ra flux (Fig. S2) were similar to Rs. Repeated measures ANOVA indicated that Rs, Rh, and Ra flux differed significantly across the sampling times ($P < 0.0001$), while only Rh exhibited significant treatment effect ($P = 0.006$).

Annual Rs was increased from 187 g C m$^{-2}$ in CK to 195–215 g C m$^{-2}$ in the N-fertilized treatments (Table 2). Rs was significantly higher in IN1 than CK and IN4, but did not significantly differ among the other treatments. All ON-fertilized treatments exhibited a significant increment in Rh in comparison with CK and IN4 (except IN0 vs. IN4). The highest Rh was measured in IN1 with a ratio of IN to ON application rate at 1:3. Regression analysis showed that annual Rh increased with the percent of applied N as ON, reached the maximum when this
percent was ∼60% and then decreased (Fig. S3). Ra varied with a range of 63.4–70.6 g C m$^{-2}$, and no significant differences were found among treatments. The contribution of annual Rh to Rs was 62.9% for CK and 63.3% for IN4, and significantly increased to 69.2–71.1% in the ON-fertilized treatments. N fertilization, particularly ON, had positive effects on Rs, Rh, and Rh/Rs; while Ra decreased with increasing IN and ON addition rate (Table 1).

The response ratio (RR) of annual Rs to N fertilizer application was highest in the IN1 treatment, similar to that in IN2 and significantly higher than that in other N-fertilized treatments (Fig. 3). The RR of annual Rh was increased in the following order: IN4 < IN0 ≤ IN3 < IN2 ≤ IN1. The RR of Ra was not significantly different among treatments. The RR of annual Rh/Rs was significantly higher in ON-fertilized treatments than IN4.

### 3.3. Environmental controls on soil respiration and its components

Rs and Rh flux significantly correlated to air or soil temperature in each treatment (Tables S1 and S2); while significant correlation between Ra flux and temperature was only observed in IN2 (Table S3). Combining data from each of the six treatments, the Rs, Rh, and Ra flux all exponentially increased with increasing soil temperature, however the $R^2$ values (0.08–0.11) were extremely low. We calculated the $Q_{10}$ values after removing the data from 31 July to 27 August 2013, when rain storm caused soil waterlogging, which significantly increased the model fitness. Rs and Rh were found to have higher $Q_{10}$ values than Ra (Table 3). N fertilization decreased the $Q_{10}$ of Rs and Rh, and the inhibition effect was greater for IN than ON (Table 1). In contrast, the $Q_{10}$ value of Ra was increased by N fertilization, significantly for IN3 and IN2.

Rs and Rh had stronger correlations to precipitation than Ra (Tables

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**Table 1**

Regression analysis assessing the effects of the application rate of inorganic (IN) and organic (ON) nitrogen fertilizer on different variables with the function of $y = a \times IN + b \times ON + c$.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$a$ (RE)</th>
<th>$b$ (RE)</th>
<th>$c$ (RE)</th>
<th>$R^2$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Annual mean NH$_4^+$</td>
<td>0.00254</td>
<td>0.000315</td>
<td>2.58</td>
<td>0.79</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Annual mean NO$_3^-$</td>
<td>0.0200</td>
<td>0.00576</td>
<td>7.81</td>
<td>0.84</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Annual mean EON</td>
<td>0.00645</td>
<td>2.87</td>
<td>0.95</td>
<td>&lt; 0.0001</td>
<td></td>
</tr>
<tr>
<td>Annual mean TEN</td>
<td>0.0261</td>
<td>0.0125</td>
<td>13.3</td>
<td>0.89</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Annual mean DOC</td>
<td>0.0110</td>
<td>0.0219</td>
<td>48.0</td>
<td>0.95</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Annual Rs</td>
<td>0.0670</td>
<td>0.123</td>
<td>187</td>
<td>0.49</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Annual Rh</td>
<td>0.0953</td>
<td>0.189</td>
<td>118</td>
<td>0.62</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Annual Ra</td>
<td>−0.00851</td>
<td>−0.0206</td>
<td>70.3</td>
<td>0.16</td>
<td>0.01</td>
</tr>
<tr>
<td>Annual Rh/Rs</td>
<td>0.0272</td>
<td>0.0547</td>
<td>62.9</td>
<td>0.54</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Q$_{10}$ of Rs</td>
<td>−0.00688</td>
<td>−0.00147</td>
<td>3.81</td>
<td>0.96</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Q$_{10}$ of Rh</td>
<td>−0.00369</td>
<td>−0.00100</td>
<td>3.28</td>
<td>0.94</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Q$_{10}$ of Ra</td>
<td>0.00213</td>
<td>0.00160</td>
<td>1.51</td>
<td>0.41</td>
<td>0.0007</td>
</tr>
<tr>
<td>Grain yield</td>
<td>−2.50</td>
<td>6.62</td>
<td>8033</td>
<td>0.97</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

