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# Dissolved organic nitrogen distribution in differently fertilized paddy soil profiles: Implications for its potential loss



San'an Nie<sup>a,b,1</sup>, Lixia Zhao<sup>b,1</sup>, Xiumei Lei<sup>b</sup>, Rubab Sarfraz<sup>b</sup>, Shihe Xing<sup>b,\*</sup>

<sup>a</sup> College of Life Sciences, Fujian Agriculture and Forestry University, Fuzhou, 350002, China

<sup>b</sup> College of Resources and Environment, Fujian Agriculture and Forestry University, Fuzhou, 350002, China

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#### ABSTRACT

Dissolved organic nitrogen (DON) is recognized as an important nitrogen (N) pool in soil N cycling, but its role in the N cycling of paddy soils, which are intensively fertilized, is not fully predicted. In this study, we investigated DON in flooded layer and soil solution along soil profiles with suction cups in fertilized paddy fields. The DON concentration showed a relative decrease in the deeper layer of paddy soil, while free amino acid N (FAA-N) exhibited a drastic increase along with nutrient profiles of soil. In the upper layer (0–20 cm), DON accounted for 54–64% of total dissolved N (TDN), but this value increased up to 63–97% in the deeper layer (40–60 cm). Low concentrations (9.6–15.0  $\mu$ g L<sup>-1</sup>) of FAA-N and low percentage of FAA-N/DON (0.1–0.2%) were observed in the upper layer, but higher concentrations (111–307  $\mu$ g L<sup>-1</sup>) and increased percentage (8–36%) were examined in the deeper layer. The high percentage of DON/TDN indicated that DON was the predominant N pool in the deeper layer. Concentrations of DON were significantly and positively correlated with organic matter, total N, and electrical conductivity (EC), while negatively related to soil pH. Additionally, capillary porosity, air porosity, bulk density and particle density were also found to be significantly associated with DON. We suggest the DON and FAA in the paddy field could be an important source for N leaching, which is most strongly related with soil nutrient profiles and physical properties. It is estimated that a total loss of 4.0 kg N ha<sup>-1</sup> yr<sup>-1</sup> is potentially linked to DON in the paddy field, which implied that ca. 3.35% of the applied N fertilizers could be lost via DON.

#### 1. Introduction

High loads of N fertilizers in agricultural fields cause various environmental problems and alter various ecosystems (Guo et al., 2010). With the rapid increasing use of various fertilizers in agricultural ecosystems, high N loss is becoming an important issue from both environmental and agronomic perspectives (Nie et al., 2015). Rice is a globally important food that grows on about 115 million hectare (ha) of the Earth's surface (Zhu et al., 2011). The N fertilizers have been applied intensively in paddy soils to increase rice yields, especially in China, which is one of the largest rice producing countries and consumers of N fertilizer in the word (FAO 2014, available at www.fao. org/publications/sofa.). However, the excessive use of N fertilizers has not only decreased the N use efficiency, but also resulted in large amounts of N pollution in atmosphere and water systems (Xing and Zhu, 2000; Zhao et al., 2009).

Most of the N input into the soil is in the form of polymers, which first have to be broken down into smaller units by enzymes (i.e., protease) (Schimel and Bennett, 2004; Geisseler et al., 2010). Of these, proteolysis is considered to be the rate-limiting step in both the release of amino acid and N mineralization (Weintraub and Schimel, 2005). The small organic N released by enzyme which is soluble in water and can pass through 0.45  $\mu$ m membrane is recognized as dissolved organic N (DON) (Westerhoff and Mash, 2002). DON is likely a complex mixture of N-containing organic compounds with a wide range of sources (Westerhoff and Mash, 2002). Despite the small proportion, DON is the most active chemical N component in soil (Nasholm et al., 1998). It can be inter-changed with other components of soil N under certain conditions, and is easy to be decomposed by microorganism which is an important source of soil nutrients (Perakis and Hedin, 2002). In comparison to inorganic N, DON often are the dominant N pool in many soils and freshwaters, representing a key component of biogeochemical cycles (Kalbitz et al., 2000).

At present, a considerable amount of research into DON have been conducted in marine (Guldberg et al., 2002; Berg et al., 2003; Guo et al., 2003; Somes and Oschlies, 2015; Dafner, 2016), freshwater ecosystems (Lawes, 1882; Sharp et al., 1995; Sharp et al., 2002; Westerhoff and Mash, 2002), and forest soil (Nasholm et al., 1998;

<sup>1</sup> These authors contributed equally to this work.

