



# Soil autotrophic and heterotrophic respiration in response to different N fertilization and environmental conditions from a cropland in Northeast China



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## ABSTRACT

Partitioning soil respiration ( $R_s$ ) into its heterotrophic ( $R_h$ ) and autotrophic ( $R_a$ ) components is crucial to evaluate the effects of inorganic and organic nitrogen (N) fertilization on carbon (C) cycling in agricultural ecosystems. We carried out a field experiment in a maize cropland in Northeast China using the root exclusion method to separate  $R_h$  and  $R_a$ , and investigate their responses to different fertilization regimes. These included no N fertilization (CK), inorganic N fertilizer (NPK), 75% urea N plus 25% pig (PM1) or chicken (CM1) manure N, and 50% urea N plus 50% pig (PM2) or chicken (CM2) manure N. Annual  $R_s$  was significantly increased from 314 g C m<sup>-2</sup> in CK to 389, 366, and 371 g C m<sup>-2</sup> in NPK, CM1, and PM2, respectively, and further to 420 g C m<sup>-2</sup> in PM1, whereas a similar value to CK was observed in CM2 (327 g C m<sup>-2</sup>). N-induced increases in  $R_s$  were largely attributable to the response of  $R_a$  (except CM2), which increased by 18–54% due to higher nitrate supply.  $R_h$  increased from 183 to 192–209 g C m<sup>-2</sup> in plots receiving N fertilizer, with significant increases observed in PM1 and PM2, likely due to the high ammonium and labile organic C concentrations in these treatments. Manure type and application rate had significant effects on  $R_s$  and  $R_a$ , but not  $R_h$ . Compared with CM, PM was more effective in stimulating  $R_a$  due to its greater decomposability.  $R_s$  and  $R_a$  decreased in the order of PM1 > PM2 and CM1 ≥ CM2, presumably because of the lower inorganic N supply with increasing manure application rate. The estimated C sequestration rate shifted from negative in CK and NPK to positive in the manure treatments, especially in PM2 and CM2 that gained 0.44 and 0.49 Mg C ha<sup>-1</sup> yr<sup>-1</sup>, respectively. These results suggested that combined application of half inorganic N plus half organic N might have potential to enhance soil C sequestration in cropland of Northeast China.

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## 1. Introduction

Soil respiration ( $R_s$ ), i.e., the carbon dioxide (CO<sub>2</sub>) efflux from soil, is estimated at 50–98 Pg C yr<sup>-1</sup> and is the second largest carbon (C) flux after photosynthesis between terrestrial ecosystems and the atmosphere (Bond-Lamberty and Thomson, 2010; Xu and Shang, 2016). Therefore, even small changes in  $R_s$  are expected to have a large impact on the global C cycle and its feedbacks to climate change (Schlesinger and Andrews, 2000). The large uncertainty in  $R_s$  estimation is partly because  $R_s$  is regulated by multiple abiotic and biotic factors, such as soil temperature,

moisture, nutrient availability, and plant productivity (Hanson et al., 2000; Davidson et al., 2006; Xu and Shang, 2016). Furthermore,  $R_s$  consists of two main components, heterotrophic ( $R_h$ ) and autotrophic ( $R_a$ ) respiration, which respond differently to changes in influencing factors (Bond-Lamberty et al., 2004; Savage et al., 2013; Chen et al., 2014a).  $R_h$  is derived from the decomposition of soil organic matter (SOM) and plant residues, which depends on the activity of soil microbial communities and labile substrate availability (Whitaker et al., 2014; Ding et al., 2016).  $R_a$  includes respiration by live roots and heterotrophic respiration from rhizosphere microorganisms, and is primarily regulated by the root activity and plant photosynthate supply (Tang et al., 2005; Vargas et al., 2011).

Among the various environmental factors, soil temperature and moisture are generally acknowledged as the dominant drivers of  $R_s$

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due to their direct effects that alter the activities of soil microbes and plant roots, and indirect effects through changing substrate supply and plant growth (Reichstein et al., 2003; Davidson et al., 2006; Wan et al., 2007). However, many studies have shown that soil temperature (e.g., Li et al., 2013a; Matteucci et al., 2015) or moisture (e.g., Zimmermann et al., 2010; Pang et al., 2013) have minor effects on Rs. These inconsistent results are likely related to the various responses of Rh and Ra. For example, Ra has been found to be more sensitive to variations in soil temperature and moisture than Rh (Mäkiranta et al., 2008; Zhang et al., 2013). Balogh et al. (2016) reported that Ra was repressed by drought more than Rh in a grassland in central Europe. In contrast, other studies demonstrated that Rh was more strongly controlled by soil temperature and moisture than Ra, which was more tightly coupled with plant physiological activity (Hartley et al., 2007; Li et al., 2010). Baldocchi et al. (2006) found that the summer rain events could cause a pulse in the flux of Rh but not Ra. Thus, partitioning Rs into its components and assessing their responses to soil temperature and moisture are essential to improve our mechanistic knowledge and model prediction of Rs under various environmental conditions and management practices (Subke et al., 2006; Hartley et al., 2007; Hopkins et al., 2013).