Values are means ± SE (n = 4). Different letters within the same column indicate significant differences among treatments at $P < 0.05$. 

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**Table 2**

Annual soil respiration (Rs), heterotrophic (Rh) and autotrophic (Ra) respiration, contribution of annual Rh to Rs (Rh/Rs), and the proportion of decomposed manure carbon ($P_{dmc}$).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Rs (g C m$^{-2}$)</th>
<th>Rh (%)</th>
<th>Ra (%)</th>
<th>Rh/Rs (%)</th>
<th>$P_{dmc}$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK</td>
<td>187 ± 3 b</td>
<td>118 ± 4 c</td>
<td>70.3 ± 4.0 a</td>
<td>62.9 ± 1.3 b</td>
<td>=</td>
</tr>
<tr>
<td>IN4</td>
<td>195 ± 6 b</td>
<td>123 ± 5 be</td>
<td>70.4 ± 5.8 a</td>
<td>63.3 ± 1.1 b</td>
<td>=</td>
</tr>
<tr>
<td>IN3</td>
<td>197 ± 7 ab</td>
<td>140 ± 4 a</td>
<td>69.0 ± 6.5 a</td>
<td>71.1 ± 1.2 a</td>
<td>42.9 ± 4.6 a</td>
</tr>
<tr>
<td>IN2</td>
<td>204 ± 7 ab</td>
<td>147 ± 14 a</td>
<td>63.4 ± 5.1 a</td>
<td>72.0 ± 4.3 a</td>
<td>31.8 ± 4.1 b</td>
</tr>
<tr>
<td>IN1</td>
<td>215 ± 9 a</td>
<td>149 ± 7 a</td>
<td>70.6 ± 8.7 a</td>
<td>69.6 ± 0.8 a</td>
<td>24.4 ± 2.7 b</td>
</tr>
<tr>
<td>IN0</td>
<td>197 ± 7 ab</td>
<td>136 ± 5 ab</td>
<td>67.4 ± 5.8 a</td>
<td>69.2 ± 0.7 a</td>
<td>11.0 ± 1.2 c</td>
</tr>
</tbody>
</table>

Values are means ± SE (n = 4). Different letters within the same column indicate significant differences among treatments at $P < 0.05$. 

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**Fig. 2.** Annual mean concentration of soil ammonium (NH$_4^+$, a), nitrate (NO$_3^-$, b), extractable organic nitrogen (EON, c) and dissolved organic carbon (DOC, d). Vertical bars represent standard errors (n = 4). Different letters indicate significant differences at $P < 0.05$. 

NH$_4^+$, ammonium; NO$_3^-$, nitrate; EON, extractable organic nitrogen; TEN, total extractable nitrogen; DOC, dissolved organic carbon; Rs, soil respiration; Rh, heterotrophic respiration; Ra, autotrophic respiration; SOC, soil organic carbon.
S1–3). Correlations between the fluxes of Rs, Rh, and Ra with SWC were very weak. However, their relationships could be fitted by Gaussian functions ($R^2$ was 0.47, 0.50, and 0.10, respectively, for Rs, Rh, and Ra). Three-dimensional (3-D) functions integrating both soil temperature and moisture could explain 62%, 66%, and 33% of the variations in Rs, Rh, and Ra fluxes, respectively (Fig. 4). The $R^2$ value of the 3-D model was higher than that of the model including temperature or moisture alone, and even the sum of the $R^2$ values of these two one-variable models (0.55, 0.58, and 0.21 for Rs, Rh, and Ra, respectively). The optimum ST and SWC was 26.0 °C and 37.5–38.2%, respectively.