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<sup>\*</sup> Corresponding author.

E-mail address: fafushx@126.com (S. Xing).

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Perakis and Hedin, 2002; Yu et al., 2002; Hannam and Prescott, 2003). Recent studies have shown that DON is an important pool for N transformations and plant uptake (Turnbull et al., 1996; Murphy et al., 2000; Näsholm et al., 2000; Jones et al., 2004). Nevertheless, most of these studies are concerned with the role of DON in dry farmland ecosystems (Siemens and Kaupenjohann, 2002; Tian et al., 2010; Wohlfart et al., 2012). Few studies have addressed DON in paddy field, which could contribute to N loss in soil solution and drainage waters through runoff and leaching in agricultural soils (Lawes, 1882; Murphy et al., 2000). In particular, high N inputs in agricultural soils may increase the loss of DON (Murphy et al., 2000). So far most of the existing reports on N loss from the paddy soil were focused on NH<sub>3</sub> volatilization (Aves et al., 2011: Rochette et al., 2013: Feng et al., 2017), denitrification (Ni et al., 2007; van der Salm et al., 2007; Awale and Chatterjee, 2015; Yang et al., 2017), and newly found anaerobic oxidation of ammonium (Zhu et al., 2011; Nie et al., 2015; Yang et al., 2015). The role of DON in different fertilized paddy soil and its contributions to the N cycle are not well known at the moment.

Hence, the objectives of the present study were to investigate the role of DON, its relationship with soil property and the quantitative importance of DON in the N cycle of fertilized paddy fields in Southern China.

#### 2. Materials and methods

#### 2.1. Experimental location and background

A long-term fertilizer application experiment station, which was established in 1983, located in Basha town, Fuzhou city, China (119°04′10″ E, 26°13′31″ N) was selected for this study (Supplementary, Fig. S1). The soil was a grey yellow paddy soil formed from hilly red earth slope deposits. At the beginning of the experiment, the main chemical properties of the soil (0–20 cm) were as follows: pH (H<sub>2</sub>O 1:2.5), 4.90; total organic matter (measured by the K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>-H<sub>2</sub>SO<sub>4</sub> oxidation method), 21.6 g kg<sup>-1</sup>; alkali-hydrolysable N (alkaline-hydrolysis and diffusion method), 141 mg kg<sup>-1</sup>; available phosphorus (extracted by 0.5 M NaHCO<sub>3</sub>), 12 mg kg<sup>-1</sup>; and available potassium (extracted by 1 M NH<sub>4</sub>OAc (pH 7)), 41 mg kg<sup>-1</sup>. Double cropping of rice was planted during 1983–2004, after then single crop rice was induced.

Four treatments: control (CK), chemical fertilizer (NPK) (urea, super phosphate, and potassium chloride, respectively), manure combined chemical fertilizer (NPKM), and straw combined chemical fertilizer (NPKS) were set up. The experimental plot was  $12 \text{ m}^2$  (3 × 4 m) with 3 replicates using a randomized design (Supplementary, Fig. S2A). The application rate of fertilizers in the NPK treatment were  $103.5 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ ,  $11.0 \text{ kg P ha}^{-1} \text{ yr}^{-1}$ , and  $109.7 \text{ kg K ha}^{-1} \text{ yr}^{-1}$ . This was applied as super phosphate + 50% urea + 50% potassium chloride at seeding and the remaining 50% urea + 50% potassium chloride applied at the tillering stage of rice growth. Manure (about  $3750 \text{ kg ha}^{-1}$  as cattle) was applied into the plots in May after the harvest of rice (Table 1). Manure contained: total organic matter 394.2 g kg<sup>-1</sup>, total N 15.8 g kg<sup>-1</sup>, 3.6 g P kg<sup>-1</sup>, 9.5 g K kg<sup>-1</sup>. Rice straw (about 4500 kg ha<sup>-1</sup>, total organic matter 647.4 g kg<sup>-1</sup>, 11.0 g N kg<sup>-1</sup>, 1.7 g P kg<sup>-1</sup>, 16.6 g K kg<sup>-1</sup>, respectively) produced from NPKS plots were returned to the corresponding plots in April after the harvests of rice. Field management measures were consistent with local routine farming measures. After 75 days of rice planting, flooded water was drained. Rice varieties were rotated every 3 to 4 years, which were consistent with local cultivars, and for each experiment plot, 300 rice bushes (15 bundles and 20 clusters) were planted.

