



# Enhanced gross nitrogen transformation rates and nitrogen supply in paddy field under elevated atmospheric carbon dioxide and temperature



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## ARTICLE INFO

### Article history:

Received 18 April 2015

Received in revised form

20 November 2015

Accepted 27 November 2015

Available online 20 December 2015

### Keywords:

Elevated carbon dioxide

Gross N transformation

<sup>15</sup>N tracer model

Rice field

Global warming

## ABSTRACT

Climate change, particularly the combined effects of elevated CO<sub>2</sub> and temperature, is likely to alter the internal nitrogen (N) cycle of agricultural ecosystems. However, little is known about such phenomena in paddy soils, which are expected to expand in the near future due to population increase. A <sup>15</sup>N tracer study, with soil taken from field manipulation treatments, showed that elevated CO<sub>2</sub>, either alone or combined with elevated temperature, stimulated the mineralization of labile organic N 37-fold but decreased the mineralization of recalcitrant organic N. In contrast, elevated temperature alone accelerated the mineralization of recalcitrant organic N approximately 2-fold but had no effect on the mineralization of labile organic N. Ammonium immobilization increased under elevated CO<sub>2</sub> and elevated temperature. Gross and net NO<sub>3</sub><sup>-</sup> production decreased under elevated CO<sub>2</sub> and the combined treatments, whereas elevated temperature caused an increase in both rates. Dissimilatory reduction of NO<sub>3</sub><sup>-</sup> to NH<sub>4</sub><sup>+</sup> increased under elevated CO<sub>2</sub> but decreased with elevated temperature. Our findings suggest that progressive N limitation can be alleviated by increasing gross N transformation rates under each climate change treatment and that counteraction will dominate the interactive responses of CO<sub>2</sub> and temperature. Because we expect a concomitant increase in both CO<sub>2</sub> and temperature, we only expect minor effects of these particular factors arising as a result of climate on soil N dynamics in paddy soils.

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## 1. Introduction

Global rice production has increased from 115.4 Mha in 1961 to 165 Mha in 2013 (FAOSTAT, 2013) and is expected to expand further upon population increase. Elevated atmospheric carbon dioxide (CO<sub>2</sub>) generally increases rice yield by increasing grain mass and the number of panicles and grains (Ainsworth, 2008). However, due to increased carbon (C) flow into the rhizosphere, nitrogen (N) consumption by microbes and plants can also be enhanced and can possibly lead to progressive nitrogen limitation (PNL) in the long term (Luo et al., 2004), resulting in even more adverse effects on growth and yield. External N inputs have been suggested to remediate the potential PNL for the sustainable growth of plants

(Luo et al., 2004). Because large amounts of N fertilizer input into paddy fields has caused serious environmental issues (Ju et al., 2009), a suitable N management plan that takes into account the effect of climate change on the potential PNL in paddy fields would be required. In China, flooded rice is typically cultivated in a rotation scheme with upland crops such as wheat. Because of the dramatically changing soil moisture conditions throughout the cultivation period, it is expected that the underlying microbially mediated soil N dynamics in paddy fields are quite different compared to those in upland ecosystems (Cookson et al., 2007). Although previous limited field studies showed that PNL might be alleviated in upland ecosystems such as grassland (Müller et al., 2009; Rütting et al., 2010) and heathland ecosystems (Björnsne et al., 2014) by increasing soil organic matter (SOM) mineralization rates, the response of soil N dynamics in paddy fields to climate change has not been studied so far.

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To understand actual N transformation processes under global climate change scenarios, it is important to quantify individual gross N transformation rates because the gross rather than the net N transformation rates reflect the actual N transformation process (Murphy et al., 2003). The most widely used method for quantifying gross N transformation rates is the  $^{15}\text{N}$  pool dilution technique (Stark, 2000). The theory for calculating gross transformation rates for N mineralization, nitrification and N immobilization was first described by Kirkham and Bartholomew (1954). Recently, numerical  $^{15}\text{N}$  tracer models (e.g., Müller et al., 2007) have been developed that consider the simultaneous individual N rates, such as mineralization and immobilization of labile and recalcitrant organic N pools, autotrophic and heterotrophic nitrification, and dissimilatory reduction of  $\text{NO}_3^-$  to  $\text{NH}_4^+$  (DNRA) (Rütting and Müller, 2007).