No significant correlation between Rs, Rh, and Ra to NH$_4^+$, NO$_3^-$, or DOC was observed in each treatment (Tables S1–3). The ln-transformed Rh flux exhibited parabolic responses to NH$_4^+$ and NO$_3^-$, and linear responses to EON and DOC during the growing season (Fig. 5). Consistently, annual Rs and Rh initially exponentially increased with increasing annual mean NH$_4^+$ and NO$_3^-$ concentration and then decreased after the maximums, and exponentially increased with increasing annual mean EON and DOC concentration (Fig. 6).

### Table 3

The Q$_{10}$ values of soil respiration (Rs), heterotrophic (Rh) and autotrophic respiration (Ra).

<table>
<thead>
<tr>
<th></th>
<th>Rs</th>
<th>Rh</th>
<th>Ra</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK</td>
<td>3.81 ± 0.06 a</td>
<td>3.28 ± 0.11 a</td>
<td>1.51 ± 0.06 c</td>
</tr>
<tr>
<td>IN4</td>
<td>2.76 ± 0.06 c</td>
<td>2.67 ± 0.09 c</td>
<td>1.69 ± 0.03 bc</td>
</tr>
<tr>
<td>IN3</td>
<td>3.03 ± 0.13 bc</td>
<td>2.84 ± 0.06 bc</td>
<td>1.84 ± 0.02 ab</td>
</tr>
<tr>
<td>IN2</td>
<td>3.21 ± 0.13 b</td>
<td>3.01 ± 0.15 ab</td>
<td>2.05 ± 0.17 a</td>
</tr>
<tr>
<td>IN1</td>
<td>3.24 ± 0.09 b</td>
<td>3.04 ± 0.05 ab</td>
<td>1.73 ± 0.05 bc</td>
</tr>
<tr>
<td>IN0</td>
<td>3.67 ± 0.08 a</td>
<td>3.07 ± 0.07 ab</td>
<td>1.65 ± 0.02 bc</td>
</tr>
</tbody>
</table>

The Q$_{10}$ values were calculated after removing the data from 31 July to 27 August in 2013 when the soil CO$_2$ fluxes were inhibited by high moisture. Values are means ± SE (n = 4). Different letters within the same column indicate significant differences among treatments at $P < 0.05$.

3.4. Plant biomass, manure decomposition, and carbon budget

Maize grain yield and straw biomass had similar variation trends among different treatments, i.e., significantly decreased by IN fertilizer addition alone and gradually increased with increasing ON application rate (Table 4). Regression analysis revealed that grain yield and aboveground biomass was significantly reversedly related to annual mean concentration of NH$_4^+$ and marginally reversely (0.05 < $P < 0.10$) to that of NO$_3^-$ (Fig. 7).

The proportion of decomposed manure C ($P_{dmc}$) decreased with increasing application rate of ON fertilizer, from 42.9% for IN3 to 11.0% for IN0 (Table 4). Both the $C_{input}$ and $C_{storage}$ were significantly lower in IN4 than CK, and linearly increased with increasing addition rate of ON ($P < 0.001$). Comparing with CK, $C_{input}$ and $C_{storage}$ were higher in the ON-fertilized treatments, significantly so for IN2, IN1, and IN0.

