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Changes in soil microbial biomass and functional diversity with a nitrogen gradient in soil columns

Fangliang Li[a,b], Ming Liu[a], Zhongpei Li[a,b,*], Chunyu Jiang[a], Fengxiang Han[c], Yuping Che[a]

[a] State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences, Nanjing 210008, China
[b] Graduate University of Chinese Academy of Sciences, Beijing 100049, China
[c] Department of Chemistry and Biochemistry, Jackson State University, 1400 J. R. Lynch St., Jackson, MS 39217, USA

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ABSTRACT

Fertilization generates nutrient patches that may impact soil microbial activity. In this study, nitrogen patches were generated by adding ammonium sulfate or urea to soil columns (length 25 cm; internal diameter 7.2 cm). Changes in nitrogen transformation, soil microbial biomass, and microbial functional diversity with the nitrogen gradients were investigated to evaluate the response of microbial activity to chemical fertilizer nutrient patches. After applying of ammonium sulfate or urea, the added nitrogen migrated about 7 cm. Microbial biomass carbon (MBC) was lower in fertilized soil than in the control (CK) treatment at the same soil layers. MBC increased with soil depth while microbial biomass nitrogen (MBN) decreased. BIOLOG analysis indicated that the average well color development (AWCD) and functional diversity indices of the microbial communities were lower in the 1 cm and 2 cm soil layers after application of ammonium sulfate; the highest values were in the 3 cm soil layer. AWCD and Shannon indices from the 1 to 5 cm soil layers were higher than those from other soil layers under urea application. Both principal component analysis and carbon substrate utilization analysis showed significant separation of soil microbial communities among different soil layers under application of ammonium sulfate or urea. Microbial activity was substantially decreased when NH_4^+-N concentration was higher than 528.5 mg kg^{-1} (1–3 cm soil layer under ammonium sulfate application) or 536.8 mg kg^{-1} (1 cm soil layer under urea application). These findings indicated that changes in soil microbial biomass and microbial functional diversity can occur with a nitrogen gradient. The extent of changes depends on the nitrogen concentration and the form of inorganic fertilizer.

1. Introduction

Nitrogen (N) is one of the most important nutrients in natural and agricultural ecosystems (Krivtsov et al., 2011). Most previous results indicated that fertilizer added to the soil is a theoretical particle that reacts completely and synchronously with soil components (Xue et al., 2006). However, nutrient patches (at a fine scale) and fertilizer bands (at a coarse scale) are formed when granular chemical nitrogen fertilizers are used intensively (Robison et al., 1999; Hodge et al., 2000; Wang et al., 2010). Patches may create special physical, chemical, and biological conditions that affect soil productivity and plant nutrient uptake. Nitrogen migration and transformation are not well understood at the micro-scale (Wang et al., 2010). Therefore, analyzing processes associated with fertilizer at the micro-scale will improve understanding of nitrogen utilization efficiency and nitrogen losses in soil.

As one of the essential components in terrestrial ecosystems, soil microorganisms play important roles in soil nutrient biogeochemical cycling, particularly in nitrogen transformation (Rich and Myrold, 2004; Shen et al., 2010). Nitrogen addition can change soil microbial communities in a relatively short time compared to plant communities (Bradley et al., 2006; Zhang et al., 2008). Given the important roles of soil microorganisms, monitoring the influence of soil nitrogen enrichment on soil microbial communities is essential for understanding changes in ecosystem structure and function (Zhang et al., 2008). However, only a limited number of studies have been reported in this aspect. Lee and Jose (2003) found that microbial biomass carbon decreased in soils with increasing soil nitrogen concentration. Wang et al. (2010) reported that fungi (18:2ω6, 9) showed the least sensitivity to high concentrations of NO_2^--N and NO_3^--N. On the other hand, Shen et al. (2010) noted significantly lower diversity indices under the highest nitrogen rate in the same vegetable season, suggesting that microbial functional diversity decreased with an increase in urea application rate. Zhong and

* Corresponding author at: P.O. Box 821, Institute of Soil Science, Chinese Academy of Sciences, No 71, East Beijing Road, Nanjing 210008, China.
Tel.: +86 25 86881505; fax: +86 25 86881000.
E-mail addresses: mliu@issas.ac.cn (M. Liu), zhpli@issas.ac.cn (Z. Li).

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Cai (2007) found that the application of nitrogen did not directly affect microbial diversity in soil, but did so indirectly by increasing crop yields, which promoted the accumulation of soil organic matter. However, little is known about how soil microbial properties respond to high nitrogen concentrations and different nitrogen fertilizers (Wang et al., 2010).

In the present study, an incubation experiment was carried out with ammonium sulfate and urea applied to the surface of soil columns. Our objectives were to: (1) determine the changes in NH_4^+-N and NO_3^--N concentration, (2) analyze shifts of soil microbial biomass and functional diversity with a nitrogen gradient, and (3) examine the relationship between soil microbial properties and nitrogen concentrations.

2. Materials and methods

2.1. Experimental design

Soil samples (Ultisols and Oxisols in US Soil Taxonomy) were obtained from paddy fields at the Ecological Station of Red Soil, Chinese Academy of Sciences, in Yingtan city, Jiangxi Province of China (28°15′30″N, 116°55′30″E). Soil properties were as follows: pH 4.5, organic carbon 21.30 g kg^{-1}, total nitrogen (N) 1.89 g kg^{-1}, total phosphorus (P) 0.64 g kg^{-1}, total potassium (K) 6.02 g kg^{-1}, alkaline hydrolysis N 160.58 mg kg^{-1}, available P 52.04 mg kg^{-1}, and available K 175 mg kg^{-1}. Air-dried soil was sieved through a 2-mm mesh and homogenized.

