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Irrigation frequency alters the abundance and community structure of ammonia-oxidizing archaea and bacteria in a northern Chinese upland soil

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ABSTRACT

Ammonia oxidation, the first and rate-limiting step in nitrification, is a key process in biogeochemical nitrogen cycling. However, little is known about how irrigation affects ammonia oxidizing microorganisms in agroecosystems. In this study, effects of irrigation frequency on soil potential nitrification activity (PNA), the abundance and composition of ammonia-oxidizing archaea (AOA) and bacteria (AOB) were investigated using real-time PCR (qPCR), terminal restriction fragment length polymorphism (T-RFLP), and clone libraries for characterizing the ammonium monooxygenase genes (*amoA*) in a northern Chinese soil. Results showed that irrigation significantly increased soil PNA and archaeal *amoA* gene abundance but decreased bacterial *amoA* gene abundance. Soil PNA was positively correlated with archaeal *amoA* gene abundance and negatively correlated with bacterial. T-RFLP and PCA results showed that irrigation greatly changed the AOA and AOB community structures by altering the relative abundances of four T-RFs of AOA and four other T-RFs of AOB, respectively. Phylogenetic analysis revealed that all archaeal sequences fell into Group 1.1b, and the bacterial clones were dominated by *Nitrosospira*-like sequences within Cluster 3a.2. Irrigation induced the appearance of two archaeal and two bacterial OTUs and increased the relative abundance of another two archaeal and four bacterial OTUs, respectively. Additionally, soil moisture, soil pH, NH_4^+-N, and NO_3^--N correlated significantly with the AOA community, and soil pH, total nitrogen (TN), and NO_3^--N were significantly correlated with the AOB community. Our results demonstrate that irrigation greatly affected the abundance and community structure of AOA and AOB and that AOA appeared to play a more important role in nitrification in the study soil.

1. Introduction

China is experiencing a serious challenge to its water resource within the last few decades. On a per capita basis, water availability per annum is one-third and one-fourth of the average of the developing countries and world, respectively [1]. Due to uneven distribution of precipitation and greater demands for domestic water and agricultural production, people in northern China suffer greatly from water scarcity issues [2]. How to enhance the utilization efficiency of water in agriculture has attracted significant attentions, and reducing irrigation during the wheat growing season is a frequently used option in this area. However, the reduction in irrigation frequency could have critical impacts on soil microbial communities and their ecosystem services.

Nitrification is a microbiologically mediated process and an essential component of the global nitrogen cycling [3] that regulates the use efficiency of fertilizers and leads to N losses from the soil through leaching of nitrogen into groundwater and emissions of greenhouse gases [4,5]. Ammonia oxidation, the primary and rate-limiting step of nitrification, is known to be performed by two different prokaryotic groups: i) chemoautotrophic ammonia-oxidizing bacteria (AOB) from the phylum Proteobacteria, class β-but also class γ-Proteobacteria [6,7]; and ii) ammonia-oxidizing archaea (AOA) belonging to the Thaumarchaeota phylum [8–10]. The *amoA* genes, which encode the α-subunit of ammonia monooxygenase (AMO) that catalyzes the ammonia oxidation process. They are found in both AOA and AOB and are frequently used as biomarkers for studying the abundance and diversity of ammonia oxidizing microorganisms [6,11].

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Table 1
Soil properties and potential nitrification activity (PNA) in different irrigation treatments.

Treatment	Moisture (%)	pH (H_2O)	SOC/(g kg^{-1})	TN/(g kg^{-1})	NH_4^+-N/(mg kg^{-1})	NO_3^--N/(mg kg^{-1})	PNA/(mg NO_3^--N kg^{-1} h^{-1})
CK	11.83 ± 0.08 c	7.25 ± 0.02 c	14.43 ± 0.22 a	1.51 ± 0.03 b	3.35 ± 0.28 b	1.47 ± 0.33 c	2.53 ± 0.13 c
I1	12.14 ± 0.02 b	7.58 ± 0.01 b	14.25 ± 0.10 a	1.64 ± 0.03 a	4.28 ± 0.20 a	5.42 ± 0.27 b	3.48 ± 0.04 b
I2	16.34 ± 0.03 a	7.73 ± 0.01 a	14.42 ± 0.31 a	1.69 ± 0.04 a	1.33 ± 0.03 c	8.03 ± 1.06 a	3.84 ± 0.10 a

CK, no irrigation; ***I1***, irrigated once at the jointing stage of wheat; and ***I2***, irrigated twice at the jointing and filling stages of wheat.
Values are mean ± standard deviation ($n = 3$). The different letters indicate significant differences at the 0.05 probability level.

AOA are widespread in most ecosystems and frequently dominate the population over AOB [12–14], suggesting that AOA may play a more important role in nitrification in these ecosystems. Normally, numerical dominance is associated with functional importance, but some studies [15,16] suggest that soils can be quantitatively dominated by AOA but functionally dominated by AOB. Soils typically contain a large number of microbial cells which have no active contributions to ecosystem functioning [17]. Therefore, the relationship between AOA and AOB abundances and their relative contributions to nitrification may differ greatly according to the soil conditions.