4. Discussion

4.1. Effects of moisture and temperature on Rs, Rh, and Ra

Annual Rs was 187–215 g C m$^{-2}$ in all treatments (Table 2), which was lower than those measured from maize-plant soils in Ohio, USA (550–660 g C m$^{-2}$; Ussiri and Lal, 2009) and in the North China Plain (461–498 g C m$^{-2}$; Ding et al., 2010). Apart from the climate conditions, Rs can be largely affected by soil properties, especially the SOC content, and management practices (Buragien et al., 2015; Xu and Shang, 2016). The SOC content was higher in our site than the above-mentioned studies (28.3 vs. 5.4–12.1 g kg$^{-1}$). Furthermore, Rs in this study was also greatly lower than that (314–420 g C m$^{-2}$) measured at the same site in our previous study which had the same field management as the present study (Chen et al., 2017b). Therefore, the lower Rs in our study was presumably mainly attributed to the large reduction in Rh and Ra by rain storm caused high soil moisture (Fig. 1 and Fig. S2).

The fluxes of Rs, Rh, and Ra increased with soil moisture when SWC was below 38%; however, above the threshold value, increasing moisture led to decreases in Rs, Rh, and Ra (Fig. 4). Consistently,
Knowles et al. (2015) reported a threshold of 38% SWC for Rs and Zhang et al. (2013) found that the optimum SWC for Rh and Ra was 30–40%. Under suboptimal moisture condition, increasing moisture can improve substrate diffusion, increase enzyme activities, and thus stimulate Rh (Moyano et al., 2013). Ra can be increased as a result of promoted root growth and nutrient uptake, and more supply of carbohydrates from aboveground plant (Gavrichkova and Kuyzakov, 2008). When moisture exceeds the optimum level, the oxygen availability can be greatly reduced (Moyano et al., 2013), and the net production of adenosine triphosphate as respiration can decrease from 36 to 2 mol mol\(^{-1}\) hexose (Bhattarai et al., 2005). Accordingly, CO\(_2\) production from the respiration processes declines in cells (Bhattarai et al., 2005). Therefore, oxygen limitation is regarded as the main reason for inhibited microbial and root respiration under high moisture condition (Castellano et al., 2011; Vicca et al., 2014).

In addition to soil moisture, temperature is another critical factor controlling the seasonal variation of Rs and its components (Davidson and Janssens, 2006). The \(Q_{10}\) values of Rs were 2.76–3.81 (Table 3), falling well within the range of 2–5 for temperate and boreal sites as synthesized by Bahn et al. (2010). Interestingly, Rh had higher \(Q_{10}\) than Ra in each treatment, which could be supported by the findings of Bhupinderpal et al. (2003) and Hartley et al. (2007). It has been acknowledged that Ra is tightly coupled with aboveground plant photosynthetic activity and is largely controlled by belowground allocation of recent photosynthates (Högberg et al., 2001; Vargas et al., 2011). Many studies have revealed that photosynthetic rate is nonresponsive to temperature change at a given growth temperature spanning a wide range of plant types (Smith and Dukes, 2013). In addition, higher \(Q_{10}\) has been observed under lower temperature (e.g. Carey et al., 2016). We further calculated the \(Q_{10}\) value during the growing season, and found it was lower for Rh than Ra (1.40 vs. 1.56). Therefore, higher \(Q_{10}\) value of Rh than Ra in an annual perspective might be also due to greater response of Rh to temperature during the cold nongrowing season.

Models including both soil moisture and temperature yielded higher \(R^2\) value than that of one-variable models and even their sum for Rs, Rh, or Ra. These results indicated an important synergetic effect of soil moisture and temperature on soil respiration and its two components (Wiaux et al., 2014). Thus, considering the interactive effects of multifactor is necessary in model prediction of various soil C fluxes, and well-designed experiments with multiple environmental factors are needed to evaluate the responses of soil C processes to climate change (Zhou et al., 2016).