#### 2.2. Soil sampling

Three soil cores (0–20 cm, 20–40 cm, and 40–60 cm, respectively) were collected from each plot by a stainless steel soil drill (5 cm

Table 1					
Fertilization	applied	into	the	paddy	fields

Treatment	Fertilizing specie(s)	Amount of pure N kg ha <sup>-1</sup> yr <sup>-1</sup>	Total amount of pure N kg ha <sup><math>-1</math></sup> yr <sup><math>-1</math></sup>
NPK	Chemical fertilizer	103.5	103.5
NPKM	Chemical fertilizer	103.5	119.3
	Manure	15.8	
NPKS	Chemical fertilizer	103.5	114.5
	Straw	11.0	

Chemical fertilizer was applied as urea, super phosphate, and potassium chloride.

diameter and 100 cm depth) in June 2016, prior to rice planting. Then the soil was delivered to the laboratory, homogenized, air-dried and sieved (< 2 mm) for determining of pH, total N, organic matter, salinity (determined as EC) and protease activity. Meanwhile, bulk density circle was used for in situ soil sampling for the determining of bulk density, particle density, capillary porosity and air porosity.

Suction cups (30  $\mu$ m sand filter, 3.2 cm inner diameter, 1 m height) were buried in each plot for in situ sampling of soil solution at three layers (0–20 cm, 20–40 cm, and 40–60 cm, respectively) prior to the rice planting (Supplementary, Fig. S2B). For each depth layer, two suction cups were buried at opposite angles and soil solutions were mixed together. The suction cup allowed smaller molecular substrates to penetrate but prohibited penetration by roots or soils (Supplementary, Fig. S2C). Surface water and soil solution were sampled with an electric pumping device (S30, MND, Guangzhou, China) at 0d (soil solution only), 5d, 10d, 15d, 20d, 25d, 35d, 45d, 60d, and 75d, respectively, during the growth period of rice. Then the liquids were filtered by microporous membrane (0.25  $\mu$ m) for further analysis of total N, NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub><sup>-</sup>, NO<sub>2</sub><sup>-</sup> and FAA-N.

#### 2.3. Soil analysis

#### 2.3.1. Soil parameters

Soil pH was determined using the potentiometric method by a pHmeter (PHS-3E, INESA Scientific Instrument Co., Ltd, Shanghai, China) in a 1:2.5 soil/H<sub>2</sub>O suspension. Soil total N and organic matter were determined using a total carbon analyzer (TOC-V CPH, SHIMADZU, Japan). Soil salinity samples were analyzed using a 1:5 (m/V) extract by a Conductivity Meter (DDSJ-308F, INESA Scientific Instrument Co., Ltd, Shanghai, China). Protease activity analysis was determined per the method of Ladd and Butler (1972). Briefly, 5 g of soil was weighed into a glass vial and 5 mL of Tris buffer (0.05 M, pH 8.1) and 0.5 mL of methylbenzene were added. Fifteen min later, 5 mL of a 2% Na-caseinate solution were added. Then the capped vials were incubated in an incubator at 50 °C for 2 h. After the incubation, the remaining casein was precipitated with 5 mL 15% trichloroacetic acid. The solution was pipetted into a microcentrifuge tube and centrifuged at 10, 000 rpm for 10 min. The clear supernatant was mixed with  $5 \text{ mL Na}_2\text{CO}_3$  (0.4 M) and 1 mL of threefold diluted Folin-Ciocalteu. The tyrosine concentration was measured colorimetrically at 680 nm after exactly 5 min at 37 °C. Soil bulk density, particle density, capillary porosity and air porosity determination was followed as per the description of Lu Lu (2000).

#### 2.3.2. Soil solution parameters

Dissolved inorganic N (DIN), including ammonium  $(NH_4^+)$ , nitrate  $(NO_3^-)$  and nitrite  $(NO_2^-)$ , were determined by a flow injection analyzer (FIA, QC8500, Lachat, Loveland, CO, USA). Total dissolved N in soil solution (TDN) was analyzed using a total carbon analyzer (TOC-L, CPH/CPN, SHIMADZU, Japan). The concentration of DON was

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