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Increasing atmospheric deposition nitrogen and ammonium reduced microbial activity and changed the bacterial community composition of red paddy soil

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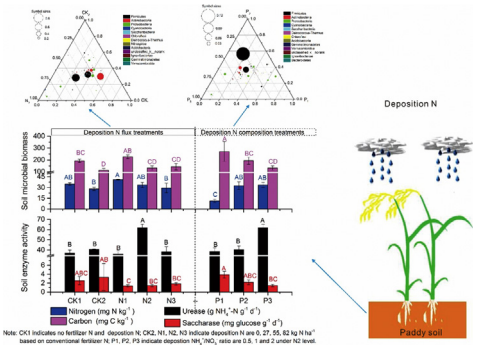
HIGHLIGHTS

- Paddy soil microbial responses to nitrogen deposition were done for the first time.
- Soil bacteria had stronger answers to deposition nitrogen form rather than flux.
- Increased NH_4^+ composition altered soil microbial biomass, diversity and community.
- Deposition nitrogen flux and composition affected *Dyella* and *Rhodoblastus*, respectively.
- The possible threshold was 55 kg N ha^{-1} and key point of NH_4^+/NO_3^- ratio was 1:1.

GRAPHICAL ABSTRACT



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ABSTRACT

Atmospheric deposition nitrogen (ADN) increases the N content in soil and subsequently impacts microbial activity of soil. However, the effects of ADN on paddy soil microbial activity have not been well characterized. In this study, we studied how red paddy soil microbial activity responses to different contents of ADN through a 10-months ADN simulation on well managed pot experiments. Results showed that all tested contents of ADN fluxes (27, 55, and 82 kg N ha^{-1} when its ratio of NH_4^+/NO_3^--N (R_N) was 2:1) enhanced the soil enzyme activity and microbial biomass carbon and nitrogen and 27 kg N ha^{-1} ADN had maximum effects while comparing with the fertilizer treatment. Generally, increasing of both ADN flux and R_N (1:2, 1:1 and 2:1 with the ADN flux of 55 kg N ha^{-1}) had similar reduced effects on microbial activity. Furthermore, both ADN flux and R_N significantly reduced soil bacterial alpha diversity ($p < 0.05$) and altered bacterial community structure (e.g., the relative abundances of genera *Dyella* and *Rhodoblastus* affiliated to *Proteobacteria* increased). Redundancy analysis demonstrated that ADN flux and R_N were the main drivers in shaping paddy soil bacteria community. Overall, the results have indicated that increasing ADN flux and ammonium reduced soil microbial activity and changed

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the soil bacterial community. The finding highlights how paddy soil microbial community response to ADN and provides information for N management in paddy soil.

1. Introduction

Human activities associated with industrialization, urbanization and agriculture have greatly altered global nitrogen (N) cycle and atmospheric N deposition (ADN) (Galloway et al., 2004; Vet et al., 2014). Increased ADN could have negative impacts on air quality, human health, and ecosystem (Wang et al., 2013; Cui et al., 2014a; Vet et al., 2014; Boutin et al., 2017). Generally, ADN was reported to have a higher NH_4^+-N/NO_3^--N ratio (R_N) in the world and an increasing trend in Asia (Larssen et al., 2011; Kros et al., 2015; Vet et al., 2014; Kanakidou et al., 2016; WallisDeVries and Bobbink, 2017). China has been witnessed severe atmospheric N pollution since late 1970s and has become a global ADN hotspot (Hu et al., 2010; Liu et al., 2011; Vet et al., 2014). Based on the satellite-retrieved data of NH_3 and NO_2 columns, the R_N was increasing during the period of 2011–2014 (Liu et al., 2017). It is therefore important to study ADN especially for the R_N increase on soil microorganisms to support improved predictions of carbon (C) and N cycling.

To date, most studies have been focusing on ADN impacts on natural and semi-natural ecosystems such as forest, grass and aquatic systems (Clark and Tilman, 2008; Janssens et al., 2010; Larssen et al., 2011; Zhao et al., 2013; Xiong et al., 2016), however the impacts on agricultural ecosystems are little studied for substantial use of N fertilizers (Liu et al., 2006; Cui et al., 2015). In fact, only wet deposition inorganic N in agricultural regions of Eastern and Southern China have reached 30–40 kg N ha^{-1} yr^{-1} (Liu et al., 2013; Cui et al., 2014a), exceeding the critical loads (25 kg N ha^{-1} yr^{-1}; Kim et al., 2009). Our group have found that the increase of ADN including the R_N induced soil pH values in a dryland of Southeastern China (Cui et al., 2014a, 2015). It is well know that microorganisms and enzymes are sensitive to soil pH (Nicol et al., 2008; Dimitriu and Grayston, 2010; Shen et al., 2013). Zhang et al. (2017), from a forest field experiment over four years, suggested that microorganisms and enzymes were more affected by NH_4^+-N than those by NO_3^--N in ADN. Also, other studies have showed that N form has strongly affected soil enzyme activities and microbial community (Hofmockel et al., 2007; Guo et al., 2011a; Li et al., 2014; Wang et al., 2017b; Xiao et al., 2017), but this has not been a case in agro-ecosystems (Wang et al., 2013; Wang et al., 2017a). Thus, it is necessary to know how ADN especially for its composition affects microorganisms and enzymes in farmland ecosystems.