4.2. Effects of nitrogen fertilization on Rs, Rh, and Ra

Nitrogen fertilization increased annual Rs by 4.3–15.0%, with a significant effect only observed in the IN1 treatment (Table 2). Similarly, Li et al. (2013) showed that Rs was enhanced slightly by IN fertilization, and significantly by ON fertilization at the same study region as ours. However, Jones et al. (2005) reported that both IN and ON application significantly increase Rs from agricultural soils. Low response of Rs to N addition in our study was largely because the autotrophic component of Rs was independent on N fertilization. Ra is highly dependent on the supply of carbohydrates from photosynthesis and can increase with increasing plant productivity (Xu and Shang, 2016). Yan et al. (2010) found that N addition significantly stimulated Ra by increasing plant biomass. However, in this study, the aboveground biomass was somehow significantly decreased in IN4 and did not significantly change in other N-fertilized treatments in comparison with CK (Table 4). And no significant relationship between plant biomass and Ra was found. A previous study at the same site in a year with much less precipitation (544 mm) showed that Ra, accompanied with plant biomass, was significantly increased by N fertilization (Chen et al., 2017b). Accordingly, it was conjectured that excessive moisture condition might inhibit the response of plant productivity, and

![Fig. 4. Three-dimensional images of the relationships between the fluxes of soil respiration (Rs, a), heterotrophic (Rh, b) and autotrophic (Ra, c) respiration and soil temperature (ST) or volumetric soil water content (SWC) for all treatments.](image-url)
Accordingly, Ra to N supply in this wetter year. Unexpectedly, Ra decreased with increasing rate of IN and ON as shown by the regression analysis (Table 1). Increase in N availability can reduce the need for plant to invest C resources to absorb N, leading to a shift in C allocation more to aboveground (Janssens et al., 2010). Therefore, N fertilization may decrease the biomass and thus respiration of roots, and also the rhizosphere C flux and thus rhizo-microbial respiration (Hasselquist et al., 2012). As a result, Ra can be inhibited by N addition, especially at high rate (Sun et al., 2014). Root biomass was not measured in this study, and should be included in future research. In addition, study-separating Ra into respiration by plant roots and rhizosphere microorganisms is needed in order to clarify their respective response to different rates and forms of N supply.

Compared with CK, annual Rh and Rh/Rs were similar in IN4, but significantly higher in the ON-fertilized treatments (Table 2). The RR of annual Rh and Rh/Rs were significantly higher in the ON-fertilized treatments than in IN4 (Fig. 3). Moreover, Rh and Rh/Rs increased more rapidly with increasing addition rate of ON than IN (Table 1). These results indicated that ON fertilizer application more effectively stimulated Rh than IN. Consistently, Chen et al. (2017a) showed that soil microbial respiration was higher under ON or mixed ON and IN addition than under IN alone. In this study, soil NH$_4^+$ and NO$_3^-$ concentrations were more elevated by IN than ON fertilization (Fig. 2 and Table 1). Rh was found to initially increase with increasing soil NH$_4^+$ and NO$_3^-$ but then decrease after the maximum (Figs. 5 and 6). Likewise, Wang et al. (2015) reported that low level addition of NH$_4^+$ or NO$_3^-$ increased soil CO$_2$ flux, and total and fungal phospholipid fatty acid content, whereas high level addition had the opposite effects. Excessive inorganic N can also decline the labile organic C availability and microbial activity, and lead to an accumulation of recalcitrant C compounds (Xu et al., 2017). Different from inorganic form of N, Rh was found to always increase with increasing soil EON (Figs. 5c and 6g), which was higher in the treatment applied with more ON fertilizer (Fig. 2). Du et al. (2014) showed that in contrast to IN, ON deposition increased soil cellulase and polyphenol oxidase activities, labile organic C content, and thus Rh. Chen et al. (2017a) suggested that there was a costimulation of soil glycosidase activity and Rh by N addition, and this stimulation was greater by ON than IN.