Realistic climate change scenarios should consider both elevated  $\text{CO}_2$  and elevated temperature because they are expected to increase concurrently (Dieleman et al., 2012). Several meta-analyses have highlighted that elevated  $\text{CO}_2$  increases gross immobilization (Hu et al., 2006; de Graaff et al., 2006) and net nitrification but does not affect gross nitrification (Barnard et al., 2005), net mineralization or gross mineralization (de Graaff et al., 2006). However, elevated temperature has been shown to increase both net mineralization (Rustad et al., 2001; Bai et al., 2013) and net nitrification (Bai et al., 2013) but not gross nitrification and immobilization (Bai et al., 2013). Thus, a realistic estimate of how N dynamics will change under future climate conditions should consider the interactive effects of elevated  $\text{CO}_2$  and increasing air temperature. The effects of elevated  $\text{CO}_2$  and temperature on microbial communities and soil processes can result in either counteractive or additive effects, as shown in temperate agricultural soils, for instance (French et al., 2009). Furthermore, a clear counteractive effect between elevated atmospheric  $\text{CO}_2$  and increased air temperature on soil N processes was shown in a Danish heathland (Larsen et al., 2011). Thus, the interactive effects of climate change factors on soil N dynamics call for specific research in paddy fields, as paddy fields experience different water regimes than other agricultural fields.

Based on the above-mentioned studies, we tested the following hypotheses: (1) PNL will be alleviated in paddy fields under climate changes as a result of enhanced gross N transformation rates, and (2) elevated temperature would counteract the elevated  $\text{CO}_2$  impact on soil gross N transformations in paddy fields. To evaluate these hypotheses, we performed a  $^{15}\text{N}$  tracer laboratory study to determine gross N transformation rates in a multifactor Free Air Carbon dioxide Enrichment and elevated Temperature (T-FACE) experiment within an open paddy field in central eastern China.

## 2. Materials and methods

### 2.1. Site description and field experiment

Since 2011, a Free Air Carbon dioxide Enrichment and elevated Temperature system (T-FACE) field study has been conducted within an annual rice–wheat rotation. The field site is situated in Changshu County (31°30' N, 120°33' E), Jiangsu Province, China (Liu et al., 2014), an important crop region for summer rice (from early June to November) and winter wheat (from November to late May). The soil type is a Gleyic-Stagnic Anthrosol (WRB-FAO) developed from lake sediments. Located in the center of the Tai Lake plain, the area has a subtropical monsoon climate with a mean annual temperature of 16 °C and annual precipitation of 1100–1200 mm during 2004–2013. The properties of the topsoil before the onset of the study in 2011 were as follows: a soil pH (1:2.5  $\text{H}_2\text{O}$ ) of 7.0, an organic C content of 16 g  $\text{kg}^{-1}$ , a total N content of 1.9 g  $\text{kg}^{-1}$ , and a bulk density of 1.2 g  $\text{cm}^{-3}$ .