China has always been an agricultural country and rice has been cultivated in China for >5000 years. Early studies almost have paid more attention to rice yield factors especially for N fertilizers (Yan et al., 2003; Guo et al., 2010). In fact, ADN also is an important N input factor in paddy soil ecosystems. Wang (2007) found that ADN was the second largest factor of N input in a subtropical paddy soil. However, microbiological properties of paddy soil in response to increased N flux and R_N of ADN is little studied and remains largely unknown. In the subtropical region including parts of Southern and Eastern China, there are experiencing increased ADN and the R_N (Cui et al., 2012, 2014a). To facilitate an exploration of the effects of ADN flux and its R_N on the paddy soil, we established relevant experiments including plot and field in Jiangxi province, a typical rice-cultivated red soil region. As a primary agricultural region, Jiangxi province in southern China has total agriculture lands of 1.08×10^6 ha (Liu and He, 1991) with primary red soil ecosystem (major soil system in southern China) and a long history of rice planting. In 2014, rice planting area ranked the second and total rice output ranked the third in China, with a steady increase of paddy field during the period of 2004–2014 (Guan et al., 2017).

In this study, we made a pot experiment to examine the effects of ADN flux and its composition on soil enzymatic activity, bacterial community composition and diversity in the red paddy soil of southeastern China, further for understanding the ADN agro-ecological effects and regional N management in rice-cultivated region.

2. Materials and methods

2.1. Experimental procedures

A red paddy topsoil (0–20 cm) was collected into one field in Qiujia County (117°14′51″E, 28°20′34″N) of Yingtan city of Jiangxi province, Southern China. The collected soil was air dried, mixed thoroughly, passed through a 2-mm sieve, and placed into a plastic pot (25 cm diameter, 30 cm high) under a plastic roof in 2016. The selected topsoil physiochemical properties are shown in Table 1.

Based on long-term monitoring data during 2004–2011 (Cui et al., 2012, 2014b), average of annual precipitation, rainfall pH, ADN and R_N in the study region were 1650 mm, 4.5, 31 kg N ha^{-1} yr^{-1} and 2.5, respectively, and the bulk dry inorganic N deposition flux was estimated at 92 kg N ha^{-1} yr^{-1} (Cui et al., 2009). The experiment consisted of treatments: 1) three R_N of 1:2, 1:1 and 2:1 under with constant simulated ADN of 60 kg N ha^{-1} yr^{-1} ((P_1-P_3) and 2) three simulated ADN of 30, 60 and 90 kg N ha^{-1} yr^{-1} N under constant R_N of 2:1 (N_1-N_3). Two controls included no fertilizer N (CK_1) and conventional N application (CK_2, 100 kg ha^{-1} N). All treatment pots were arranged in a randomized complete block design with three replications of each and received same conventional rate of N application as CK_2.

The ADN solution was prepared at pH 4.5 from $^{15}NH_4Cl$, $^{15}NH_4^{15}NO_3$, $Na^{15}NO_3$, $CaSO_4$, $MgSO_4$, K_2SO_4, $CaCl_2$, Li_2SO_4, HCl and DI (deionized) water. The abundance of N isotopes was 10% in the ^{15}N-labelled chemicals. In addition, the solutions for CK_1 and CK_2 control were the same and prepared at pH 4.5 from only $CaSO_4$, $MgSO_4$, K_2SO_4, $CaCl_2$, Li_2SO_4, HCl and DI water. The ADN solution was added into a sprayer at the middle of each month with total 16.5 L per year. The corresponding monthly solution added were 0.80, 1.30, 1.85, 2.35, 2.80, 2.80, 0.90, 1.20, 0.60, 0.45, 0.80 and 0.65 L from January to December, respectively. The monthly amounts of ADN simulated per treatment are shown in Table 2. During the period of ten-month experiment, the ADN input was 27, 55 and 82 kg N ha^{-1} yr^{-1} for N_1, N_2 and N_3, respectively.

2.2. Samples collections and analysis

Red paddy soils (0–20 cm) were collected on 6th November 2017 post ten-month experiment, and then stored at 4 °C immediately and analyzed within one week after vacuum freeze-drying.

2.2.1. Soil microbial biomass

Soil microbial biomass carbon (MBC) and nitrogen (MBN) were measured using the chloroform-fumigation extraction method (Brookes et al., 1985). Soil was suspended in 0.5 mol L^{-1} potassium sulfate solution, shaken for 30 min at 300r min^{-1}, and filtered through a 0.45um membrane (JinTeng Inc., TianJing, China). The extracted solutions were determined for dissolved organic carbon and nitrogen (DOC and DON) by a multi N/C 3100 analyzer (AG. Ltd., Germany). Correction factors of MBC were 0.38 (Vance et al., 1987) while MBN was 0.45 (Brookes et al., 1985).