However, a decrease of Rh was found when the percent of added N as ON increased from 75% to 100%, i.e., Rh was lower in IN0 than IN1, albeit not significantly (Table 2). Rh was found to be highest when ON contributed to ~60% of total applied N (Fig. S3). Similarly, Li et al. (2014) reported that the positive effect of N deposition on the activities of enzymes involved in litter decomposition was greatest when the percent of added N as ON was 80%. The proportion of decomposed manure C (P$_{dmc}$) decreased with increasing application rate (Table 2), which was in line with Niklasch and Joergensen (2001). In our study, fertilizers were applied in bands at the ridges, so the manure was applied deeper in soil and might form a larger volume under higher application rate. Accordingly, the diffusion rate of oxygen from air to soil could decrease (Zhu et al., 2015), and the contact between manure and soil became poorer (Henriksen and Breland, 2002). This might impede the colonization and growth of soil microorganisms and lower enzyme activities (Henriksen and Breland, 2002; Mann et al., 2014). Decreased P$_{dmc}$ indicated less N release from manure mineralization (Chen et al., 2014). As a result, Rh decreased when the percent of applied N as ON exceeded the optimum.

Additionally, Rh increased with increasing soil DOC concentration (Figs. 5d and 6h), which was higher in the treatments with higher ON addition rate (Fig. 2d). Thus, higher Rh in the ON-fertilized treatments was also likely due to more labile substrate supply to soil microorganisms. Because organic N and C were simultaneously added into soil by organic N fertilizers, it was hard to assess their separate effects on Rh. Studies focusing on soil microbial possesses after addition of various forms of N and/or C compounds at different addition rates are recommended in future studies to further address this issue.

Interestingly, the Q$_{10}$ of Rs and Rh were decreased by N fertilization, particularly by IN (Tables 1 and 3). Previous studies also reported lower Q$_{10}$ values of Rs and Rh after N fertilization (e.g. Zhai et al., 2017). On the contrary, the Q$_{10}$ of Ra was increased by N fertilization, which agreed with the results of Yan et al. (2010). Because there was no fertilization treatment effects on soil temperature, changes in Q$_{10}$
values of Rh and Ra were likely due to the shifts in metabolic processes of soil microorganisms and plant roots, respectively. In addition, alteration in soil microbial community structure and substrate quality by different fertilization regimes might also influence the $Q_{10}$ of Rh (Davidson et al., 2006).

It should be noted that although the root exclusion method is widely used to separate Rs components, it may cause some biases due to likely changes in soil microclimate conditions and alteration of microbial community structure and activity (Subke et al., 2006), and neglect of rhizosphere priming effect on SOC decomposition (Shahzad et al., 2015). Thus, cautions should be taken when extrapolating the contribution of Rh or Ra to Rs, and their responses to N fertilization observed here. It has been suggested that organic matter mineralization and its temperature sensitivity depend on the C quality (Lefèvre et al., 2014). Therefore, studies including more kinds of organic N fertilizers with different chemical compositions are needed to address their effects on Rs components and the $Q_{10}$ values. Additionally, Rs and its components generally have large spatio-temporal variation, which necessitates long-term and cross-site studies to further confirm our findings.

Fig. 6. Relationships between annual soil respiration (Rs), heterotrophic respiration (Rh) and annual mean soil ammonium (NH$_4^+$, a and e), nitrate (NO$_3^-$, b and f), extractable organic nitrogen (EON, c and g) or dissolved organic carbon (DOC, d and h) concentration among different treatments. Bars represent standard errors (n = 4).
4.3. Plant biomass and carbon balance

It was unexpected to observe that grain yield and aboveground biomass were significantly decreased by IN fertilizer addition alone and then gradually increased with increasing addition rate of ON fertilizer (Table 4). Moreover, grain yield and aboveground biomass decreased with increasing soil NH$_4$$^+$ and NO$_3$$^-$ concentration, in particular with the former (Fig. 7). The rain storm occurring on 30 July 2013 led to soil waterlogging for about one month (Fig. 1a). During this period, hypoxia condition might have formed in soil as indicated by large reductions in both Rh and Ra as discussed above. It has been acknowledged that hypoxia or anoxia, even lasting for a short period, can decrease leaf chlorophyll concentration, inhibit plant photosynthesis and reduce the shoot and root biomass (Bhattarai et al., 2005). Ashraf and Athar (1999) reported that the negative effect of waterlogging on maize was greater under higher N supply. After soil was waterlogged, a sharp increase of soil NH$_4$$^+$ was observed in each treatment, probably produced through the process of dissimilatory nitrate reduction to ammonium (Chen et al., 2016). High NH$_4$$^+$ can have toxicity to maize plant, particularly under the anaerobic condition (Britto and Kronzucker, 2002). Manipulation experiment is necessary to get deep insights into the response of maize plant performance to NH$_4$$^+$ and NO$_3$$^-$ supply with a gradient rate under waterlogging condition.

