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Soil Biology and Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

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

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ARTICLE INFO

Keywords: Autotrophic respiration Heterotrophic respiration Inorganic nitrogen Organic nitrogen Q¹⁰ value Soil respiration

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 (2.67–3.28) had higher Q₁₀ values than Ra (1.51–2.05). Application of N fertilizer, especially IN, enhanced the Q₁₀ 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.

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\)](#page--1-0). Global carbon (C) and N cycles are tightly coupled, and thus the widespread N enrichment can considerably alter the terrestrial C cycle [\(Gruber and Galloway, 2008\)](#page--1-1). Soil respiration (Rs), i.e., soil carbon dioxide (CO₂) flux, is estimated at 98 \pm 12 Pg C yr−¹ and is the largest C flux from terrestrial ecosystems to the atmosphere ([Bond-Lamberty and Thomson, 2010](#page--1-2)). 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](#page--1-3)). Cropland contains ∼10% of global soil organic C (SOC) and has a large potential in C sequestration ([Lal, 2004](#page--1-4)). Meanwhile, 60% of global anthropogenic reactive N is input to agricultural soils through nitrogenous fertilizers application [\(Galloway et al., 2008\)](#page--1-0). 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\)](#page--1-5). 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;](#page--1-6)

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<http://dx.doi.org/10.1016/j.soilbio.2017.10.029>

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Received 26 June 2017; Received in revised form 23 October 2017; Accepted 24 October 2017 0038-0717/ © 2017 Elsevier Ltd. All rights reserved.

[Comeau et al., 2016\)](#page--1-6).

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 [\(Janssens et al., 2010; Zhou et al.,](#page--1-7) [2016\)](#page--1-7). For example, [Tu et al. \(2013\)](#page--1-8) found that N addition stimulated Rs as a result of increases in both Rh and Ra. [Comeau et al. \(2016\)](#page--1-9) showed that N fertilizer application enhanced Rs due to the increase of Rh but not Ra. While, [Yan et al. \(2010\)](#page--1-0) 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. \(2005\).](#page--1-10) However, other researchers suggested that the decreased Rs by N supply was mainly because of the reduction of Rh ([Burton et al., 2004](#page--1-11)) or Ra [\(Sun](#page--1-12) [et al., 2014\)](#page--1-12). 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](#page--1-13)). The temperature sensitivity of Rs is commonly assessed by the Q_{10} value, the factor by which Rs increase with a 10 °C temperature rise ([Davidson and](#page--1-14) [Janssens, 2006](#page--1-14)). Despite numerous studies, response of the Q_{10} value of Rs to N addition remains controversial, and little is known on the effect of N fertilization on the Q_{10} of Rs components ([Sun et al., 2014](#page--1-12)).

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](#page--1-15)). [Zhai et al.](#page--1-16) [\(2017\)](#page--1-16) 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](#page--1-17)). However, excessive N loading may inhibit soil extracellular enzyme activities and fungi biomass, resulting in a decrease in Rh (Maaroufi [et al., 2015; Jian](#page--1-18) [et al., 2016](#page--1-18)), and may reduce the belowground allocation of plant C, root biomass and thus Ra [\(Högberg et al., 2010; Hasselquist et al.,](#page--1-19) [2012\)](#page--1-19). [Du et al. \(2014\)](#page--1-20) and [Li et al. \(2014\)](#page--1-21) 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. \(2017a\)](#page--1-22) 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](#page--1-16) [Kuzyakov, 2008; Jian et al., 2016\)](#page--1-16). However, [Ramirez et al. \(2010\)](#page--1-23) 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\)](#page--1-24). 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.,](#page--1-3) [2006\)](#page--1-3). 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 Agroecological 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 NH $_4\,+$, NO_3 ⁻, and DOC concentrations were measured as described below.

2.2. Experimental design

The experiment used a complete random block design with six treatments replicated four times. Twenty-four 4.2 \times 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 ONfertilized 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 NH_4 $^+$ 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 \times 2.0 m² in each plot was left unplanted. Preemergence 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 aboveground 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.

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