Irrigation water quality greatly affects the AOB community in cultivated plots [18,19] and precipitation dramatically influences the abundance and composition of the AOB community in a typical steppe [20], as water addition increased AOA but not AOB abundance in dry sub-humid ecosystems [21,22]. Considering physiological differences toward oxygen [7], water managements may have discrepant impacts on ammonia oxidizing microorganisms in different soil conditions.

In this study, we therefore investigated soil potential nitrification activity (PNA) and the abundance and structure of the AOA and AOB communities in soils exposed to three different irrigation regimes. We aimed to investigate: i) how soil potential nitrification activity (PNA) and the AOA and AOB communities respond to irrigation frequency; and ii) which soil properties would be the key factors affecting the AOA and AOB communities.

2. Materials and methods

2.1. Field site and experimental description

The irrigation experiment was performed at the Wuqiao Experimental Station of China Agricultural University (37°37′N, 116°23′E), Hebei Province, China. The study location has a warm temperate semi-humid continental monsoon climate with a mean annual frost-free period of 201 d. Average annual precipitation, temperature, and sunshine time are 562 mm, 12.6 °C, and 2724.8 h, respectively. The soil is classified as Calcaric Fluvisol [23] with a sandy clay loam texture.

The experiment on irrigation frequency was conducted in a wheat-maize rotation system dating from 2000 and that has maintained the same irrigation regime every year since then during the wheat growing season and no irrigation during the maize growing season. Three treatments with three replicates for each treatment were administered in a random plot design. The treatments were as follows: i) control without irrigation, CK; ii) irrigated once at the jointing stage of wheat, I1; and iii) irrigated twice at the jointing and filling stages of wheat, I2. All treatments received a volume of 750 m^3 ha^{-1} irrigation before the wheat was sowed and at each irrigation time. The fertilizers were 225 kg urea [$(NH_2)_2CO$] ha^{-1}, 300 kg diammonium phosphate [$(NH_4)_2HPO_4$] ha^{-1} and 150 kg potassium sulfate [K_2SO_4] ha^{-1} for all treatments per year, and all fertilizers were all applied as basal fertilizer.

2.2. Soil sampling

Soil samples (0–20 cm) were collected from each replicate plot in June 2015 after the wheat was harvested. Five soil cores were randomly taken from each plot and mixed together to form one composite sample for each replicate. All soil samples were passed through a 2.0-mm sieve to remove wheat root and then divided into two parts; one part was used for analyses of soil chemical properties, and the other part was stored at −80 °C for DNA extraction.

2.3. Soil chemical properties analysis

Soil moisture was measured by oven-drying at 105 °C for 24 h. Soil pH was measured at a water to soil ratio of 2.5:1 using a pH meter (FE28, Mettler Toledo, USA). Soil nitrate and ammonium were extracted from fresh soil with 2 M KCl extraction buffer at a solution to soil ratio of 2.5:1 and determined by a Continuous Flow Analyzer (Skalar + Analytical, Holland, The Netherlands). Soil organic carbon (SOC) and total nitrogen (TN) contents were determined by the $K_2Cr_2O_7$ oxidation-reduction titration method and Kjeldahl digestion method [24], respectively.

2.4. Soil potential nitrification activity

Soil potential nitrification activity (PNA) was determined according to the standard protocol described by Hart et al. [25]. Briefly, 10 g of fresh soil was placed in a 250-mL Erlenmeyer flask with 100 mL of a 1.5 mM NH_4^+ and 1 mM phosphate buffer with the pH adjusted to 7.2. The slurry was shaken on a shaker in the dark at 180 rpm for 24 h at 25 °C to maintain aeration. Aliquots of 5 mL were subsequently removed using a pipette at 2, 6, 12 and 24 h after the start of the incubation. The aliquots were then centrifuged, and the supernatant was filtered and stored at −80 °C until analysis. The NO_3^--N concentrations were determined using a Continuous Flow Analyzer (Skalar + Analytical, Holland, The Netherlands) after which the PNA was calculated from the rate of linear regression of NO_3^--N concentrations over time (mg NO_3^--N g^{-1} h^{-1}).

Table 2
Pearson correlation coefficients among soil properties, potential nitrification activity (PNA), and archaeal and bacterial *amoA* gene abundances.

Item	Moisture	pH	TN	NH_4^+-N	NO_3^--N	SOC	AOB abundance	AOA abundance
AOA abundance	**0.931****	**0.837****	**0.803****	**−0.790***	**0.898****	0.235	−0.476	–
AOB abundance	−0.414	**−0.829****	**−0.812****	0.080	**−0.734***	0.150	–	−0.476
PNA	**0.744***	**0.991****	**0.956****	−0.439	0.359	−0.071	**−0.865****	**0.828****

**$P < 0.01$; *$P < 0.05$. Significant correlations ($P < 0.05$) are highlighted in bold.