The IN fertilization promoted $C_{\text{output}}$ in the form of Rh, and significantly decreased the $C_{\text{input}}$ as a result of lower plant biomass. Hence, the $C_{\text{storage}}$ was significantly lower in IN4 than CK (Table 4). In contrast, the ON-fertilized treatments exhibited higher $C_{\text{storage}}$ than CK mainly due to the application of exogenous organic C. The $C_{\text{storage}}$ increased from 120 to 296 g C m$^{-2}$ yr$^{-1}$ with increasing ON fertilizer application rate. Similarly, based on a meta-analysis, Aguilera et al. (2013) showed that the increase in SOC storage linearly increased with the increasing applying amounts of C inputs. Considering large uncertainties existing in our estimation of C budget with this one-year experiment, multi-year study is needed to make a direct evaluation of the changes in SOC storage under different N fertilization regimes.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Aboveground biomass (kg ha$^{-1}$)</th>
<th>$C_{\text{input}}$</th>
<th>$C_{\text{storage}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Grain</td>
<td>Straw</td>
<td>Total</td>
</tr>
<tr>
<td>CK</td>
<td>8033 ± 330 ab</td>
<td>8277 ± 341 b</td>
<td>16310 ± 671 ab</td>
</tr>
<tr>
<td>IN4</td>
<td>6920 ± 520 c</td>
<td>6662 ± 500 c</td>
<td>13582 ± 1020 c</td>
</tr>
<tr>
<td>IN3</td>
<td>7263 ± 513 bc</td>
<td>8015 ± 566 b</td>
<td>15278 ± 1080 bc</td>
</tr>
<tr>
<td>IN2</td>
<td>8152 ± 312 ab</td>
<td>8254 ± 315 b</td>
<td>16406 ± 627 ab</td>
</tr>
<tr>
<td>IN1</td>
<td>8600 ± 251 a</td>
<td>9006 ± 263 ab</td>
<td>17606 ± 514 ab</td>
</tr>
<tr>
<td>IN0</td>
<td>8899 ± 389 a</td>
<td>9728 ± 425 a</td>
<td>18627 ± 815 a</td>
</tr>
</tbody>
</table>

Values are means ± SE (n = 4). Different letters within the same column indicate significant differences among treatments at $P < 0.05$.  

5. Conclusions

In conclusion, our study demonstrated that N fertilization increased Rh but not Ra from a cropland of northeast China. Rh increased with increasing soil EON and DOC, but can be inhibited by excessive NH$_4$$^+$ and NO$_3$$^-$ supply. Thus, a greater role of organic over inorganic N fertilizer application was observed in stimulating Rh. In contrast, inorganic N fertilization more effectively decreased the $Q_{10}$ of Rs and Rh and increased that of Ra than organic N. These results implied that the form and addition rate of applied N fertilizers played an important role on the effects of N fertilization on soil C cycling and its feedback to climate warming.

Fig. 7. Relationships between maize grain yield (a, b), aboveground biomass (c and d) and annual mean soil ammonium (NH$_4$$^+$) or nitrate (NO$_3$$^-$) concentration for different treatments.

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Table 4

Aboveground biomass, total carbon input to soil ($C_{\text{input}}$), and the increase in soil organic carbon storage ($C_{\text{storage}}$) in different fertilization treatments.
plantation on peat respond to nitrogen fertilizer application? Geoderma 268, 41–51.
respiration regardless of the form of nitrogen applied. Soil Biology and Biochemistry 42, 2333–2342.