Nitrogen (N) enrichment can have profound effects on C cycling in terrestrial ecosystems (Janssens and Luysaert, 2009). Although numerous experiments have been conducted, the impact of N addition on Rs is still debated in the literature, mainly due to the different responses of each of the Rs components (Janssens et al., 2010; Zhou et al., 2014). N fertilization can stimulate Ra due to increased plant productivity (Cleveland and Townsend, 2006; Tu et al., 2013), and can enhance Rh as a result of higher soil microbial biomass or activity (Gong et al., 2012; Xu et al., 2016). In contrast, Phillips and Fahey (2007) found that Ra was lower after fertilization because of a reduction in root biomass and mycorrhizal colonization in a hardwood forest soil. It was suggested that belowground C allocation might be reduced after N addition as a result of decreased C and energy costs of foraging for nutrients (Olsson et al., 2005). Likewise, N addition may reduce Rh by suppressing soil microbial biomass and oxidative enzyme activities, promoting recalcitrant compound formation or inducing soil acidification (Treseder, 2008; Janssens et al., 2010). Therefore, it is essential to investigate the different responses of Rh and Ra to N addition to fully evaluate and understand soil C dynamics with increasing N inputs.

Croplands store more than 10% of global soil organic C (SOC) and have a large potential for C sequestration and climate change mitigation (Jobbágy and Jackson, 2000; Amundson et al., 2015). Cultivated Mollisols of Northeast China are highly fertile and cover ~300 000 km<sup>2</sup> of farmland that is largely devoted to grain production, but these soils have experienced significant SOC loss in the past decades (Sun et al., 2010). Application of organic fertilizer along with inorganic N fertilizer has been recommended to increase SOC content and simultaneously ensure N supply to crop growth (Ding et al., 2012). Organic amendments have been widely demonstrated to increase Rs (e.g., Brye et al., 2006; Ding et al., 2007b; McMullen et al., 2015). However, Mejjide et al. (2010) showed that organic fertilizers could increase, decrease, or have no effect on Rs depending on fertilization types. Brye et al. (2006) revealed that Rs was unaffected by the form of poultry litter, but generally affected by its application rate. Conversely, Li et al. (2013b) pointed out that the impact of organic input on Rs was primarily related to the type of organic amendments but not the application rate. It is very likely that the composition and application rate of organic materials can exert an impact on C decomposition and N mineralization, and thus microbial substrate availability and plant N supply (Romanyà et al., 2012; Weber et al.,

2014). As a consequence, the heterotrophic and autotrophic component of Rs will be altered, which may complicate the responses of total Rs. However, evidence of the effects of organic amendments on Rh and Ra is scarce.

The root exclusion method has been widely used to separate Rh and Ra because it is easier and cheaper to implement compared with other methods, such as the isotopic (<sup>13</sup>C or <sup>14</sup>C) technique (Hanson et al., 2000; Hopkins et al., 2013). Nevertheless, this method may underestimate Rh because organic C inputs to soil from roots are excluded and the rhizosphere priming effect on SOM decomposition is absent. In addition, soil temperature and moisture may be altered due to the lack of roots. Any method to partition Rs has unavoidable biases (Subke et al., 2006) but uncertainty associated with Rs components could be lower when roots are excluded without disturbance of the soil environment, such as by leaving unplanted areas in cropland (Suleau et al., 2011). In the present study, we conducted a field experiment to measure the annual fluxes of Rs, Rh, and Ra with the root exclusion method, from a cropland under different fertilization regimes. The main aims were to investigate how Rs and its components respond to the variation of environmental factors, specific to soil temperature and moisture, and combined application of inorganic N fertilizer and manure with different types and application rates.

## 2. Material and methods

### 2.1. Study site

This study was conducted in a rainfed maize-cultivated cropland at the Hailun National Agro-ecological Experimental Station, Heilongjiang Province, China (47°26'N, 126°38'E). The climate of this region is temperate sub-humid and is influenced by the continental monsoon, characterized by a short hot summer and long cold winter. From 1953 to 2013, the mean annual air temperature was 1.9 °C, and the monthly mean air temperature was lowest in January (−21.6 °C) and highest in July (21.6 °C). The long-term mean annual precipitation is 556 mm, of which 87% occurs during the crop growing season from May to October. Prior to the establishment of our experiment, the field was continuously cultivated under a maize-soybean rotation. The soil is derived from loamy loess and classified as Typic Hapludoll in the USDA soil taxonomy. The soil texture is clay loam with 8% sand, 72% silt, and 20% clay. The bulk density is 1.0 g cm<sup>−3</sup>, and other soil properties are listed in Table 1.

### 2.2. Experimental design

In May 2011, eighteen plots (12 m × 4.2 m) were established in three blocks (= replicates). Six treatments were assigned randomly per block: no N fertilizer as control (CK), 100% urea N (NPK), 75% urea N plus 25% pig (PM1) or chicken (CM1) manure N, and 50% urea N plus 50% pig (PM2) or chicken (CM2) manure N (Table S1). The N fertilizer application rate was 150 kg N ha<sup>−1</sup> in each fertilized treatment. As a common practice, N fertilizers were split into pre-planting and post-emergence fertilization with a ratio of 1:1 for N application rate. In pre-planting fertilization, 75, 37.5, and 37.5 kg N ha<sup>−1</sup> urea was applied for NPK, PM1, and CM1, respectively; manure was applied at 37.5 kg N ha<sup>−1</sup> for PM1 and CM1, and 75 kg N ha<sup>−1</sup> for PM2 and CM2. For the post-emergence fertilization, 75 kg N ha<sup>−1</sup> urea was side-dressed in all fertilized treatments. Organic fertilizers were obtained from commercial companies and their main characteristics are given in Table 1. Ammonium (NH<sub>4</sub><sup>+</sup>) and nitrate (NO<sub>3</sub><sup>−</sup>) content in the manure were measured using the MgO-Devarda alloy distillation method (Lu, 2000). The hemicellulose (HEM), cellulose (CEL), and lignin (LIG) fractions were

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