The T-FACE system included four treatments, each with three replicates (i.e., 12 rings, each with a 50  $\text{m}^2$  area). Treatments included an ambient control and three global climate change scenarios: (1) elevated atmospheric  $\text{CO}_2$  concentration of 500  $\mu\text{mol}/\text{mol}$  (EC), (2) canopy air temperature elevated by 2 °C (ET) and (3) elevated  $\text{CO}_2$  combined with elevated temperature (ECT). Each ring was surrounded by an appropriate buffer strip planted with rice. For the EC and ECT treatments, the atmospheric  $\text{CO}_2$  concentration was set to 500  $\mu\text{mol}/\text{mol}$ , which corresponds to the atmospheric concentration in approximately 30–40 years, depending on the RCP scenario (IPCC, 2007). Pure  $\text{CO}_2$  from a liquid tank was injected into the ring plot through perforated pipes that surrounded the ring. The consistency of the  $\text{CO}_2$  concentration throughout the ring was controlled with automatic adjustments for wind direction and velocity. Li-COR  $\text{CO}_2$  sensors and thermometers (Li-820, USA) were installed both over the canopy and around the ring to control the release of  $\text{CO}_2$  and the heating of the canopy air, respectively. To elevate the temperature in the ET and ECT treatments, four infrared lights were set over each ring to heat the air uniformly. The observed near-canopy increase in air temperature was  $2 \pm 0.4$  °C for the ET and ECT plots, and the enriched atmospheric  $\text{CO}_2$  concentration was  $505 \pm 26$   $\mu\text{mol}/\text{mol}$  for the EC and ECT plots.

The management practices followed local conventional crop practices and were kept the same for all four treatments. The water regime was as follows: flooding from the rice seedling to tilling stages, intermittent irrigation during the rice heading stage and drained conditions for rice ripening, while no irrigation receiving only precipitation during the period of wheat growth.

### 2.2. $^{15}\text{N}$ tracer study

Soil was collected from the plowed layer (0–20 cm) in each T-FACE ring after the harvest of the winter wheat crop in May 2014 (i.e., after three cycles of the rice–wheat rotation). The physico-chemical properties of the soils from each treatment are presented in Table 1. Soils from the three replicates were homogenized and subsequently split into triplicate composite samples. Fresh soil was partially air-dried for one day, sieved through a 2 mm mesh and stored at 4 °C for about one month until the start of the incubation experiment.

Following Zhang et al. (2011), the equivalent of 30 g of oven-dried soil was added to each 250 mL Erlenmeyer flask and was pre-incubated at 25 °C for 24 h before the addition of the  $^{15}\text{N}$  tracer. There were two batches of  $\text{NH}_4\text{NO}_3$  tracer incubation: in the first batch, the  $\text{NH}_4^+$  pool was labeled using  $^{15}\text{NH}_4\text{NO}_3$  at 10.28 atom% excess, and in the second batch, the  $\text{NO}_3^-$  pool was labeled using  $\text{NH}_4^{15}\text{NO}_3$  at 10.23 atom% excess. A total of 96 flasks (4 field climate change treatments  $\times$  2  $^{15}\text{N}$  batches  $\times$  3 reps  $\times$  4 times) were prepared so that soil could be extracted at 1, 24, 48 and 72 h after  $^{15}\text{N}$  labeling. A 2 mL volume of the  $^{15}\text{NH}_4\text{NO}_3$  or  $\text{NH}_4^{15}\text{NO}_3$  solution was uniformly applied using a pipette over the soil surface in each flask to obtain a final concentration of 100 mg N  $\text{kg}^{-1}$  dry soil (50 mg N  $\text{kg}^{-1}$  as  $\text{NH}_4^+$  and 50 mg N  $\text{kg}^{-1}$  as  $\text{NO}_3^-$ ). Subsequently, distilled water was sprayed onto the soil to adjust the water content to 60% water-holding capacity. Finally, all flasks were sealed with screw-on gas-tight PVC lids to prevent water loss, and flasks were then incubated in the dark at 25 °C for 72 h. Each day, the jars were opened for 15 min for aeration. The flasks were weighed daily, and any water lost by evaporation was replaced.

### 2.3. Sample analyses

Following Lu (2000), the SOM content was determined using wet digestion with  $\text{H}_2\text{SO}_4\text{--K}_2\text{Cr}_2\text{O}_7$ , and the total N content was determined using semi-micro Kjeldahl digestion and Nessler's

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