The experiment had three treatments, each with three replications. The treatments were as follows: control (CK) without fertilization, ammonium sulfate, and urea. The procedures of the experiment were as follows: (1) microcosm columns made of PVC (polyvinyl-chloride) pipe (length 25 cm, internal diameter 7.2 cm) were filled with soil (soil bulk density 1.25 g cm^{-3}); (2) the soil columns were pre-incubated for 7 days at 25 °C and 30% water-holding capacity (WHC) to allow microbial activity to decline after the initial disturbance (Jenkinson, 1988); (3) ammonium sulfate or urea was applied uniformly on the surface of pre-incubated soils at a rate of 120 mg N kg^{-1} dry soil, respectively, and no fertilizer was applied in the CK; (4) the soil moisture was adjusted to 60% of WHC with deionized water and incubated at 25 °C; (5) all soil columns were covered with plastic film perforated with several pinholes to ensure gas exchange and to avoid rapid water evaporation. Deionized water was added to soil columns once a week during incubation to compensate for water lost through evaporation.

Soil columns were sampled after 35 days of incubation. The different soil layers, i.e. layers at 1, 2, 3, 4, 5, 6, 7, 10, 15, and 20 cm, from the top to the bottom of each soil column were taken using a slice-cutting device.

2.2. Analyses and statistics

Soil organic carbon was determined by the Tyurin method, total N by the semimicro Kjeldahl method, pH by the potentiometric method, and total P and K were digested by HF–$HClO_4$ and determined by molybdenum-blue colorimetric and flame photometry, respectively. Available P and K in the soil were extracted with sodium bicarbonate and ammonium acetate, respectively (Lu, 1999). The NH_4^+-N and NO_3^--N concentrations of the soils were extracted with 2 mol l^{-1} KCl and measured by a colorimetric method (Lu, 1999) using a continuous-flow auto-analyzer (Auto Analyzer III, Bran + Luebbe GmbH, Germany).

Soil microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) were measured by the fumigation-extraction method using an automatic analyzer (Multi N/C 3100 TOC/TN, Jena, Germany) (Vance et al., 1987).

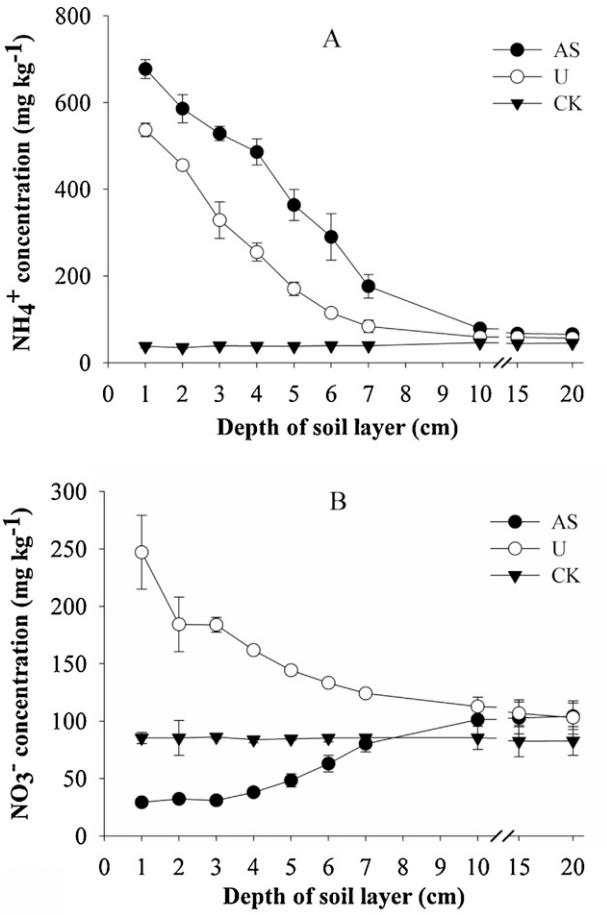

Fig. 1. Changes in NH_4^+-N (A) and NO_3^--N (B) concentrations in soil layers under ammonium sulfate or urea application (AS, ammonium sulfate; U, urea; CK, control). Error bars represent the standard deviations of the means ($n = 3$).

Functional diversity of the microbial communities in soil was estimated by the BIOLOG method (Garland and Mills, 1991; Zak et al., 1994; Campbell et al., 1997; Insam, 1997a, 1997b). Five grams of fresh soil were suspended in 50 ml of 0.85% sterile NaCl solution, shaken for 30 min on a reciprocal shaker, and diluted 200-fold. Aliquots of 150 μl of the diluted sample were inoculated directly into EcoPlates (BIOLOG, Hayward, CA, USA) and incubated at 25 °C in the dark without shaking. Plates were read every 24 h at 590 nm for 168 h using the Microlog Re1 4.2 software. The average well color development and functional diversity were calculated according to Harch et al. (1997).

All results were expressed on the basis of the oven-dry soil weight. All statistical analyses were conducted using SPSS 13.0 for Windows. Means and standard deviations were compared by one-way analysis of variance (ANOVA), and significant differences were analyzed by Duncan's method. The representation and graphical fits of experimental data were obtained using SigmaPlot 10.0.

3. Results

3.1. Changes in inorganic nitrogen concentrations in soil columns

After fertilizer application on the surface of soil columns, nitrogen migration and transformation caused changes in the concentration of NH_4^+-N and NO_3^--N in soil layers (Fig. 1). NH_4^+-N and NO_3^--N concentrations in the control (CK) treatment did not differ significantly ($P > 0.05$) across any soil layer (Fig. 1A and B). After application of ammonium sulfate (AS) or urea (U), the NH_4^+-N